EFFECTS OF MACRONUTRIENTS AND PHYSICOCHEMICAL PROPERTIES OF CARBOHYDRATES ON GLYCAEMIC INDICES (GI) OF SOME SRI LANKAN FOODS

By

Usha Pushkala Kumari Hettiaratchi

Ph.D. 2009
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By

Usha Pushkala Kumari Hettiaratchi

Thesis submitted to the University of Sri Jayewardenepura for the award of the degree of Doctor of Philosophy in Biochemistry on 02\textsuperscript{nd} July, 2009.
DECLARATION

The work described in this thesis was carried out by me under the supervision of Dr. S. Ekanayake and Prof. J. Welihinda and a report on this has not been submitted in whole or in part to any University or any other Institution for another Degree/Diploma.

U.P.K. Hettiaratchi

Date

We certify that the candidate has incorporated all possible corrections, amendments and additions recommended by the examiners.

Dr. Sagarika Ekanayake
Prof. J. Welihinda
CERTIFICATION

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

Dr. S. Ekanayake

Prof. J. Welihinda
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<tr>
<td>AUC</td>
<td>Area under curve</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
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<tr>
<td>CVD</td>
<td>Coronary vascular disease</td>
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<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>FAO</td>
<td>Food and Agricultural Organization</td>
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<tr>
<td>FFA</td>
<td>Free fatty acids</td>
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<tr>
<td>FSG</td>
<td>Free sugar glucose</td>
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<tr>
<td>GER</td>
<td>Gastric emptying rate</td>
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<tr>
<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<tr>
<td>GLP-1</td>
<td>Glucagons-like peptide 1</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose dependent insulinitrophic peptide</td>
</tr>
<tr>
<td>GI</td>
<td>Glycaemic index</td>
</tr>
<tr>
<td>GL</td>
<td>Glycaemic load</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HI</td>
<td>Hydrolysis index</td>
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<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<td>IAUC</td>
<td>Incremental area under curve</td>
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<td>IDF</td>
<td>Insoluble dietary fibre</td>
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<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>IDDM</td>
<td>Insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin like growth factor-1</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>II</td>
<td>Insulinaemic index</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
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<tr>
<td>NIDDM</td>
<td>Non insulin dependent diabetes mellitus</td>
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<td>RAG</td>
<td>Rapidly available glucose</td>
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<tr>
<td>RDS</td>
<td>Rapidly digestible starch</td>
</tr>
<tr>
<td>RS</td>
<td>Resistant starch</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>SAG</td>
<td>Slowly available glucose</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Slowly digestible starch</td>
</tr>
<tr>
<td>SDF</td>
<td>Soluble dietary fibre</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>TDF</td>
<td>Total dietary fibre</td>
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<td>WAI</td>
<td>Water absorption index</td>
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<td>WSI</td>
<td>Water solubility index</td>
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<td>WHO</td>
<td>World Health Organization</td>
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ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor Dr. Sagarika Ekanayake, Department of Biochemistry, Faculty of Medical Sciences; University of Sri Jayewardenepura for her continuous support, guidance and advice given throughout my Ph.D. programme. She was a great source of strength to me during carrying out experimental work, solving practical problems, and writing up processes. I am very grateful for her patience, enthusiasm and motivation during supervision as well as all other times.

My greatest gratitude to my supervisor, Prof. Jayantha Welihinda, Department of Biochemistry and Molecular Biology, University of Colombo for his support, guidance, advice and motivation given during my postgraduate degree programme.

I wish to express my gratitude to everyone who volunteered to participate in this study for devoting their time and being patient throughout the period. Without their selfless contribution this achievement would not have been possible.

I wish to acknowledge the financial assistance given by IFS E3941/1 grant, Sweden, NSF RG/2005/AG/10 grant, NRC 05-03 grant and IPICS Sri-07 grant.

I wish to express my gratitude to Emeritus Professor E.R. Jansz for the encouragement and all the advice given especially at the initial stages of this project.
I wish to thank Prof. Hemantha Peiris, Head/Department of Biochemistry for arranging for study leave to complete my PhD study programme and all academic and non academic staff of Department of Biochemistry for the support given at needed times.

I wish to extend my gratitude to Dr. W.M.A.D.B. Wickramasinghe (Rice Research Institute, Batalagoda) for providing me with the rice samples.

I wish to thank Prof. Renu Wickramasinghe, Head, Department of Parasitology, FMS, USJP for giving permission to use the Parasitology laboratory and facilities and Mr. W.D.I Thilakarathna for the assistance given when using the microscope.

I wish to acknowledge Prof. M.S.A Perera, Department of Family Medicine, FMS, USJP for the assisting given in enrolling type 2 diabetic patients and allowing me to use Family Practice Centre for the study. I would like thank Dr. Malkanthi Galhena, Mrs. L.K.D.T. Dassanayake, Mrs. Amitha Jayawardena and other non academic staff members of Family Practice Centre, FMS, USJP for the kind cooperation given during my study at Family Practice Centre.

Also I wish to acknowledge the consultant medical officer and the staff of Diabetes Clinic, Colombo South Teaching Hospital for their assistance given in enrolling type 2 diabetic patients for the study.
I wish to acknowledge Mr. Samanatha, National Research Council for the assistance given in developing the web site.

I wish to sincerely thank all my research colleagues at the Research Lab, Department of Biochemistry, FMS for their support and sharing their valuable time at critical stages.

Finally I would like to thank my parents and my husband for their unconditional support, encouragement given throughout the study. I wish to especially thank my little son for tolerating me through this difficult period, when he was not getting enough attention from his mother.

I dedicate this thesis to my family.

U.P.K. Hettiaratchi

June, 2009.
ABSTRACT

EFFECTS OF MACRONUTRIENTS AND PHYSICOCHEMICAL PROPERTIES OF CARBOHYDRATES ON GLYCAEMIC INDICES (GI) OF SOME SRI LANKAN FOODS

U.P.K. Hettiaratchi

The glycaemic index (GI) concept ranks basic foods and mixed meals according to the blood glucose response following ingestion of foods. Low GI foods with slow and prolonged glycaemic responses are reported to be beneficial for diabetic patients and in general for non-diabetic individuals. The recent reports imply a markedly high prevalence of diabetes mellitus (DM) in Sri Lanka among both urban (16.4%) and rural populations (8.7%) with one in five adults having either diabetes or pre-diabetes.

Currently, GI values of only basic foods are available with little or no information regarding frequently consumed Sri Lankan mixed meals. The availability of such data will be of value for clinicians and dieticians in planning meals for diabetic patients. The present study was therefore designed to determine the GI values of frequently consumed Sri Lankan foods and mixed meals with healthy individuals. Further, the physicochemical properties (factors) contributing to the differences in GI of foods and the effect of edible portion of fibre on GI were studied. An in vitro method was also used to predict the glycaemic responses of mixed meals. The present study further determined the glycaemic and insulinaemic responses to selected foods with type 2 diabetic patients and the second
meal effect of breakfast meals on a subsequent lunch meal as the data regarding glucose and insulin responses to Sri Lankan foods with diabetic patients are not available. GI values of foods were determined according to Brouns et al., (2005), FAO/WHO, (1998), physicochemical factors by AOAC, standard methods and rapidly and slowly available glucose and starch fractions using a modified method of Englyst et al., (2000). In vitro starch hydrolysis was carried out according to Granfeldt et al., (1992).

GI of foods with healthy individuals were as follows: bread varieties [wholemeal bread (103±11), ordinary bakery bread (114±9)], mixed meals [wholemeal bread & lentil curry meal (87±6), red rice & kiri hodi meal (99±10), red rice mixed meal (60±5), string hopper meal (wheat flour) (104±7), string hopper meal (red rice flour) (103±11), manioc meal (120±9), jackfruit meal (75±11)], and bananas [kolikuttu (61±5), embul (61±5), anamalu (67±7), seeni kesel (69±9)]. The red rice mixed meal, jackfruit meal and bananas can be categorized as low GI, wholemeal bread & lentil curry meal as medium GI and all other foods as high GI foods. The rice mixed meal yielded the lowest GI among the foods analyzed.

The GI values obtained using a bread of Sri Lankan origin as the standard can be converted to GI values expected against glucose by a conversion factor of 1.34. This enables reporting GI data with respect to glucose for comparison with the internationally reported values.

The GI values determined with the conventional enzymatic kit method were not significantly different (p>0.05) to that of the GI values calculated using a glucometer...
(Accu-Check Active, Roche Diagnostics GmbH, Germany) thus indicating the potential to use this particular glucometer when determining GI values.

Significant negative correlations ($p<0.05$) were observed with GI & insoluble dietary fibre (IDF) ($p=0.032$), soluble dietary fibre (SDF) ($p=0.010$) and total dietary fibre (TDF) ($p=0.038$) contents of 50 g available carbohydrate portions of basic foods and mixed meals.

Protein and amylose contents of portions given for determination of GI indicated non significant negative relationships ($p=0.165$ and $p=0.054$ respectively) with GI values.

The wet heat processed foods analyzed in this study that elicited high GI values had disintegrated starch granules compared with the raw flour. The observed effect on the starch granules of the foods studied in this thesis was due to the gelatinization of starch granules during wet processing highlighting the impact of processing methods on GI for the foods concerned.

When the rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS) and slowly digestible starch (SDS) contents of portions given for determination of GI in basic foods and mixed meals were correlated with GI values, significant positive correlations ($p<0.05$) were observed with RAG ($p=0.023$), RDS ($p=0.011$) fractions and significant negative correlation with SAG/RAG ($p=0.036$) ratio. This indicates that the rapidly available carbohydrates of a food/meal could be taken as a food related determinant of GI.

As a novel approach a standard $in vitro$ method was used to predict GI values of mixed meals by estimating the hydrolysis indices (HI) of composite meals. The $in vitro$ HI values
indicated a significant positive correlation (p=0.0001) with in vivo GI values of both basic foods and mixed meals.

The GI of a rice meal declined by 9% with an increase of 7.2% TDF content as meal accompaniments of a part of a rice meal.

Three breakfast meals (chickpea, red rice meal, atta roti) were given to type 2 diabetics. GI values of three meals were 40±7 (low GI), 64±11 (low GI), 88±9 (medium GI) and the insulinaemic indices (II) were 76±13, 90±20 and 115±28 respectively. The glycaemic responses of the meals analyzed indicated a positive linear relationship (r=0.9838) with the corresponding insulinaemic responses. The GI values of the meals with diabetic patients were not significantly different (p>0.05) from healthy individuals but slightly higher than the values reported with healthy individuals. The peak serum glucose levels of chickpea, rice and roti declined by 27%, 13%, 4% respectively compared with the standard (bread).

The effects of above breakfast meals on the glycaemic and insulinaemic responses of a standard rice lunch meal (second meal effect) were analyzed. According to the results obtained none of the breakfast meals elicited an effect on the subsequent lunch.
1. INTRODUCTION

Carbohydrates constitute a major source of energy in the diet of people in developing countries. The contribution to total energy from carbohydrates worldwide vary from 40-80% (Englyst and Cummings, 1986). However, recent recommendations insist on a 55% total energy content from complex carbohydrates representing different starchy sources (FAO/WHO, 1998).

Carbohydrates in a meal are recognized to be the main factor contributing to the postprandial glycaemic responses. However, foods containing identical amounts of carbohydrates have elicited different postprandial glycaemic responses (Arvidsson-Lennner et al., 2004). These differences have been attributed to various intrinsic food factors and extrinsic physiological factors (Vosloo, 2005).

Thus, to categorize carbohydrate rich foods according to the postprandial glycaemic responses the “Glycaemic Index” (GI) concept was introduced in 1981 (Jenkins et al., 1981). This concept allows categorization of foods as low, medium and high GI foods (Beals, 2005).

Consumption of high GI foods for long durations is reported to be responsible for the development of insulin resistance leading to type 2 diabetes (Wolever et al., 1993). That could further lead to the development of diseases such as cardiovascular disease, renal disease, retinopathy and cancer.

The prevalence of diabetes mellitus (DM) is rapidly increasing in the world with 171 million reported cases of adults in 2000. The figures are expected to rise to 366 million by
2030 (Wild et al., 2004) with a more prominent rise in developing (170%) rather than in developed countries (42%) (King et al., 1998; Hossain et al., 2007). The majority of individuals affected in developing countries is in the age range of 45-64 years (Wild et al., 2004) who are still in their productive years.

This health issue is a burden on the economy of Sri Lanka with a markedly high prevalence observed in both the urban (16.4%) and rural (8.7%) populations. Further, the prevalence of pre-diabetes among urban and rural populations are 13.6% and 11.0% respectively (Katulanda et al., 2008).

Thus, preventive measures of DM are necessary. Some recognized options, mainly for type 2 DM patients are healthy life style, diet and increased physical activity (Chowdhury et al., 2003). Type 2 DM patients undergo dietary manipulations and are advised to avoid foods which give rapid and high blood glucose responses as these foods will in turn stimulate high insulin responses. Therefore, knowledge of the blood glucose raising potentials of commonly consumed basic foods and mixed meals is essential to avoid excessive blood glucose response. This practice will be beneficial not only for diabetic (Venn and Green, 2007) but also for non diabetic individuals (Vosloo, 2005) to avoid an excessive weight gain.

Short term studies have shown that low GI diets are beneficial in improving blood glucose control in both type 1 and type 2 DM patients (Jenkins et al., 1981; Jarvi et al., 1999; Wolever et al., 1993). These studies have also been shown to reduce serum lipids in diabetic, non diabetic, hypertriglyceridemic individuals, (Wolever et al., 1992; Bornet et al., 1987) as well as improve insulin sensitivity (Frost et al., 1994).
Although there are data on GI of certain basic Sri Lankan foods (Hettiarachchi et al., 2001; Widanagamage, 2007) sufficient information is not available on mixed Sri Lankan meals making it difficult for the medical practitioners and dieticians to plan meals for the impaired glucose tolerant and diabetic individuals in Sri Lanka. Thus, the present study attempted to cover this inadequacy of GI data on typical Sri Lankan mixed meals with healthy individuals, type 2 diabetic patients and to correlate factors responsible for variations in GI values. Further, as a novel approach an in vitro method was used to predict the glycaemic responses of mixed meals.

1.1 Scope and objectives of the study:

The work carried out in this thesis is categorized into four general objectives.

General objective 1:

To determine the in vivo glycaemic indices of a variety of foods with healthy individuals.

Specific objectives:

1.1 To determine the GI of basic foods (02 bread varieties), mixed meals [red rice & kiri hodi meal, red rice mixed meal, wholemeal bread & lentil curry meal, string hopper meal (wheat flour), string hopper meal (red rice flour), manioc meal, jackfruit meal] and banana varieties (kolikutu, embul, anamalu, seeni kesel).

1.2 To calculate the conversion factor that can be used to convert GI values obtained with one standard (bread) to the values expected with the other standard (glucose).
1.3 To study the feasibility of using a glucometer to determine GI values.

1.4 To establish a database containing GI values of Sri Lankan foods, in the absence of such a database.

General objective 2:
To determine the effect of physicochemical properties on the differences in GI values of selected Sri Lankan foods.

Specific objectives:

2.1 To determine the physical properties [Water solubility index, water absorption index, starch granules (microscopic study)] of foods involved in the current study.

2.2 To determine the chemical properties [moisture, ash, digestible starch, undigestible starch, soluble dietary fibre, insoluble dietary fibre, protein, fat, amylose, amylopectin] of foods involved in the current study and glucose or starch fractions [rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS), slowly digestible starch (SDS)] of selected basic foods and mixed meals.

2.3 To identify the relationships between physicochemical factors contributing towards low or high GI values.

2.4 To study the effect of dietary fibre from different sources in the same meal (by maintaining edible portion sizes) on GI.
General objective 3:
To determine the applicability of using an *in vitro* method to determine glycaemic responses of basic foods and mixed meals.

Specific objectives:
3.1 To use an existing *in vitro* starch hydrolysis procedure to determine the rate of hydrolysis of starch of basic foods.
3.2 As a novel approach, to apply this method to predict glycaemic responses of mixed meals.

General objective 4:
To determine the glycaemic and insulinaemic responses to selected foods with type 2 diabetic patients.

Specific objectives:
4.1 To determine the postprandial glycaemic and insulinaemic responses to breakfast meals i.e., chickpea, atta roti, red rice meal.
4.2 To determine the effect of above breakfast meals on a standard lunch meal with the same category of patients (second meal effect).
2. LITERATURE REVIEW

2.1 Glycaemic Index

In early 1900s it was observed that the postprandial glycaemic response to a starchy meal was similar to a meal rich in sugars (Maclean and de Wesselow, 1921). However, for more than 50 years, it was assumed that the postprandial blood glucose responses to complex starchy foods (polysaccharides) were less than that for the food items containing simple sugars such as mono or disaccharides (Bornet et al., 1997). Later in 1970s and 80s it was reported that identical quantities of different complex carbohydrates elicit markedly different postprandial glucose and insulin responses (Crapo et al., 1977; Crapo et al., 1981). These observations led to the introduction of the “Glycaemic Index” (GI) concept in 1981 (Jenkins et al., 1981). GI is defined as “the Incremental Area Under the blood glucose Curve (IAUC) after the ingestion of a 50 g available carbohydrate load of a test food, expressed as a percentage of the IAUC of an equal amount of a standard (generally glucose or white bread)” (Beals, 2005).

GI estimates the blood glucose increasing potentials of carbohydrate rich foods as all carbohydrates (starch, lactose, sucrose, maltose) are metabolized to glucose mainly to give rise to a transient increase in blood glucose levels. However, the rate of increase of blood glucose varies with different foods. This led to categorization of foods as low, medium and high GI foods. Foods that have a low GI yield a slow and prolonged glycaemic response. The dietary fibre hypothesis put forward in 1977 (Burkitt and Trowell, 1977) stressed the fact that foods that are slowly absorbed might have benefits with regard to metabolic
diseases such as diabetes and coronary heart disease (CHD). The GI concept was also an extension of the above mentioned dietary fibre hypothesis (Jenkins et al., 2002).

2.2 Determination of Glycaemic Index values of foods in vivo

In order to obtain the maximum practical use of GI values the in vivo methodology was standardized while allowing certain options within the standard protocol (Brouns et al., 2005).

2.2.1 Quantity of carbohydrate

Following many experiments based on glycaemic responses of foods the quantity of carbohydrate for GI determination was standardized as either a 25 or 50 g available carbohydrate portion. A portion containing 50 g carbohydrate load is given except when the portion size is too large to be consumed (Bornet et al., 1997; Brouns et al., 2005).

The 50 g available carbohydrate portion is calculated excluding carbohydrates that cannot be digested and absorbed in the human small intestine (i.e., dietary fibre, resistant starch and other carbohydrates) (Wolever et al., 2003; Beals, 2005; Brouns et al., 2005) while all the carbohydrates that provide sugars for absorption in the small intestine are taken into account (Brouns et al., 2005).

2.2.2 Time of the day to carry out GI study

The duration of fasting prior to GI testing has been recommended as 10-12 hours overnight fast, preferably starting the test before 10 am (Wolever et al., 1988; Brouns et al., 2005).
This time period has been proposed to reduce the intra individual variations at different times of the day and the influences of the previous meal (breakfast) on lunch meal (Brouns et al., 2005).

2.2.3 Number of subjects to be employed in a GI study

The GI measurement with less than 10 subjects had indicated a higher margin of error compared with ≥10 individuals. The margin of error was a plateau when 10-40 individuals were included (Brouns et al., 2005). Thus, it is recommended to include 10 subjects (preferably >10).

2.2.4 Health status of the subjects

The GI values obtained with non-diabetic, insulin dependent diabetes mellitus patients (IDDM—type 1 DM), and non insulin dependent diabetes mellitus (NIDDM—type 2 DM) patients (Wolever et al., 1991; Bornet et al., 1997) have not shown significant differences in GI values for similar foods. Thus, healthy, non-diabetic individuals, both males and females (Wolever et al., 2003) are recommended for routine determination of GI (Brouns et al., 2005). When estimating GI, subject characteristics such as age, body mass index (BMI), sex or ethnicity are not recognized as factors that need to be controlled (Wolever et al., 2008).
2.2.5 Duration an individual could be included in the study

With regard to the individuals participating in the study, the maximum duration an individual can be recruited is stated as 4 months. Thus, to represent the variations within this period (up to 4 months) the standard should be given initially and at 6-8 weeks later (Wolever et al., 1991; Brouns et al., 2005). However, if any individual participates for a further period of time the standard should be given accordingly.

2.2.6 Preparation of subjects

Different dinner meals, physical exercises on the day prior to GI testing have shown to influence glycaemic responses to foods. Certain low GI dinner meals have produced better glucose tolerance in the following morning compared with high GI dinner meals (Wolever et al., 1988; Thorburn et al., 1993) due to the colonic fermentation of low GI foods (Nilsson et al., 2008c). However, some low GI foods have not shown this effect (Brouns et al., 2005; Granfeldt et al., 2005). Acute physical exercise on the day prior to the test has shown to improve insulin sensitivity for 48 hours (Mikines et al., 1988). However, this did not have any effect on systemic fasting plasma glucose levels which was shown to differ significantly in post exercise compared to rest (Brouns et al., 2005). Alcohol consumption may have an effect on glucose homeostasis as moderate consumption of alcohol has shown to inhibit gluconeogenesis and hepatic glucose output (Shelmett et al., 1988; Brand-Miller et al., 2007). However, a randomised crossover study carried out to analyze the effect of moderate alcohol consumption on the night before glycaemic testing had not shown significantly different (p>0.05) glycaemic responses with or without alcohol (Godley et al., 2005).
Cigarette smoking is reported to cause insulin resistance (Attvall et al., 1993; Frati et al., 1996) and increase the glycaemic response. Thus, to minimize variations of GI values it was recommended to control unusual vigorous activity on the day before and subjects to have their normal diets (Wolever et al., 2008).

2.2.7 Standard foods given in GI studies

Glucose was the first standard used in determination of GI (Jenkins et al., 1981; Wolever et al., 1985). At present, local white bread is also used as a standard. Bread is preferred as the standard against glucose as bread follows the physiological digestion and absorption in the human gastrointestinal tract as other test foods (represent a true meal) (Wolever et al., 2003) and glucose has a high osmolarity (Jenkins et al., 1981; Brouns et al., 2005) and can cause nausea in some individuals. Thus, the GI values can be determined with either glucose or local white bread and interconverted with a ratio of 1.4.

\[
\text{GI values (obtained with bread)} = 1.4 \times \text{GI values (expected with glucose)}
\]

This ratio ranges over 1.22-1.58 because of the differences in composition and preparation of bread used in different parts of the world (Bornet et al., 1997). However, the inter laboratory study carried out using local bread from different countries observed similar GI values for the foods analyzed (Wolever et al., 2003). Regardless of the standard used, the GI values are advised to be expressed with reference to glucose for the easiness of
comparison of similar foods from different parts of the world (FAO/WHO, 1998; Brouns et al., 2005). Rice also had been considered in using as a standard in determining GI values (Sugiyama et al., 2003). However, it is not commonly used as a standard at present because of the wide variations of rice varieties available and differences in preparation.

2.2.8 Number of times the standard should be given
As GI to test foods depend on the responses of the standard, it is recommended to give the standard to individuals at least twice. This is mainly to reduce the intra individual day to day variations (Brouns et al., 2005).

2.2.9 Other components of the meal
When considering other components of the meal given for determination of GI, a drink of 250 mL is recommended to be included with the test and the standard. Although the drink can be water, tea or coffee (Wolever et al., 2008) water is recommended as ingestion of caffeine (375 mg) had significantly increased c-peptide, insulin and glucose IAUC by 24% thereby decreasing insulin sensitivity (Brouns et al., 2005). However, daily consumption of coffee (~ 4 cups) and black tea (~1 cup) have shown to lower the risk of type 2 diabetes (Odegaard et al., 2008).

2.2.10 Time of ingestion of food
The time of ingestion of liquid foods is recommended as 5-10 minutes while for solid foods 10-20 minutes. The time given for ingestion of food affects the glycaemic response
in both normal and diabetic individuals as with increased time blood glucose response become flattened (Jenkins et al., 1992).

2.2.11 Blood sampling time

The collection of postprandial blood samples are timed after taking the first bite or sip of the meal (Brouns et al., 2005). It is recommended to take blood samples every 15-30 minutes (fasting, 15, 30, 45, 60, 90, 120 min) for two hours following ingestion in non diabetic subjects (Beals, 2005). When blood samples are taken for less than 2 hours in non diabetic subjects it had significantly influenced the mean and coefficient of variation (CV) of area under curve (AUC) values (Wolever, 2004; Brouns et al., 2005). However, three hours are recommended for subjects with diabetes at 30, 60, 90, 120, 150, 180 minute intervals (Wolever et al., 1991; Brouns et al., 2005).

2.2.12 Source of blood

The blood samples can be sampled either from a venous or capillary. The variation of glucose concentration in venous is reported to vary widely with a CV of >50% compared with <30% in capillary blood (Wolever, 2004; Brouns et al., 2005). Thus, the sensitivity of glucose measurement is supposed to be greater with capillary blood sampling (Granfeldt et al., 1995b). However, the site from which capillary blood is taken will also show variations in blood glucose values. Fingertip capillary blood samples show higher values when glucose concentrations are rising compared to other sites including forearm, thigh and abdomen (Ellison et al., 2002; Jungheim and Koschinsky, 2002) and lower values with
decreasing blood glucose concentrations (Jungheim and Koschinsky, 2002). Although, fingertip capillary blood samples are preferred to other sites (Brouns et al., 2005) even the use of venous blood is accepted (FAO/WHO, 1998).

2.2.13 Analysis of glucose
Glucose concentrations of collected blood samples can be estimated using laboratory enzymatic methods with whole blood or serum or with another enzymatic method employing glucometers. However, the CV of the method used should not be >3% when used for a scientific purpose (Brouns et al., 2005).

2.2.14 Area under the curve
When calculating the area of the blood glucose response curve only the area above the fasting level was taken into account (IAUC) (Bornet et al., 1997; FAO/WHO, 1998; Wolever et al., 2003). The area under the curve includes a balance between exogenous/endogenous glucose and the uptake of glucose by peripheral cells (Bornet et al., 1997). However, the basis of taking only glucose values above fasting levels had been questioned by Pi-Sunyer, (2002) as all the glucose molecules are the same whether above fasting or below fasting level (Beals, 2005).
2.2.15 Calculation of GI

The GI of each test food for each individual is calculated using the equation given below:

\[
\text{GI} = \frac{\text{IAUC of test food}}{\text{IAUC of standard food}} \times 100
\]

(FAO/WHO, 1998; Brouns et al., 2005).

The GI values of the total number of individuals are averaged to take the GI of the particular food. This method is referred to as the mean of the ratios (Brouns et al., 2005).

2.3 Insulin responses to starchy foods

Carbohydrate foods of diverse nature are digested at different rates releasing glucose into the blood stream. This process stimulates secretion of insulin to clear the blood glucose levels. Insulin is an anabolic polypeptide hormone produced by β-cells of islets of Langerhans of pancreas. The precursor molecule of insulin is preproinsulin (108 amino acids) which first cleaves to proinsulin (86 amino acids). The active hormone insulin and C-peptide are released from proinsulin. Glucose is the main stimulus for secretion of insulin while amino acids (arginine, leucine) and gastric peptides also stimulate the release. Insulin stimulates peripheral cells to take up glucose from blood, store it as glycogen in the liver and muscle, and prevent use of fat as an energy source. In the absence of insulin (or low), body begins to use fat as an energy source (Murray et al., 1996).
A correlation between glycaemic and insulinaemic responses has been shown with many carbohydrate rich foods (Holt et al., 1997; Englyst et al., 2003). However, foods with similar glucose responses have shown to give different plasma insulin responses (Chew et al., 1988). Chew et al., (1988) studied the plasma insulin responses to a solution of glucose and several normal meals and observed higher insulin responses to meals compared with the glucose solution. This indicates the ability of protein, fat and other components in a meal to act as insulin secretagogues (Bornet et al., 1987; Chew et al., 1988). Studies have shown that meals with higher protein contents compared to less protein or only carbohydrates increase the insulin more rapidly during the initial 20 min postprandial period (Schenk et al., 2003). However, the secretion of insulin with respect to increase in protein content is not linear (Wolever et al., 1996). Adding 16 g protein to a liquid test meal reduced the glucose response by 40% and doubled the insulin response. When protein was increased to 50 g the glucose response was reduced by a further 40% but the insulin response was the same as seen previously (Spiller et al., 1987).

2.3.1 Determination of Insulinaemic Index

The ratio between the insulin response to test food and to the standard is referred to as the Insulinaemic Index (II) (Wolever et al., 1985; Bornet et al., 1997; Holt et al., 1997). It focuses on the postprandial secretion of insulin which is clinically significant in non-diabetic as well as in type 2 diabetic as hyperinsulinaemia is recognized as a risk factor for many health issues (Stout, 1979).
2.4 Effect of glucagon on foods

Another hormone apart from insulin that plays a role in carbohydrate metabolism is glucagon which is an antagonist to insulin. The increased glucagon levels (excess) act against the response of insulin and stimulate gluconeogenesis and glycogenolysis (Fanelli et al., 2006) thereby causing hyperglycaemia (Cryer, 2008). Thus, the differences in glucagon levels might also be another factor that gives rise to diverse glycaemic responses with different sources of starch among a group of individuals (Crapo et al., 1980).

2.5 Glycaemic Indices of foods

2.5.1 Glycaemic Indices of basic foods

GI values of foods are ranked as low (<55), medium or high (>70) according to the classification with respect to glucose as the standard (Beals, 2005). At present 2487 data on GI of foods are tabulated (Atkinson et al., 2008).

The GI values of certain basic Sri Lankan foods are known (Hettiarachchi et al., 2001; Widanagamage, 2007). However, sufficient information is not available on mixed Sri Lankan meals. Studies done on GI values of Indian foods show higher GI values for South Indian fast foods compared with the traditional South Asian snacks (Mani et al., 1997).

The GI values of same foods from different locations reported in the literature have elicited similar or different GI values. Differences may be due to different botanical origins, composition of foods, and the processing/preparation methods (Bornet et al., 1997). The foods belonging to similar botanical categorization had also yielded different GI values
(Vosloo, 2005). These could be due to the various intrinsic factors characteristic to the specific food.

Most fruits and vegetables are categorized as medium or high GI foods. However, increased consumption of fruits and vegetables is recommended to control and prevent various health issues (FAO/WHO, 1998). Thus, it is not advisable to reduce the intake of fruits and vegetables by only referring to GI values as these are rich sources of most other micronutrients and dietary fibre. However, the edible portions of fruits and vegetables are less than the portions given for determination of GI. Thus, indicating the importance of Glycaemic Load (see section 2.7).

Milk and dairy products also share low or medium GI values with high postprandial insulinaemic responses reported with both regular and fermented milk products and low GI pasta meals containing milk (Arvidsson-Lenner et al., 2004). Milk proteins have demonstrated insulinotrophic properties with the whey component (soluble milk proteins) exhibiting the highest insulin secretagogue properties (Nilsson et al., 2004). This was attributed to the presence of amino acids (leucine, phenylalanine, and tyrosine) that stimulate insulin release (insulinogenic amino acids) (Nilsson et al., 2007) and the incretin hormone [especially GLP-1 (glucagon-like peptide 1)] activities (Nilsson et al., 2004; Frid et al., 2005). The incretins are reported to stimulate insulin secretion via cAMP signal transduction pathway (by activating cAMP dependent protein kinase A) (Nilsson et al., 2007).
2.5.2 Glycaemic Indices of mixed meals

When several carbohydrate sources are included in a mixed meal the beneficial effect of the carbohydrate with the lower GI will be masked by the quantity of the higher GI carbohydrates or vice versa. Different components of a mixed meal: fat (Collier et al., 1984), protein (Granfeldt and Bjorck, 1991), fibre (Vosloo, 2005; Venn and Green, 2007) anti nutritional factors (enzyme inhibitors) (Thorne et al., 1983) and acidity/acidic compounds (Liljeberg et al., 1995; Liljeberg and Bjorck, 1996) also affect the response of the carbohydrate load (Brouns et al., 2005). However, studies have shown that the normal amounts of protein and fat content in meals generally produce negligible effects on postprandial glucose and insulin responses (Wolever et al., 1996).

Some studies have shown that GI of a composite meal can be predicted from GI values of carbohydrate foods included in the meal (Wolever et al., 1985; Chew et al., 1988). The method by which GI of a mixed meal can be calculated from its individual counterparts is given in Table 2.1.

However, different studies showed contradictory observations on this approach as differences in GI between foods had lessened when incorporated in composite meals (Coulston et al., 1984). The disagreements between the predicted and observed GI values might had been due to the application of incorrect GI values of the food components included in the meal (Flint et al., 2004) as the preparation methods and processing techniques of the same foods can yield different GI values.
Table 2.1: Calculation of GI of meals from individual components

<table>
<thead>
<tr>
<th>Food</th>
<th>CHO per 100 g (g)</th>
<th>Portion size (g)</th>
<th>CHO per portion (g)</th>
<th>Proportion of total CHO (c=a/b)</th>
<th>Food GI² (d)</th>
<th>Meal GI C=Σ (cxd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>4.9</td>
<td>250</td>
<td>(a) 12.3</td>
<td>(c) 0.24</td>
<td></td>
<td>51</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>78.6</td>
<td>25</td>
<td>(a) 19.7</td>
<td>(c) 0.38</td>
<td></td>
<td>116</td>
</tr>
<tr>
<td>White bread</td>
<td>48.8</td>
<td>40</td>
<td>(a) 19.5</td>
<td>(c) 0.38</td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>Total</td>
<td>(b) 51.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94</td>
</tr>
</tbody>
</table>

Source: (Arvidsson-Lenner et al., 2004); CHO - Carbohydrates
2.6 Mechanisms of low Glycaemic Index diets in improving glycaemic response

The plasma glucose concentration is a balance between rate of absorption of glucose into the systemic circulation and rate of clearance from it. Both these processes are influenced by a variety of factors. Absorption of glucose into the circulation is affected by intrinsic factors of the food (Bjorck et al., 1994) as well as extrinsic factors including the insulin hormone which affects the clearance of glucose from the circulation (DeFronzo and Ferrannini, 1982). Glucose is the primary factor that stimulates release of insulin, however protein when ingested with glucose augment the process of release of insulin (Nuttall et al., 1984; van Loon et al., 2000). Thus, a food can have a low GI either having a low absorption of glucose into the systemic circulation or high clearance of glucose (Schenk et al., 2003). The exact reason can only be known by measuring the plasma glucose kinetics.

Proposed mechanisms by which low GI foods improve postprandial glycaemic responses are given below:

(i) The consumption of low GI carbohydrate foods results in a reduced rate of glucose absorption with a reduced rise in insulin (Wolever et al., 1988; Jenkins et al., 1990; Nilsson et al., 2007). The slow absorption of carbohydrates will maintain a lower blood glucose concentration while suppressing the post-meal free fatty acid (FFA) levels (Wolever et al., 1988; Jenkins et al., 1990; Nilsson et al., 2007). With the reduction of FFA over a period of time the glucose is cleared from the circulation at a higher rate leading the blood glucose concentrations to maintain at the baseline levels. When these processes are taking place, glucose is continuously being absorbed from the small
intestine. As the glucose is absorbed and rapidly cleared from the circulation, the peak postprandial glucose levels will be lower as well as the IAUC of the blood glucose response curves (Jenkins et al., 2002).

(ii) Incretins (gut hormones) are released with the presence of digested foods in the upper gastrointestinal tract (Hellstrom and Naslund, 2001). Those are glucose dependent insulino trophic peptide (GIP) and GLP-1 (Nilsson et al., 2007). Secretion of GIP from the upper small intestine is stimulated by absorbed carbohydrates and lipids. GLP-1 is secreted from the lower small intestine in response to the availability of nutrients in the gut lumen (Juul and Cathrine, 2001) and GLP-1 stimulates release of insulin (Milton et al., 2007; Greenfield et al., 2009). GLP-1 lowers the gastric emptying rate, and inhibits appetite. As the meals with slowly absorbable nutrients show a prominent effect on the secretion of GLP-1 than with the rapidly absorbed ones this could be another mechanism by which low GI foods improve postprandial glucose response (Milton et al., 2007).

(iii) Low-GI foods generally contain higher amounts of dietary fibre and resistant starch that escape digestion and/or absorption in the small intestine. Thus, the amount of carbohydrate entering the colon and undergoing colonic fermentation and short chain fatty acid (SCFA-acetate, propionate, and butyrate) production also relatively increase and slow the passage of food in the upper gastrointestinal tract (Wong and Jenkins, 2007).
2.7 Glycaemic Load

The GI compares equal quantities of available carbohydrate in foods and assesses the quality of carbohydrate. However, glycaemic response to a food or a meal is influenced by both the quality and quantity of carbohydrate (Barclay et al., 2005). For certain foods the 50 g available carbohydrate portion given for GI determination do not represent the edible portion, i.e., fruits (in order to obtain 50 g available carbohydrates one would have to consume in excess of edible portion) (Table 2.2). In practice, the actual carbohydrate load of a normal portion size varies considerably between food products. In order to address this problem the concept of Glycaemic Load (GL) was introduced (Salmeron et al., 1997) as this focuses on the glycaemic responses of “normal” servings of carbohydrate rich foods (Foster-Powell et al., 2002; Arvidsson-Lenner et al., 2004). This was put forward in 1997 by nutritional epidemiologists at the Harvard School of Public Health to stress the idea that not only the amount of carbohydrate alone but the overall glycaemic effect of a diet is also related to disease risk (Salmeron et al., 1997; Liu et al., 2000). Thus, quality and quantity of carbohydrates are both reflected in GL.

GL is the product of a food’s GI and its total available carbohydrate content. Thus, it is calculated as given below:

\[
\text{Glycaemic Load} = \frac{\text{GI} \times \text{available carbohydrate}}{100}
\]

(Barclay et al., 2005)
According to the GL values, foods are classified as low (1-10), medium (11-19) and high (≥20) GL foods against glucose as the standard (Brand-Miller et al., 2003; Barclay et al., 2005). Thus, both low GI, high carbohydrate foods or high GI, low carbohydrate foods can have the same GL.

2.7.1 Relationship between Glycaemic Load with glucose and insulin responses

A dose dependent glycaemia and insulinaemia was clearly observed (Brand-Miller et al., 2003) at lower carbohydrate doses (equivalent to one, two and three slices of bread). However, when high amount of carbohydrate (equivalent to four and six slices of bread) were ingested the glycaemia reaches a plateau or levels off. This indicates that regardless of the dose, healthy individuals were able to control glycaemia by increasing the amount of insulin secreted (DeFronzo and Ferrannini, 1992).

Stepwise increase in GL produced insulin responses that were directly proportional to the load and the insulin AUC showed no sign of a threshold as was the case for glycaemia (Brand-Miller et al., 2003). This shows a marked effect of high GL in predicting the risk of type 2 diabetes as increased demand for insulin is expected to exhaust pancreatic β-cells mainly in individuals with high risk i.e., individuals with family history or who had gestational diabetes mellitus (DeFronzo and Ferrannini, 1992).
2.8 Second meal effect of starchy foods

Studies have shown certain foods ingested at breakfast can improve glycaemia and insulinaemia of a subsequent lunch meal (4 hours later) (Jenkins et al., 1982; Wolever et al., 1996; Liljeberg et al., 1999). This was recognized as the “second meal effect” (Jenkins et al., 1982; Liljeberg et al., 1999). The same effect of dinner meals on breakfast (overnight second meal effects) have also been demonstrated (Wolever et al., 1988). The colonic fermentation of dietary fibre (present mostly in low GI foods) or suppression of the FFA levels (see section 2.6) is reported to be responsible for the improved glycaemic response (Nilsson et al., 2008a; Nilsson et al., 2008b; Nilsson et al., 2008c). However, all low GI foods do not show a second meal effect as some studies have not observed any improvement in the glycaemic, insulinaemic and FFA concentrations after the post lunch meal (Clark et al., 2006). Identifying meals that produce second meal effects will be of use in the long-term dietary management of impaired glucose tolerant (IGT) and type 2 diabetic patients (Arvidsson-Lenner et al., 2004).
Table 2.2: GI and GL of commonly consumed foods

<table>
<thead>
<tr>
<th>Food</th>
<th>GI (with glucose as the standard)</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Wheat bread (mean)</td>
<td>75 ± 2</td>
<td>11</td>
</tr>
<tr>
<td>*Wholemeal bread (mean)</td>
<td>74 ± 2</td>
<td>9</td>
</tr>
<tr>
<td>*Corn flakes (mean)</td>
<td>81 ± 3</td>
<td>20</td>
</tr>
<tr>
<td>Chickpea</td>
<td>22-38</td>
<td>3-11</td>
</tr>
<tr>
<td>Mung beans</td>
<td>22-53</td>
<td>5-9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>68</td>
<td>7</td>
</tr>
<tr>
<td>Watermelon</td>
<td>35 - 80</td>
<td>4-5</td>
</tr>
<tr>
<td>Bananas</td>
<td>47-70</td>
<td>11-16</td>
</tr>
<tr>
<td>Spaghetti</td>
<td>47-68</td>
<td>17-25</td>
</tr>
<tr>
<td>Instant “two minute” noodles, Maggie®</td>
<td>46-52</td>
<td>11-13</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>51-75</td>
<td>3-12</td>
</tr>
<tr>
<td>*Carrots (mean)</td>
<td>39 ± 4</td>
<td>2</td>
</tr>
<tr>
<td>Potato</td>
<td>56-101</td>
<td>9-25</td>
</tr>
</tbody>
</table>

Source: (Foster-Powell et al., 2002; Atkinson et al., 2008); * values are given as mean ± SEM (Standard error of mean); other values are given as a range.
2.9 Factors influencing Glycaemic Index

Foods with identical amounts of available carbohydrate contents had elicited different glycaemic and insulinaemic responses. This has been attributed to the intrinsic factors of the food and the extrinsic physiological factors (Thorne et al., 1983; Bornet et al., 1997; Bjorck et al., 2000; Augustin et al., 2002a).

2.9.1 Factors associated with food

The characteristics of raw food and processing conditions of foods affect the rate of delivery of glucose to blood. There are three main levels/stages of the gastrointestinal tract at which food factors influence the blood glucose response, i.e., lowering of the gastric emptying rate (GER), lowering of the rate of digestion of starch from starchy foods and lowering of the rate of absorption. Some factors affect at more than one of these levels.

2.9.1.1 Nutrients present in meals

Inclusion of protein, fat and dietary fibre in a carbohydrate rich food /meal have shown to influence the glycaemic and insulinaemic responses (Nuttal et al., 1984; Pi-Sunyer, 2002; Sahyoun et al., 2008) while protein and fat also increasing the calorie value of a food. However, it is not advisable to increase the calorie value of a food or a meal for the purpose of reducing the glycaemic index (Beals, 2005). Although fat and protein influence gastric emptying and insulin secretion, the actual effects on reducing the GI is not observed unless relatively large amounts of protein (about 30 g per 50 g carbohydrates) and fat (50 g fat per 50 g carbohydrates) are consumed (Wolever et al., 1994).
2.9.1.1(i) Proteins

A significant negative relationship between GI and protein has been reported (Jenkins et al., 1981). This is attributed to the ability of dietary proteins to stimulate secretion of insulin and thereby increase uptake of glucose into peripheral cells (Jenkins et al., 1981; Bornet et al., 1987; Nilsson et al., 2007). Foods with natural gluten contents (pastas, bread) have shown to yield lower glycaemic responses compared to foods with added gluten content (Jenkins et al., 1987).

2.9.1.1(ii) Lipids

Lipid content of a meal and GI have also shown a significant negative relationship (Jenkins et al., 1981). Lipids reduce postprandial glycaemia by slowing down the gastric emptying (Thomas, 1957). The lipids are reported to reduce the glycaemic response without affecting the insulinaemic response (Latge et al., 1994). When considering the different types of fat, monounsaturated fat containing diets have shown to improve glucose tolerance during short term (6 months) studies (Due et al., 2008).

2.9.1.1 (iii) Dietary fibre

The potential health benefits of dietary fibre in the prevention and control of various diseases, including diabetes is widely discussed (Jenkins et al., 2003; Key and Spencer, 2007). The American Diabetes Association (ADA) recommends a 20-35 g of dietary fibre intake for healthy adults (2000 cal/8400 KJ diet with a 25 g fibre per day) (Chandalia et al., 2000).
The fibre content in a meal elicit an inverse association on the postprandial glycaemic response (Wolever, 1990; Bjorck et al., 1994; Liljeberg and Bjorck, 1994). The soluble dietary fibre (SDF) fraction lower postprandial blood glucose response (Jenkins et al., 1981; Braaten et al., 1991) more than the insoluble counterpart (insoluble dietary fibre – IDF). The high viscosity with SDF is expected to 1) delay gastric emptying 2) slow down intestinal absorption (Hallfrisch and Behall, 2000), 3) alter the motility of the small intestine, or 4) reduce the accessibility of α-amylase to its substrate due to the high viscosity of the gut content (Leclere et al., 1994). The beneficial effects of SDF are more pronounced in hypercholesterolemic, older, obese, or NIDDM subjects for whom lowering glucose and insulin is an improvement. Less significant effects are observed with young and subjects with normal glucose and insulin responses (Hallfrisch and Behall, 2000).

Uronic acids (found mainly in hemicellulose) present in green vegetables are recognized as a major component of IDF (Chen and Anderson, 1981). The cellulose content and uronic acid amounts have also shown a significant negative relationship (p<0.01) with GI (Wolever, 1990). This indicates certain low GI foods to have a strong cell wall with high amounts of cellulose and hemicellulose (Wolever, 1990). The consumption of a high fibre meal (containing 33 g IDF) 75 min prior to the main meal has also shown to reduce the glycaemic response as well as the appetite (Samra and Anderson, 2007).

The established health benefits of fibre are well documented and it is advisable to include many fibre rich foods in the diet. However, the long term benefits of foods with naturally
occurring fibre or foods manufactured with increased fibre contents (with Non starch polysaccharides) will have to be determined with long term studies. (Mann, 2007).

2.9.1.2 Different sources of carbohydrates

The postprandial glycaemic or insulinaemic responses following ingestion of a carbohydrate meal vary with the source of carbohydrate, i.e. glucose solution, tubers, cereals and legumes (Crapo et al., 1977; Crapo et al., 1980; Jenkins et al., 1981; Thorne et al., 1983; Jenkins et al., 1988). The responses to similar carbohydrate doses of glucose solution and potato were rapid and similar while that of fructose, rice, corn and legumes were much slower (Coulston, 1980; Crapo et al., 1980; Jenkins et al., 1981). The different glycaemic responses to various starchy sources were observed not only in healthy individuals but also in IGT, type 1 and type 2 DM patients (Crapo et al., 1980; Crapo et al., 1981; Wolever and Jenkins, 1986). The magnitude of differences in postprandial glycaemic responses to different sources of starch in normal subjects is related to each individual’s degree of glucose tolerance, i.e., the most glucose tolerant individuals exhibiting the smallest differences in postprandial glycaemic responses whereas the least glucose tolerant subjects exhibiting the greatest difference (Crapo et al., 1980).

The differences in postprandial glycaemic responses to different sources of starch are high in individuals with deficiencies in clearing glucose less efficiently from the circulation. In individuals with IGT peak glucose and insulin values were delayed (15 min) as compared to healthy individuals (Crapo et al., 1977).
2.9.1.3 Amylose-amylopectin content

Starch is a homo polysaccharide made up of D-glucose units with both α-1,4 and α-1,6 linkages and comprises of two different components namely amylose and amylopectin. Amylose is a straight chain of α-1,4 glucose linkages while amylopectin is a branched chain with both α-1,4 and α-1,6 glucose linkages (Behall et al., 1988). Thus, hydrolysis of amylose gives rise to fewer glucose molecules than hydrolysis of amylopectin. The straight chain portion of amylose and amylopectin have a helical structure stabilized by hydrogen bonding (Behall et al., 1988). Due to the branching in amylopectin the surface area per molecule and the area available for amylolytic attack is increased (Behall et al., 1988).

Amylose content and GI is reported to have an inverse relationship (Andersen et al., 1981; Goddard et al., 1984; Granfeldt et al., 1995a). However, it is an exponential relationship rather than linear (Brand-Miller et al., 1992).

Studies with meals containing high percentage of amylose have shown significant reductions in glucose and insulin responses than consumption of high amylopectin meals (Goddard et al., 1984; Behall et al., 1988). This could be due to the formation of amylose-lipid complexes which slows the digestion or absorption of the high amylose foods or formation of more hard gels that are less accessible to digestion by the hydrolytic enzymes. The hard gels formed by retrograded amylose can be reversed by cooking at a higher temperature as high as 120 °C (Behall et al., 1988). Amylopectin forms softer gels which can be reversed at a lower temperature (50-85 °C) (Behall et al., 1988).
Legumes contain a higher percentage of starch as amylose (30-40%) compared to the levels found in most other carbohydrate sources (15-30%) (Thorne et al., 1983). This could be one factor contributing towards the low GI observed with legumes. When a high amylose meal is consumed the amount of insulin needed to clear an equivalent amount of plasma glucose was less compared with the amyllopectin meal. Thus, the meals containing high percentage of amylose might be beneficial for individuals with glucose intolerance, carbohydrate-sensitivity, or diabetic as well as healthy and obese individuals (Behall et al., 1988). However, there were instances that high amylose foods did not elicit the expected lower glycaemic responses (Vosloo, 2005). This had been attributed to the hypothesis that “high amylose content may affect glucose and insulin responses only if the food matrix is not readily accessible to enzymatic attack during cooking or processing” (Weststrate and van Amelsvoort, 1994).

2.9.1.4 Fate of the starch granule with processing

Starch is packaged in the form of granules in plants. The size, shape and morphology of starch granules vary with the botanical source and contain different amounts of protein and lipids in addition to the polysaccharide portion (Imam, 1989; Vasanthan and Hoover, 1992).

When an aqueous solution containing starch is heated, starch granules swell with increased temperature. At a particular temperature, granules undergo a transition from an organized to a disorganized structure and this phenomenon is known as “gelatinization”. The process of gelatinization makes starch to be more available for digestion by amylolytic enzymes.
The gelatinization temperature increases with the amylose content (Colonna and Mercier, 1985). Swelling of the starch granule continues with further heating and leaching of amylose and some amylopectin occurs producing a viscous suspension. When this suspension is cooled a gel is formed and this process is referred to as “retrogradation”. However, retrogradation of amylose is more rapid than amylopectin (Russel, 1987; Biliaderis, 1991). This is due to the linear structure of amylose which facilitates cross linkage via hydrogen bonds (Russel, 1987). When amylose and amylopectin retrogrades with repeated cooling they become resistant to enzymic digestion (Englyst and Cummings, 1987). The realignment of chains of amylose and short chains of amylopectin can occur with time.

When starch granules swell during cooking interactions with other macromolecules are also possible, i.e., proteins. One such example is pasta. When pasta is cooked starch granules get trapped in a structured matrix (Bornet et al., 1990b; Colonna et al., 1990) which makes the starch-protein interactions less susceptible to α-amylase action and slows down the starch digestion in the gastrointestinal tract.

The cell enclosed starch granules present in legumes are reported to undergo limited swelling thereby yielding a lower GI (Golay et al., 1986).

2.9.1.5 Types of sugars

The different monosaccharides/carbohydrates present in a food metabolize differently (Vosloo, 2005) and affect the rate of absorption of each other, i.e., the rate of absorption of fructose has shown to increase when given with glucose or starch (Riby et al., 1993). The
GI of glucose, sucrose, lactose and fructose are 140, 85, 65, and 30 respectively (Bornet et al., 1997; Arvidsson-Lenner et al., 2004). An animal study has shown that small amounts of orally administered fructose or sucrose was useful in lowering the postprandial glucose response to a carbohydrate challenge (Wolf et al., 2002). Fructose has demonstrated to play an active role in the augmentation of hepatic glucose uptake (Ribu et al., 1993; Heacock et al., 2002). Thus, it is used as an alternative sweetener for diabetics. However, certain negative effects had been observed with high intake of fructose (Wolf et al., 2002) in increasing the fasting and postprandial plasma triglyceride concentrations (Bantle et al., 2000). Sucrose taken in moderate quantities (10-15% of calorie intake) with a meal has shown no significant effect on postprandial glycaemia (Slama et al., 1984). Certain studies have shown connections between high levels of sucrose and lactose with increased risk of colorectal and ovarian cancer (Key and Spencer, 2007).

2.9.1.6 Particle size

Studies done with wheat flour breads containing different particle sizes have shown “higher glycaemic responses and lower satiety scores” for bread made with fine flour compared with others, i.e., whole grain, cracked grain, coarse wholmeal flour (Holm and Bjorck, 1992; Holt et al., 1994). Whole kernels and larger particle sizes are associated with lower glucose and insulin responses for a variety of other grain sources as well (Hallfrisch and Behall, 2000).
2.9.1.7 Cooking method/processing

The processing techniques influence the glycaemic responses to foods, i.e., milling breaks the cell wall thereby facilitating the digestion of starch granules by amylase enzyme (Bjorck et al., 1994). Grinding of food and heat treatment (boiling) have also shown to increase the GI values (Arvidsson-Lenner et al., 2004).

The differences in glycaemic responses between the whole vs. parboiled grains and ground vs. polished grains is more obvious than when comparing white rice and red rice (O'Dea et al., 1980).

The highly ordered crystalline structure in native starch granules forms a barrier to enzymatic attack, and lowers the rate of digestion (Bornet et al., 1989). During processing certain structures may form which are less readily hydrolyzed by amylases, or in some cases totally resistant (Englyst and Cummings, 1985; Englyst and Cummings, 1987).

Pasta is a food with low GI due to its processing technique (Bornet et al., 1990b; Colonna et al., 1990b). Bread baked at pumpernickel conditions (120 °C for 20 h) (Akerberg et al., 1998) or with lactic acid (Ostman et al., 2002) have reported to elicit low glycaemic responses. Some Caribbean foods prepared with crushed and uncrushed preparations did not elicit significantly different AUC nor GI values (Ramdath et al., 2004).

The studies that showed beneficial effects of low GI or GL carbohydrate foods (cereals, vegetables, and fruit) mainly included minimally processed natural foods (Venn and Green, 2007). This stresses the significance of maintaining the intact native starch granules as a key regulatory step in modulating the glycaemic responses.
2.9.1.8 Anti nutritional compounds

Certain compounds (antinutrients/enzyme inhibitors) such as polyphenols, phytic acid (Yoon et al., 1983; Thompson et al., 1987; Kestin et al., 1990) and lectin (Thompson et al., 1984; Rea et al., 1985) slow down the digestion of digestible starch by inhibiting various enzymes and physiological processes involved in the digestion process (Thompson and Yoon, 1984; Thompson et al., 1984). Phytic acid may lower glycaemia by affecting the digestibility of starch via interacting with the amylase protein and/or binding with calcium, which is an activator of amylase enzyme (Thompson et al., 1987). Lectin interferes with the mucosal phase of digestion or by binding directly to the starch and/or the digestive enzymes (Rea et al., 1985). Polyphenols work by interacting directly with starch molecules and/or indirectly via forming protein-polyphenol interactions that first affect protein digestion and then obstruct the digestion of starch molecules within the protein network (Thompson et al., 1984b). However, the negative effect of the antinutrients on glycaemic response is minimal compared with the effect of the macronutrients.

2.9.1.9 Organic acids

Bread containing lactic acid, the sodium salt of propionate (Liljeberg and Bjorck, 1994; Liljeberg and Bjorck, 1996) and acetic acid (Brighenti et al., 1995; Liljeberg and Bjorck, 1998) elicit low postprandial glycaemic and insulinaemic responses. Acetic and propionic acids delay gastric emptying while sodium salt of propionate increase postprandial satiety
2.9.1.10 Resistant starch (Undigestible starch)

The fraction of starch that is resistant to digestion in the human small intestine is classified as resistant starch (RS). These are categorized into four groups i.e., RS\textsubscript{1} (physically protected starch), RS\textsubscript{2} (ungelatinized resistant starch), RS\textsubscript{3} (retrograded starch), RS\textsubscript{4} (chemically modified starch) (Sajilata \textit{et al.}, 2006).

The naturally occurring RS in foods can be lowered by boiling in water as boiling causes gelatinization of starch and allows easier access of digestive enzymes to starch (Snow and O'Dea, 1981; Wursch \textit{et al.}, 1986). However, cooling after heating and repeated cycles of heating and cooling increases the RS content in foods (Englyst and Cummings, 1987; Brouns \textit{et al.}, 2005). This was attributed to retrograded amylopectin and retrograded amylose (Muir and O'Dea, 1992).

RS is present in fruits and vegetables naturally with proportions changing according to the levels of ripeness, i.e., raw ones having more RS while ripe ones have less (Brouns \textit{et al.}, 2005).

RS like dietary fibre is fermented by anaerobic bacteria in the colon (Cummings and Englyst, 1987) and share the potential health benefits attributed to dietary fibre, especially in diabetes (Vinik and Jenkins, 1988), cardiovascular disease (Ullrich, 1987), and bowel disease (Cummings and Bingham, 1987).
2.9.1.11 Patterns of blood glucose curves

Two foods with the same GI can give rise to markedly different blood glucose response curves (Wolever et al., 2003; Beals, 2005). This is mainly due to the presence of various types of available carbohydrates in a food as the starting material for digestion i.e., dietary sugars (glucose and fructose) are rapidly digested and absorbed; blood glucose levels rise and falls rapidly following digestion of sucrose; starch leads to the blood glucose levels to rise and fall more gradually (Beals, 2005).

2.9.2 Extrinsic factors affecting Glycaemic Index

GI was initially recognized as an intrinsic property of a food. Thus, any given food should exhibit consistent and reproducible glycaemic responses regardless from person to person and day to day. However, GI of the same food varied between individuals and even within the same individual depending on the time of the day, previous meal and the duration of fasting (Barclay et al., 2005). This is due to the extrinsic factors or physiological factors that influence the GI of a particular food.

2.9.2.1 Inter individual variations

This indicates the variations between different individuals to the same food (Wolever et al., 2003). Inter individual variations contribute to about a 74% of the variance in determination of GI values (Bornet et al., 1997). A study done with diabetic individuals to analyze the variations to the same food has resulted in a 45% of CV for AUC and 10% CV
for GI. (Wolever, 1990). Thus, to minimize inter individual variations GI values are expressed relative to a standard.

2.9.2.2 Intra individual variations

The variations of glycaemic responses within an individual person is referred to as intra individual variations (Wolever et al., 1992; Wolever et al., 2003) This accounts for about 26% variance seen when determining GI of foods (Rasmussen, 1993). A study done with repeated tests of 50 g available carbohydrates of glucose or white bread, with type 2 diabetics, non-diabetic subjects and type 1 diabetics showed 15%, 23-25% and 30% CV of AUC for the three respective groups (Wolever et al., 1985). Effective and reliable ways to reduce intra individual variations in glycaemic responses may be the most effective strategy to improve the precision of measurement of GI values (Wolever et al., 2003). However, higher intra individual variability (43%) and lower inter individual variability (18%) have also been reported using white bread and glucose with healthy individuals (Vega-Lopez et al., 2007). The differences in inter and intra individual variations of the two sets of data could be due to the different dinner meals consumed by the subjects as certain foods have shown to elicit second meal effects.

2.9.2.3 Gastric emptying

Duration of gastric emptying depends on various factors: the physical state of the food (solid versus liquid), energy, carbohydrate, lipid, protein content of the meal, (Latge et al., 1994) the presence of viscous SDF (i.e., guar gum) (Leclere et al., 1994) and the
bioavailability of carbohydrate components (Bornet et al., 1990a). Potential health benefits of traditional spices and herbs (cinnamon, bay leaf, cloves, black and green tea) on regulating the postprandial blood glucose responses via delaying the gastric emptying rate have been observed (Hlebowicz et al., 2007). This might also have contributed towards preventing or delaying diabetes with previous generations of mainly Asians who consumed traditional meals prepared with a variety of herbs.

2.9.2.4 Meal frequency

Increased meal frequency has shown to lower postprandial glycaemic and insulinaemic responses, reduce serum low density lipoprotein (LDL) cholesterol, apo-lipoprotein B concentrations (Bornet et al., 1997) and improve glucose tolerance (Lundin et al., 2004). This reflects on the beneficial effect of less glucose entering the blood stream at a given time with the concomitantly less insulin response.

2.10 In vitro procedures used to determine glycaemic responses

Determination of GI by the standard in vivo method is very laborious, costly, time consuming and requires the corporation of motivated individuals to participate in the study. Thus, in vitro methods that mimic the physiological rate of digestion of carbohydrate foods have been developed. These in vitro procedures are established with the rationale that carbohydrate digestion by the digestive enzymes is an essential component of both in vitro and in vivo digestion procedures. Especially in vitro digestion procedures that follow the
digestion of foods using pancreatic and brush border enzymes (Englyst et al., 2003) have shown high correlations with the in vivo glycaemic responses (Brouns et al., 2005). These methods use a wide variety of enzymes; amylase only (Jenkins et al., 1980; Snow and O'Dea, 1981) or amylase with other proteolytic enzymes (Holm et al., 1985; Colonna et al., 1990). If proteolytic enzymes are used the starch-protein interactions will be broken down enabling starch to be digested (Holm and Bjorck, 1988). Some methods employ the use of dialysis bags restricting the amount and rate of digestion (Jenkins et al., 1980). The viscosity of the gastrointestinal content is also reported to influence glycaemic response (Jenkins et al., 1978). Thus, the use of dialysis bags provides a solution for this problem as the viscosity in the dialysis bags will affect the rate of appearance of digested products in the dialysate.

The in vitro studies initiate the digestion and absorption of starchy foods either by the in vivo mastication process (Granfeldt et al., 1992) or imitating mouth grinding process (Englyst et al., 1992). The chewing procedure which subjects the food to digestion by α-amylase in the mouth offers certain advantages over the traditional milling or grinding. The chewing time and the physical characteristics of the food will influence the degree of degradation of food particles and the rate of hydrolysis of starch. Read et al., (1986) have shown an increase in GI of food following chewing rather than swallowing without chewing. The in vitro procedures with chewing have shown to affect the amount of starch escaping digestion in vitro, i.e., more times a starch containing food was chewed the less starch escaped digestion in the in vitro assay system.
Most of the studies using in vitro methods had focused on analyzing basic foods available in those respective countries (Granfeldt et al., 1992; Garsetti et al., 2005) without focusing on mixed meals. This could be due to the expected influences of intrinsic properties of food and extrinsic physiological factors. However, the GI of mixed meals is calculated from the individual components in the meal (Table 2.1). Thus, the in vitro methods could also be applied to mixed meals with different accompaniments.

2.11 Carbohydrates and health issues

Higher intake of certain carbohydrates increases insulin sensitivity (Smith, 1994) while some produce insulin resistance and adverse lipid profiles (Daly et al., 1997; Frost et al., 1998). High blood sugar levels increase oxidative stress, (Hsu et al., 2007) protein glycation and the risk of development of type 2 diabetes and coronary heart disease (Hannah and Howard, 1994; Gavin, 2001).

There is an epidemic of obesity and type 2 diabetes at present in both developed and developing countries (Hossain et al., 2007). Main causative factors of this problem have been recognized as life style changes, lack of physical exercise and diet. The metabolic disorders associated with diet have been classified as metabolic syndrome/insulin resistance syndrome (Isomaa et al., 2001).

2.11.1 Metabolic syndrome

It is characterized by glucose intolerance, obesity, hypertension, low levels of high density lipoproteins (HDL) and high triacylglycerol levels (Giovannucci, 2007). An individual
with several characteristics of metabolic syndrome (mentioned above) has a higher chance of developing diabetes, cardiovascular disease or cancer.

Obesity related insulin resistance is recognized as the main factor that leads towards the development of metabolic syndrome (Hsu et al., 2007). Insulin resistance occurs when tissues become less sensitive to insulin. Adipocytes have shown to act as an endocrine cell and release a variety of molecules, leptin, adiponectin and cytokines (tumour necrosis factor-α, interleukin-6). Among which, adiponectin has been recognized as a factor linked with obesity and insulin resistance as a decrease in adiponectin receptors have been observed with obesity (Hsu et al., 2007).

2.11.2 Diabetes Mellitus

Diabetes mellitus (DM) is defined as “a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both” (Committee report, 2003).

2.11.2.1 Prevalence of diabetes

The prevalence of diabetes has risen more rapidly in Asia than any other part of the world. Among the 10 leading countries with the highest prevalence of diabetes, five are in Asia. India tops the list with 19.4 million in 1995 and 31.7 million in 2000 (King et al., 1998; Chowdhury et al., 2003; Weekly Epidemic Report, 2004; Wild et al., 2004). When focusing attention on the diabetes prevalence in Sri Lanka, the data of a cross sectional study reported a 16.4% and 8.7% incidence for urban and rural areas respectively. Further,
the prevalence of pre-diabetes among urban and rural populations is stated as 13.6% and 11.0% respectively. This study further stressed that “one in five adults in Sri Lanka has either diabetes or pre-diabetes” (Katulanda et al., 2008).

The predicted percentage increase of prevalence of DM by 2025 in the developed and developing countries are 42% and 170% respectively (Wild et al., 2004; Hossain et al., 2007). The underling factors responsible for this problem have been recognized as lifestyle changes that lead to reduced physical activity and foods with rapid release carbohydrates and excess calorie intake. In developing countries the oldest age group (≥65) includes the largest number of people with diabetes while in developing countries it is the 45-60 age group who are still in the productive years of their lives (Wild et al., 2004).

Furthermore, an increase in incidence of type 2 diabetes among children and teenagers in Asian countries extends this health issue to the next generation (Chowdhury et al., 2003; Hossain et al., 2007). South Asians in United Kingdom have the highest death rate with CHD partly due to their higher chance of developing type 2 diabetes at an earlier stage than Europeans. The increasing incidence of diabetes among South Asians has been attributed to the genetic factors as well as increasing central obesity (intra-abdominal) and hyperinsulinaemia which are more commonly seen among the South Asian population compared with others (Chowdhury et al., 2003).

These data stress the epidemic nature of diabetes in the world with increasing burden on the developing world. Thus, effective intervention methods and early prevention strategies are needed to combat this health issue before long.
2.11.2.2 Classification of diabetes

The diagnostic criteria of diabetes and pre-diabetes are depicted in Table 2.3. Diabetes is classified into two major types, i.e., type 1 and type 2 DM. Several other intermediate/transient stages have also been identified, i.e., IGT, gestational diabetes mellitus (GDM).

2.11.2.2 (i) Type 1 diabetes

Type 1 diabetes (juvenile-onset diabetes) is due to cellular mediated autoimmune destruction of the β-cells of the pancreas (Atkinson and Maclaren, 1994). Degree of β-cell destruction varies between individuals. Most individuals with this type of diabetes depend on insulin for survival. There will be very low or negligible levels of plasma C-peptide due to very little or absence of insulin secretion at the latter stages of the disease (Committee report, 2003). The increased obesity among children has been recognized as a risk factor in the development of type 1 DM.

2.11.2.2 (ii) Type 2 diabetes

This is the most common type of diabetes worldwide and this results due to different degrees of insulin deficiency and resistance (Weekly Epidemic Report, 2004). The risk factors of development of type 2 diabetes are age, obesity and lack of physical activity (Bogardus et al., 1985; King et al., 1998; Chowdhury et al., 2003). This particular type has a strong genetic predisposition than the type 1 diabetes (Committee report, 2003).
Table 2.3: Diagnostic criteria of diabetes

<table>
<thead>
<tr>
<th>Category</th>
<th>Fasting plasma glucose</th>
<th>2-hour plasma glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Healthy)</td>
<td>&lt;100 mg/dL</td>
<td>&lt;140 mg/dL</td>
</tr>
<tr>
<td></td>
<td>(&lt;5.6 mmol/L)</td>
<td>(&lt;7.8 mmol/L)</td>
</tr>
<tr>
<td>Impaired Fasting Glucose (IFG)</td>
<td>100-125 mg/dL</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(5.6-6.9 mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Impaired Glucose Tolerance (IGT)</td>
<td>-</td>
<td>140-199 mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.8-11.0 mmol/L)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>≥ 126 mg/dL</td>
<td>≥ 200 mg/dL</td>
</tr>
<tr>
<td></td>
<td>(≥7.0 mmol/L)</td>
<td>(≥ 11.1 mmol/L)</td>
</tr>
</tbody>
</table>

2.11.2.2 (ii) Type 2 diabetes

This is the most common type of diabetes worldwide and this results due to different degrees of insulin deficiency and resistance (Weekly Epidemic Report, 2004). The risk factors of development of type 2 diabetes are age, obesity and lack of physical activity (Bogardus et al., 1985; King et al., 1998; Chowdhury et al., 2003). This particular type has a strong genetic predisposition than the type 1 diabetes (Committee report, 2003).

2.11.2.2 (iii) Impaired Glucose Tolerance and Impaired Fasting Glucose

These refer to a metabolic stage intermediate between normal glucose homeostasis and diabetes and referred to as pre-diabetes (Weekly Epidemic Report, 2004). Individuals with IGT may have normal or near normal glycated haemoglobin levels (Little et al., 1988). IGT is a risk factor in the development of diabetes and coronary vascular disease (CVD) (Fuller et al., 1980). A 197 million people worldwide are reported to have IGT and an increase to 420 million is expected by 2025 (Hossain et al., 2007).

2.11.2.2 (iv) Gestational Diabetes Mellitus (GDM)

A high blood sugar level during pregnancy is referred to as gestational diabetes mellitus (GDM). The hormones produced during pregnancy are believed to be responsible for causing this transient insulin resistance (Retnakaran et al., 2006). Impaired insulin secretion and insulin sensitivity have been observed in women with GDM (Di Cianni et al., 2007). As the foetus gets the supply of glucose from the mother (through placenta) a foetus of a hyperglycaemic mother gets a high dose of glucose which will have to be
subsided by high levels of insulin produced by the foetus. High levels of insulin stimulate
growth of the foetus (high birth weight) as well as increase the susceptibility of these
babies to become insulin resistant at a later stage of their lives. GDM also increases the
risk of a female acquiring diabetes in later life, mostly type 2 diabetes due to the
progressive β-cell failure to counteract the insulin resistance (Retnakaran et al., 2008).

2.11.2.3 Complications with diabetes

The chronic hyperglycaemia is associated with complications in eyes (retinopathy),
kidneys (nephropathy), nerves (neuropathy) heart, and blood vessels (Committee report,
2003). Diabetic retinopathy is due to the micro vascular retinal changes. Over
accumulation of glucose or fructose especially in the small blood vessels damage the
vessels in the retina. Thickening of the glomerulus leads to the development of diabetic
nephropathy. During initial stages more albumin passes with urine (microalbuminuria).
With the progression of the disease more glomeruli are destroyed with concomitantly
increased amount of albumin excreted in the urine. Diabetic neuropathy is due to micro
vascular injury leading to neuronal dysfunction and development of vascular
abnormalities. The damage to various tissues that results from chronic hyperglycaemia is
expected to be due to the glycation of tissue proteins and other macromolecules
(Committee report, 2003).

Diabetic ketoacidosis is another complication mainly observed among type 1 diabetic
patients. This is due to the absence of circulating insulin which leads to increased lipolysis
and production of ketone bodies. When high levels of ketone bodies are produced, the blood pH lowers thus resulting in a life threatening condition (Murray et al., 1996).

2.11.3 Observational studies, short and long term trials of low and high Glycaemic Index diets
Several epidemiological studies have correlated the GI to disease risk. A major drawback of these studies was the usage of a dietary assessment methods not aimed to study GI when taking data (Arvidsson-Lenner et al., 2004). Studies have shown a direct relationship between increased intake of simple sugars and risk of diabetes, (Iowa women’s health study) (Meyer et al., 2000) beneficial effects of low GI diets on diabetes (Barclay et al., 2008) as well as an inverse relation between consumption of whole grains and diabetes risk (Arvidsson-Lenner et al., 2004). However, certain studies showed no relationship between intake of sugar and development of type 2 diabetes (Hu et al., 2001). The differences observed among the studies could be due to obtaining data from individuals with different levels of insulin resistance.
Prospective long term studies with low GI diets (Jenkins et al., 1988) have shown that better blood glucose control may be seen with diets that give rise to greater weight loss (Davidson et al., 1984) which was more obvious with low GI starchy foods (Jenkins et al., 1988). In most of these studies high fibre diets comprised of legumes which were low GI foods with large amounts of soluble fibre. A decrease in serum fructosamine (which is a short term indicator of blood glucose levels) (Jenkins et al., 1988) was observed when legumes were given as dietary management of diabetes (Zavoral et al., 1983). Other test
meals apart from legumes that were effective in reducing postprandial blood glucose and insulin levels were comprised of more soluble or viscous fibres (Jenkins et al., 1978; Levitt et al., 1980). Decreases in glycosylated serum proteins were seen on both high and low GI diets but more significant decreases obtained with the low GI diet. The weighed portion sizes, regular diet pattern with the high GI diet might have given similar results as low GI diet (Jenkins et al., 1988).

Follow up studies showed pre pregnancy high GI, low fibre diets to be associated with risk of developing GDM (Zhang et al., 2006). Studies carried out with low and high GI diets with GDM women showed a reduction of the number of individuals seeking insulin with low GI diet regime (Moses et al., 2009). Low GI diets had assisted in reducing the birth weight of infants compared with high GI diets. High birth weight is associated with chronic diseases later in life and experiencing a low GI diet regime from early years will be an added advantage (Moses et al., 2007).

Consumption of low GI foods by athletes 30 to 60 minutes prior to starting exercise is encouraged as it reduces being hyperglycaemic and hyperinsulinaemic at the onset of exercise and increase endurance time and fatty acid oxidation (Stevenson et al., 2009).

2.11.4 Glycaemic Indices and satiety

Studies carried out to determine the relationship between GI and satiety have elicited mixed results with some low GI foods associated with increased satiety, (Holt et al., 1992; van Amelsvoort and Weststrate, 1992; Bornet et al., 2007) delayed onset of hunger
(Ludwig et al., 1999) and some showing opposite results (Chapman et al., 1998; Lavin et al., 1998).

Low GI foods increase satiety via slower rate of digestion and absorption of food (Havel, 2001). Higher levels of gut peptide (cholecystokinin) which suppress hunger are also reported with low GI compared with high GI meals (Reynolds et al., 2008).

High GI foods are generally associated with less satiety. This might be due to rapid increase in insulin levels which will reduce both glucose and FFA sometimes even below the fasting levels, after ingestion of food. The decrease of glucose and FFA might lead to hunger (Brand-Miller et al., 2002).

Both high and low GI foods have an impact on satiety with effects being prominent at various time durations (Anderson and Woodend, 2003; Arvidsson-Lenner et al., 2004).

2.11.5 Glycaemic Indices and disease correlation

Obesity has become a major concern all over the world due to its high prevalence (van Dam and Seidell, 2007) and being a main risk factor in causing insulin resistance leading to the development of metabolic syndrome. Positive effect of high GI foods on obesity has been attributed to the satiety factor as individuals consume more to compensate and gain weight due to the positive energy balance (van Dam and Seidell, 2007).

In contrast, low GI diets promote the oxidation of fat at the expense of carbohydrates (Ludwig, 2000) and replace adipose tissue by lean tissues. (Bouche et al., 2002). A study done with a low GI diet having equal energy and macronutrient content as high GI diet in overweight men showed a reduction in the total fat mass with no difference in body weight
Bouche *et al.*, 2002). Slabber *et al.*, (1994) also showed a significant weight reduction of obese hyperinsulinaemic women when following a low GI diet.

Elevated insulin levels (hyperinsulinaemia) and insulin resistance contribute to the development of many adverse conditions, i.e., hypertension, abnormalities of plasma lipids, and atherosclerosis (DeFronzo and Ferrannini, 1991; Dickinson *et al.*, 2002). Hyperinsulinaemia promotes atherosclerosis and the development of CVD by facilitating the formation of atherosclerotic plaques. Once formed the re-absorption of these plaques are inhibited. Acute hyperglycaemia increases blood pressure in both diabetic and non diabetic subjects (Ceriello, 2000).

Reduction of LDL cholesterol and triacylglycerol concentrations have been observed with low GI diet consumed by hyperlipidaemic patients (Jenkins *et al.*, 1987a). In type 2 diabetics with a low GI diet given for 2 days in a crossover design resulted in normalization of pallsminogen activator inhibitor-1 (PAI-1) which is a cardiovascular risk factor (Committee report, 2003).

SCFA produced during digestion of low GI carbohydrates (acetate, propionate) might reduce the risk of CHD by having negative effects on hyperlipidaemia. Animal studies had shown propionate to inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) and 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) enzymes (Wong and Jenkins, 2007).

However, a recent epidemiological study carried out to determine the effect of high GI diets on mortality with established CVD males showed no association between the diet and
mortality (Levitan et al., 2009). This might have been due to the severity of the disease and age of the patients participated in this study. This stresses the importance of studying the long term effects of low GI meals with CVD patients.

As diabetes increases the risk of CVD, (Jenkins et al., 2003) the dietary advice given in the treatment of coronary diseases should be focused on preventing or controlling diabetes as well, if that is the primary cause (Jenkins et al., 2003).

Several long and short term studies proved the beneficial effects of low GI diets in improving renal function (Jenkins et al., 1985; Jenkins et al., 1987b) and reducing the urinary C-peptide to creatinine ratio. Low GI diets have shown to reduce renal perfusion (Jenkins et al., 1987) and this might be another advantage of low GI foods in diabetes where renal damage is a relatively common complication (Jenkins et al., 1988).

High GI foods that lead to insulin resistance are reported to be associated with risk of development of colon, colorectal cancer, (Augustin et al., 2001; Franceschi et al., 2001; Giovannucci, 2007) breast, (Augustin et al., 2001; Xue and Michels, 2007) prostate and ovarian cancers (Jenkins et al., 2002). During population studies the insulin resistance and insulin like growth factors (IGF-1) have been connected with diet related cancers (Giovannucci, 1999; Stoll, 1999; Augustin et al., 2001). High IGF-1 levels increase cell growth, proliferation, differentiation (Giovannucci, 2007) and accumulate cells with genetic damage which will lead towards carcinogenesis (Pollak, 2007) via inhibiting
apoptosis (Brand-Miller et al., 2005). IGF-1 promotes cell growth via stimulating tyrosine-specific protein kinase activity (Augustin et al., 2002).

SCFA (butyrate) produced by low GI foods have shown to play a key role in regulation of cell proliferation and differentiation in colonic epithelial cells by controlling the cell cycle at G1 checkpoint via p21WAF1/Cip1 protein. Butyrate, acetate and propionate also induce apoptosis of colorectal tumour cell lines (Wong and Jenkins, 2007).

Prolonged exposure of the enamel to low pH is recognized as a factor responsible in causing dental caries. The degree of fall of pH varies with different starchy foods, and a strong correlation between high GI foods and dental caries is reported (Petti, 2005).

2.11.6 Relationship between Glycaemic Load and diseases

Research done over the past 10 years have shown that overall Glycaemic Load is also a risk factor of type 2 diabetes (Salmeron et al., 1997), cardiovascular morbidity and mortality (Liu et al., 2000) and certain types of cancers (Franceschi, 1996; Franceschi et al., 2001). In addition fasting triglyceride concentrations (Liu et al., 2001) and C-reactive protein levels (Liu et al., 2002) have been correlated with overall GL as these will increase the risk of CHD particularly in individuals with insulin resistance (Brand-Miller et al., 2003).
2.12 Dietary recommendations regarding Glycaemic Index

The dietary recommendations regarding GI vary from country to country and organization to organization (Arvidsson-Lenner et al., 2004). GI values are primarily used to guide selection of foods for diabetic patients (Venn and Green, 2007) but is also beneficial for healthy individuals (Vosloo, 2005). The Food and Agricultural Organization (FAO) suggested the intake of low GI foods for hyperglycaemic, hyperlipidaemic, obese as well as healthy persons (FAO/WHO, 1998).

The Diabetes Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD) also recommends the carbohydrate containing foods that are rich in dietary fibre or low GI foods for patients with diabetes (Arvidsson-Lenner et al., 2004).

2.13 Dietary recommendations for diabetes

The increasing prevalence of diabetes throughout the world is partly related to fast-release nature of the staple carbohydrate foods (Chew et al., 1988). In contrast the slow-release nature of traditional foods of Asia, India, Oceania and Southern Europe had been protective against diabetes (Thorburn et al., 1987) in spite of these populations showing a genetic predisposition to being hyperinsulinaemic.

Diets high in complex carbohydrate and dietary fibre and low in fat are reported to be beneficial in improving carbohydrate metabolism in individuals with diabetes (Mann, 1984). Dietary management of diabetes requires a sound knowledge of plasma glucose and insulin responses to meals as the treatment targets on the reduction of postprandial hyperglycaemic and hyperinsulinaemic levels. Thus, the inclusion of low GI foods, i.e.,
legumes to diets had shown to reduce both the postprandial and 24 hour glucose profiles in diabetics (Simpson et al., 1981). When preparing meals to reduce the plasma glucose loads the fat content must also be considered.

Vegetarian diets have shown promising effects on reduction of diseases discussed earlier as these diets contain high fibre, cause high satiety and increase the bulk of the meal (Jenkins et al., 2003). Apart from reduction of the susceptibility to various diseases the weight loss that can be achieved is another advantage of a vegetarian diet (Jenkins et al., 2003).

However, Brand-Miller et al., (1992) stressed the importance of both GI and insulinaemic index (II) in determining the optimal carbohydrate foods for individuals with diabetes as insulin concentration is a major determinant of blood cholesterol and triglyceride concentrations and influence the progression of CHD (Zavaroni et al., 1989). All the foods with high glycaemic responses will not necessarily have a high insulin response and vice versa (Brand-Miller et al., 1992).

Studies revealed a reduction of serum antioxidant levels with increasing postprandial glycaemia. (Ceriello et al., 1998; Rao and Agarwal, 1999). Thus, intake of vitamin E have shown to improve glycaemic control in the high risk groups (Paolisso et al., 1993).

Diabetes which is considered as an age related disease can be delayed or prevented by consuming a diet with special emphasis to the GI starting from an early age and also prior to and during pregnancy in order to give birth to a healthy child (Everitt et al., 2006).
2.14 Nutrient density

Nutrient density is defined as the nutrient content of a food relative to its calorie value [nutrient density – nutrients (g) in total energy content (kilocalories or joules)]. Fruits and vegetables are foods with high nutrient density and provide substantial amounts of vitamins and minerals. Certain high GI foods such as potatoes, whole wheat breads, other grains, water melon, dates and carrots are sometimes very high in nutrient density. On the contrary, there are high calorie, non-nutrient dense foods such as chocolate, ice cream and cookies which are low in GI (Beals, 2005). Thus, this aspect also must be considered when planning meals for diabetics as well as selecting diets for non diabetic individuals.

2.15 Food items analyzed in the present study

2.15.1 Rice

Rice is a staple diet of many around the world and is the second most commonly consumed cereal. Domesticated rice comprises of two species, Oryza sativa (Asian), and Oryza glaberrima (African). The rice husk is removed using a rice huller giving rise to brown rice. At this stage rice can be steamed or parboiled to retain the nutrients. This category of rice is referred to as “parboiled rice”. If the rice is further processed to remove the bran it is referred to as “white rice or polished rice”. Rice is a good source of protein. However, it does not contain all the essential amino acids in adequate quantities. Thus, the supplementary dishes should be included. The preparation methods vary widely from country to country and even in the same country (Wickramanayake, 1998).
2.15.2 Bread

It is one of the oldest foods consumed by many and currently a staple food of mainly western countries. Bread is made by baking dough of flour and water with or without a leavening agent (i.e., yeast). Wheat flour is the most common grain used in making bread. However, bread is also made from other grains, i.e., rye, barley, maize and oats. Sometimes these are made in combination with wheat flour. Wheat flour contains two water insoluble proteins, i.e., glutenin and gliadin which form a structure of strands referred to as “gluten”. This gives the soft texture of the bread. White bread is made from polished wheat flour containing only the endosperm while wholemeal bread contains both the bran and the endosperm (Wickramanayake, 1998).

2.15.3 String hoppers

It is made with either wheat or rice flour and available mainly in South Asia. String hoppers are given as a breakfast or a dinner menu. String hopper dough is made with flour, water and a little salt. It is made into flat spirals by forcing through a mould and steam cooked.

2.15.4 Manioc (Manihot esculenta)

Manioc (Cassava) plant is native to South Africa. The manioc tuber is a good source of carbohydrate and is one of the largest (third largest) human carbohydrate sources in the world. Manioc tuber is poor in protein and other nutrients while the leaves are a good source, i.e. but does not contain methionine. However, the leaves contain free and bound
cyanogenic glucosides which are converted to cyanide by linamarase present in the manioc. Cooking in an open pot is needed to eliminate the toxicity. Manioc is prepared in different ways around the world (Wickramanayake, 1998).

2.15.5 Jackfruit (*Artocarpus heterophyllus*)

This plant is native to South and Southeast Asia. The fruit is one of the largest fruits in the world. Jackfruit can be eaten ripe or unripe. Jackfruit is boiled and consumed as a breakfast menu or cooked as a side dish to be eaten with rice. Jackfruit seeds are also eaten either cooked or baked (Wickramanayake, 1998).

2.15.6 Bananas (*Musa spp.*)

Bananas grow in hanging clusters. There are about 15-25 fruits in a tier and several tiers to a bunch. The fruit contains about 75% water and 25% dry matter. Bananas are good sources of vitamin B₆, vitamin C and potassium (Wickramanayake 1998). These are a non-seasonal crop and available throughout the year.

2.15.7 Meal accompaniments

Sri Lankan mixed meals include a starchy staple, vegetables, fish/meat curry and sambol/mallung prepared with green leaves. Few meal accompaniments used in the present study are discussed below:

Lentil (*Lens culinaris*) is a good source of protein (~ 26% protein in the raw) and constitutes a major component of the diet in the vegetarian menus especially in countries
like Nepal and India. When taken with rice the meal provides all the essential amino acids. Lentils are also a good source of dietary fibre and iron (Wickramanayake, 1998).

*Gotukola (Centella asiatica)* a leafy vegetable, is prepared as a sambol or mallung and is an accompaniment of rice. A highly nutritious porridge (*kola kenda*) is also made with *gotukola*. *Gotukola* is a mild antibacterial, antiviral and anti-inflammatory agent and a good source of iron (Wickramanayake, 1998). *Centella* also has anti-oxidant properties and shown to enhance mental ability.

*Kohila (Lasia spinosa)* is frequently cultivated in marshy areas in the villages for its young leaves and rhizomes. (Wickramanayake, 1998). It is prepared as curry or a sambol and eaten with rice.
3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Water
Distilled water ("Manesty" distillation unit, Manesty Machine Ltd, UK) was used for all analysis.

3.1.2 Chemicals
All chemicals were of analytical grade and purchased from BDH Laboratory Suppliers, UK except 3'5' dinitrosalicylic acid, guar (Sigma Chemical Company, USA), potato amylose (Sigma Aldrich, UK), potassium sodium tartrate-4-water (AvonChem Ltd, UK), sodium fluoride, sodium chloride and CuSO$_4$.5H$_2$O (Fisons Scientific Equipments, UK).

3.1.3 Solvents
Analytical grade solvents were used for all experiments (BDH Laboratory Suppliers, UK and Fisons Scientific Equipments, UK).

3.1.4 Enzymes
Enzymes used were α-amylase [bacterial origin (EC 3.2.1.1), Sigma (USA)], amyloglucosidase [from *Aspergillus niger* (EC 3.2.1), Roche Diagnostics GmbH, (Germany)], pepsin [porcine pepsin (EC 3.4.23.1), Sigma (USA)], pancreatin [porcine
pancreas (EC 232-468-9), Sigma (USA)], invertase [from baker’s yeast (EC 3.2.1.26), Sigma (USA)].

3.1.5 Enzyme kits
The glucose oxidase enzymatic kit (GOD-PAP) from Biolabo, France was used for the analysis of glucose concentrations of food and serum samples. Serum insulin was analyzed with Elecsys insulin reagent kit (Roche Diagnostics GmbH, Germany) using Elecsys 1010 analyzer. Insulin analysis was carried out at Ceymed Healthcare (Pvt) Ltd, Nugegoda.

3.1.6 Glucometer, lancets and needles
Glucometer used for this study was Accu-Check Active type (Roche Diagnostics GmbH, Germany). Measuring range is linear within 0.6 mmol/L – 33.3 mmol/L (10-600 mg/dL) range. Repeatability—coefficient of variation (CV) of 1.7%. Reproducibility-CV of 1.4%. Accu-Check Softclix pricking device and Softclix needles also from Roche Diagnostics GmbH, Germany were used to prick fingertips.

3.1.7 Instruments
Hot Air Sterilizer (Y Co-010 series, Gemmyss, Taiwan) was used to estimate the moisture contents of foods (3.2.2.1). Food items were dried using REMI Laboratory oven (India). Ash contents were measured by Muffle furnace, FP 32 (Japan) (3.2.2.2). The water baths, OLS 200 (Grant Instruments Cambridge Ltd, UK) and Eyeal uni thermo shaker (NTS1300, Japan) were used for incubations of samples at different temperatures. pH was measured
with Orion 410A+ pH meter (Thermoelectron Corporation, USA). The spectrophotometer, 169 (Systronic, India) was used to measure all absorbance values. Fibre analyzer, Dosi Fibre (P Selectra, Spain) was used to analyze the insoluble dietary fibre (IDF) contents of foods (3.2.2.7). The eppendorf centrifuge, Mikro 20 (Hettich, Zenttrifugen GmbH and Co, Germany) was used to separate serum samples (3.2.3.5, 3.2.7-3.2.8). Kubota 5100 centrifuge (Japan) was used for estimation of water solubility and water absorption indices (3.2.4.2) and analysis of glucose and starch fractions present in foods (3.2.4.4).

3.1.8 Human subjects

3.1.8.1 Healthy individuals

Healthy volunteers [age: 20-30 yrs, body mass index (BMI): 24±3 kg/m²] with fasting blood glucose levels 70-100 mg/dL, and not taking any medications participated in the first part of the study leading to determination of glycaemic indices (GI) of foods (3.2.3; a total of 24 individuals participated for the determination of GI of foods; n=10 for each food). Ethical Clearance was obtained from the Ethics Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (USJP) (Appendix II, letter 1, No - A 224).

3.1.8.2 Type 2 Diabetes Mellitus patients

Type 2 diabetes mellitus (DM) patients (age: 40-62 yrs, BMI: 25±2 kg/m²) with fasting blood sugar levels 126-200 mg/dL and only on the oral drug, metformin were recruited for the determination of glycaemic and insulinaemic responses of different breakfast meals and effect of these on a standard lunch meal (3.2.7 & 3.2.8). Ethical Clearance was
obtained from the Ethics Committee, Faculty of Medical Sciences, USJP (Appendix II, letter 2, No – A365/7), and Hospital Ethical Review Committee, Colombo South Teaching Hospital (Appendix II, letter 3, application No. 104).

Informed and written consents were obtained from both healthy individuals and type 2 diabetic patients before enrolling them for the study (Appendix III).

3.1.9 Raw materials

All raw materials were bought in bulk and stored at room temperature till used.

3.1.9.1 Flour varieties

Prima wheat flour (Prima Ceylon Ltd, Rajagiriya) and Ckakki brand atta flour (Food link Enterprises, Colombo 15) were bought from retail outlets.

3.1.9.2 Red rice

Red rice variety AT 353 obtained from Rice Research Institute (RRI), Batalagoda was dehulled and polished prior to use.

3.1.9.3 Bread varieties

Prima crust top bread, wholemeal bread (Ceylon Agro Industries Ltd, Seeduwa) were bought from retail outlets and ordinary bakery bread from a bakery near USJP.
3.1.9.4 Vegetables, meal accompaniments, fruits and ingredients for curry preparation

Three bulk specimens of manioc (*Manihot esculenta* from Kosgama, Horana and Ambilipitiya areas), gotukola (*Centella asiatica*), kohila (*Lasia spinosa*), snake gourd (*Trichosanthes curcumerina - pathola*), bread fruit (*Artocarpus altilis*), and specimens of four banana varieties (*Musa spp.*; kolikutu, embul, anamalu and seeni kesel (from Ambilipitiya area) were bought from Sunday market.

Three bulk specimens of Jackfruit (*Artocarpus heterophyllus*) and coconut (*Cocos nucifera*) from a single tree were obtained from Gangodawila area.

White eggs, onion (*Allium cepa* Linn), garlic (*Allium sativum* Linn), chilli (*Capsicum frutescens*), curry leaves (*Murraya koenigii* Linn), lime (*Citrus aurantifolia*), iodised salt crystals and salt powder were bought from retail outlets. Spices of Ma’s, Tropical Food processing (Pvt) Ltd, Dambulla were used for all food preparation.

3.1.9.5 Legumes

Lentils (*Lens culinaris* - myssor dhal), Chickpea (*Cicer arietinum*), mung beans (*Vigna radiata*) and cowpea (*Vigna unguiculata*) (Ariya brand, TradLanka Agricultural Enterprises, Anuradhapura) were bought from retail outlets.
3.2 Methods

3.2.1 Preparation of food and food flour

Food items were prepared according to standard Sri Lankan preparation methods and proportions of different ingredients were adjusted by considering the palatability. The methods used to prepare each food were standardized (i.e., ingredients, proportions, durations) to minimize day to day variations. Once prepared these were stored in tight containers until analyzed or served on the same day (within 1-2 hours) when determining GI.

Food flour was prepared by either air drying or oven drying at 40 °C. Dried flour was milled using an analytical mill (IKA ® A 11 basic, Brazil, 25 000 r.p.m. with 1 min pulses) and sifted through a sieve (0.315 milimicrons). Foods with high fibre or fat contents which could not be sifted were stored without sifting. Flour samples were stored in closed, air tight containers at —20 °C until analyzed.

3.2.1.1 Bread varieties

Bread slices were broken into small pieces, and dried at 40 °C in an oven.

3.2.1.2 Red rice

Rice (500 g) was cooked in a rice cooker with water (1000 mL), salt water (10 mL from 20 g salt crystals dissolved in 100 mL water). Cooked rice was first air dried and subsequently in the oven at 40 °C.
3.2.1.3 String hoppers (wheat and rice flour)

Wheat flour was steam cooked for 30 min. Flour was sifted using a household sieve once cooled.

Red rice was washed and soaked overnight in excess water. Rice flour was prepared by grinding after draining the water followed by roasting on a saucepan.

Dough was prepared by mixing flour (500 g), salt water (30 mL) and boiled and cooled water (450 mL) (to room temperature for wheat flour), slightly warm water (500 mL ~ 50-60 °C for rice flour). String hoppers were steam cooked for 10 min. Each was broken into small pieces and flour was obtained by drying at 40 °C in an oven.

3.2.1.4 Manioc

Manioc flesh was chopped into small pieces (200 g) and boiled in an open saucepan with adequate amount of water (200 mL) and salt water (20 mL) under low heat till all the water was dried. Samples were first blended and air dried. The dried gelatinized mass was crushed and dried in the oven at 40 °C. Specimens from different locations were subjected to the same treatment.

3.2.1.5 Jackfruit and bread fruit

The seeds and the flesh of jackfruit were boiled separately. Flesh was cut into small pieces (800 g), boiled in a large saucepan with water (100 mL) and salt water (20 mL) under high heat for 10 min, and under low heat till all the water was dried. Seeds were crushed. Outer covers were removed, cut into small pieces (200 g) and boiled with water (200 mL) and
salt water (20 mL) till soft. Boiled jackfruit flesh and seeds were first air dried, then crushed using a grinder into small pieces and again dried at 40 °C in the oven. Breadfruit was boiled according to the procedure given by Widanagamage et al., (2009) and flour was prepared by drying in the oven.

3.2.1.6 Lentil curry

Lentils (200 g) were boiled with water (400 mL), chilli powder (2.5 g), curry powder (2.5 g) and turmeric powder (1 g) for 10 min. Coconut milk (1st and 2nd extracts) for lentil curry and *kiri hodi* (3.2.1.7) was prepared as given below:

1st extract - coconut scrapings (100 g) was extracted with water (100 mL),
2nd extract – coconut scrapings from first extract with water (125 mL).

Lentil curry was prepared by first adding 2nd extract (100 mL), salt (20 mL), green chilli (10 g), curry leaves (5 g) and subsequently first extract (25 mL). Curry was tempered with chopped onions (10 g) and garlic (5 g). Curry was first air dried, subsequently dried in the oven at 40 °C.

3.2.1.7 *Kiri hodi*

Second coconut milk extract (125 mL) was boiled with onions (10 g), garlic (5 g), green chilli (10 g), curry leaves (5 g) and turmeric powder (1 g) for 10 min. First coconut milk extract (50 mL) was added and further boiled for 5 min. Once cooled salt (20 mL) and lime were added.
3.2.1.8 Egg

Eggs were boiled for 10 min.

3.2.1.9 Fresh salads (sambols)

Chopped *gotukola* (100 g) was mixed with coconut scrapings (50 g), onions (20 g), garlic (10 g), green chilli (10 g), salt powder (10 g) and lime. Raw *gotukola* was air dried.

Coconut sambol was prepared by grinding coconut scrapings (100 g) with chopped onions (20 g), garlic (5 g), dried chilli pieces (10 g), lime and salt powder (10 g). Coconut scrapings were first air dried, then oven dried.

Chopped onions (50 g), chilli pieces (5), salt powder (5 g) were mixed and lime was added to prepare the onion sambol.

*Kohila* and snake gourd skins were cleaned, flesh was cut into small pieces (100 g), salt water (100 mL) added and left for 5 min. Excess salt water was squeezed out. *Kohila* was immersed in hot water (80-90 °C, 100 mL) for further 5 min while snake gourd was washed with warm water. Excess water was again squeezed out and mixed with chopped onions (25 g), garlic (10 g), green chilli (10 g), salt powder (10 g) and lime. Raw *kohila* was first air dried, then oven dried.

3.2.1.10 Roti and *pittu*

Proportions of ingredients used to prepare *roti* varieties and *pittu* are given in Table 3.1. All were dried in the oven.
Table 3.1: Ingredients of roti and pittu preparations

<table>
<thead>
<tr>
<th>Food</th>
<th>Wheat flour</th>
<th>Atta flour</th>
<th>Rice flour</th>
<th>Coconut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roti (wheat flour)</td>
<td>50 g</td>
<td></td>
<td></td>
<td>50 g</td>
</tr>
<tr>
<td>Roti (atta flour)</td>
<td></td>
<td>50 g</td>
<td></td>
<td>50 g</td>
</tr>
<tr>
<td>Roti (rice flour)</td>
<td>25 g</td>
<td>25 g</td>
<td></td>
<td>50 g</td>
</tr>
<tr>
<td>Pittu (wheat flour)</td>
<td>50 g</td>
<td></td>
<td></td>
<td>50 g</td>
</tr>
</tbody>
</table>

(Widanagamage et al., 2009)

3.2.1.11 Legumes (Chickpea, mung beans, cowpea)

Legumes were washed and soaked overnight in excess water. All were boiled and excess water drained. Chickpea (200 g) was tempered with coconut oil (10 mL) and onions (10 g). All were first air dried and then in the oven (Widanagamage et al., 2009).

3.2.1.12 Banana varieties

Specimens of four varieties of banana were packaged as 10 g portions in air tight polythene bags, sealed and stored at -20 °C. Proximate analysis of each variety was carried out within ~ 8-10 days.
3.2.2 Analysis of proximate compositions of foods

3.2.2.1 Determination of moisture of fresh and dried food samples

Fresh food (edible portions) samples (1.000 g) were weighed into pre-heated, pre-weighed crucibles and heated at 105 °C until constant weight to determine moisture contents. Moisture was calculated as loss of weight on heating (AOAC, 1984a).

3.2.2.2 Determination of ash content

After determining fresh moisture contents, samples were heated at 550 °C for 6 hours. Weights of the samples (W₁) were measured once cooled to room temperature and ash was calculated as difference between W₁ and empty crucible (AOAC, 2000).

3.2.2.3 Determination of digestible starch content

Dried food flour samples (0.500 g) were digested with α-amylase (200 μL) and distilled water (15 mL) for 25 min in a boiling water bath. Once cooled to room temperature the samples were diluted with distilled water to 50 mL in volumetric flasks. Aliquots of samples (1 mL) were transferred into tubes containing 2 mL of 0.1 M sodium acetate buffer (pH 4.75). Amyloglucosidase enzyme (60 μL from 14 mg/mL stock solution) was added and incubated at 60 °C for 30 min. Samples were diluted to 10 mL with distilled water. Glucose concentrations of diluted samples (10 μL) were estimated using enzymatic kit (GOD-PAP) at 500 nm wavelength (Holm et al., 1986).
3.2.2.4 Estimation of glucose concentrations using enzymatic kit method (GOD-PAP)

Glucose oxidase reagent (500 mL) of the kit was prepared as given below:

Vial R1 [containing phosphate buffer (150 mmol/L), glucose oxidase (GOD) ≥ 20 000 UI/L, peroxidase (POD) ≥ 1000 UI/L, 4-Amino-antipyrine (PAP) (0.8 mmol/L)] was dissolved with ~ 300 mL of distilled water in the dark bottle provided and contents of vial R2 [containing chloro-4-phenol (2 mmol/L)] was added and mixed. Remaining distilled water (~ 200 mL) was added to the dark bottle and mixed. Contents were stored at 2-8 °C.

Glucose oxidase reagent (1 mL) was pipetted into three tubes, i.e., blank, standard, and test. Blank contained 10 μL distilled water, standard, 10 μL glucose standard solution (100 mg/mL), and test, 10 μL sample. All were incubated at 37 °C for 10 min and absorbance measured at 500 nm.

\[
\text{Starch} \% = \frac{\text{Abs}_{\text{sam}} \times 10 \times 50 \times 0.9 \times 100}{\text{Abs}_{\text{stand}} \times \text{weight of sample (mg)}}
\]

Abs$_{\text{sam}}$ – Absorbance of the sample

Abs$_{\text{stand}}$ – Absorbance of the standard

0.9 – Conversion factor of glucose to starch
In the cases of manioc and jackfruit, digestible starch contents of specimens from three locations were determined to analyze the variations of starch content in different locations. The specimen (in bulk) closest to the average was selected to determine the GI.

3.2.2.5 Determination of total starch content

The method of Tovar, (1992) was used to analyze the total starch content. Flour samples (0.500 g) were soaked with 10 mL of 0.1 M sodium phosphate buffer (pH 6.2) for 5 min. pH of samples were adjusted to 1.5, pepsin (0.10 g) added and incubated at 37 °C for 1 hour in a shaking water bath (shaking speed – 40 linear strokes/min). The pH was adjusted to 6.5, 10 mL of KOH (4 M) added, and incubated for 30 min at room temperature. Samples were then neutralized to pH 6-7. a-amylase (200 µL) was added, incubated in the boiling water bath for 25 min. Starch content was calculated as mentioned in 3.2.2.3.

3.2.2.6 Determination of fat content

Flour samples (2.00 g) were weighed into Majonnier tubes, moistened with 2 mL ethanol (95%), 10 mL HCl (7.7 M) added and incubated at 70-80 °C for 1 hour. Samples were cooled to room temperature, 10 mL of ethanol (95%) added and mixed. Diethyl ether (25 mL) was added and tubes were turned upside down slowly while opening the stopper every 2-3 turns to release gas. This was continued till all the gas was released. Petroleum ether (25 mL) was added and contents mixed by turning tubes upside down for 20 times. Layers were allowed to separate and supernatant layer pipetted out into a pre-weighed flask.
Contents in Majonnier tubes were washed with 30 mL diethyl ether:petroleum ether mixture (1:1) twice and supernatants were transferred into the same flasks.

Ether layers were allowed to evaporate in a fume cupboard first and in a desiccator secondly till constant weight and final weights recorded (Croon and Suchs, 1980).

3.2.2.7 Estimation of Insoluble Dietary Fibre and Soluble Dietary Fibre contents

The method of Asp et al., (1983) was used to estimate IDF and soluble dietary fibre (SDF) contents.

Flour samples (1.000 g) were digested with 25 mL of 0.1 M sodium phosphate buffer (pH 6.2) and α-amylase (100 μL) in a boiling water bath for 25 min. 0.2 M HCl (20 mL) added and left samples to reach room temperature. pH was adjusted to 1.5 and pepsin (0.10 g) added. The samples were mixed and incubated in a 40 °C water bath while shaking for 1 hour. pH was adjusted to 6.8, pancreatin (0.10 g) added, and incubated at 40 °C for further 1 hour while shaking (40 linear strokes/min). pH was adjusted to 4.5 once cooled to room temperature.

Samples were filtered with the fibre analyzer using No:2 sintered crucibles to determine IDF contents. Filtered samples were washed with 15 mL ethanol (95%) followed by acetone (15 mL) twice. Samples were dried at 105 °C till weights were constant, contents were ashed and constant weights recorded.

Once pH of the samples were adjusted to 4.5, (during the common procedure for IDF and SDF) these were filtered through No.4 filter papers (Whatman, USA) to determine the SDF contents. Absolute ethanol was added to filtered solution to have a final ethanol
concentration of 76%. Resulting mixture was incubated at 60 °C for 1 hour. Solutions were filtered with No. 4 filtration crucibles by applying pressure. Once the samples were filtered, crucibles were washed with 15 mL ethanol (95%), followed by 15 mL acetone twice. SDF contents were calculated by drying the samples at 105 °C in the oven and then at 550 °C in the muffle furnace.

3.2.2.8 Determination of protein content

Kjeldhal method (AOAC, 1984b) was used to determine the protein content. Samples were reacted with conc.H2SO4 and Hg-catalyst to form ammonium sulphate. Ammonia was released by adding NaOH and released ammonia was collected into boric acid and titrated with standard HCl.

The percentage nitrogen by mass (N%) was calculated using the equation below:

\[
N\% = \frac{(A-B) \times N_1 \times 14}{W_{\text{sam}} \times 1000}
\]

- **N1** — Normality of standard HCl
- **A** — Volume of standard HCl used for the sample (mL)
- **B** — Volume of standard HCl used for the blank (mL)
- **14** — Atomic weight of nitrogen
In order to calculate the protein content the percentage nitrogen was multiplied by 6.25 (conversion factor).

3.2.2.9 Estimation of sucrose content

Chopped pieces of bananas were crushed using mortar and pestle. Samples of 1.00 g were weighed and petroleum ether (5 mL) added to remove fat. Samples were vortexed vigorously for 5 min, ether layers decanted (twice). Excess ether was allowed to evaporate in the fume cupboard. Distilled water (20 mL) was added to samples, mixed and incubated at 85 °C for 10 min, to denature protein.

Control samples were prepared by adding 1 mL of sample to 2 mL of 0.1 M sodium acetate buffer (pH 4.5). Control sample contained free glucose. Test samples were prepared by mixing 1 mL samples with 2 mL 0.1 M sodium acetate buffer (pH 4.5) and invertase (70 mg). Control and test samples were incubated at 55 °C for 30 min. Contents were cooled to room temperature and diluted to 10 mL with distilled water. Amount of sucrose hydrolyzed to glucose was measured using the enzymatic kit (GOD-PAP) by calculating the difference between the test and the control samples (Seneviratne, 2007).

3.2.2.10 Estimation of reducing sugar content

Reducing sugar contents of banana varieties were determined by Lane-Eynon method (James, 1999). This method includes a titration of sugar solution with the Fehlings' solution.
Fehlings’ solution was prepared by mixing equal amounts of Fehlings’ solution A (69.3 g of CuSO₄·5H₂O and 1.0 mL of 1 M H₂SO₄ dissolved in 1000 mL distilled water) and Fehlings’ solution B [346 g of potassium sodium tartrate tetrahydrate (KNaC₄H₄O₆·4H₂O) and 100 g NaOH dissolved in 1000 mL distilled water] each day prior to use.

Fehlings’ solution was standardized by the following protocol given below prior to using the solution for analyzing banana samples.

Fehlings’ solution (20 mL) and distilled water (15 mL) were added to the Erlenmeyer flask followed by of 0.25% glucose solution (25 mL) from a burette (50 mL). Resulting solution was heated till boiled. Three drops (~300 μL) of 1% methylene blue indicator [methylene blue indicator (1 g) in 100 mL of distilled water] were added to the Erlenmeyer flask and titrated till blue colour disappeared and red copper oxide colour persisted. Approximate titre reading was recorded.

Titration was repeated using the values given below to obtain the accurate titre reading:

Fehlings’ solution = 20 mL

Sample volume = (Approximate titre reading from the first titration − 0.5 mL)

Distilled water = [75- (20 + sample volume)] mL

Accurate titration reading should be ~ 40 mL

Sample weights of bananas needed to carry out the titration procedure were finalized following trials to obtain approximate titre readings in the range of 25-50 mL. When the titre readings were less than 25 mL, original samples were further diluted and when titre readings were more than 50 mL samples were made more concentrated.
Sample weights of *kolikuttu*, *embul*, *anamalu* and *seeni kesel* used for titrations were 7.2, 7.5, 12.5 and 6.5 g respectively. Samples were crushed using mortar and pestle, suspended in 100 mL distilled water and homogenized for 20 min. Samples were filtered and diluted to 500 mL. Titration was carried out to obtain the approximate titre reading as mentioned earlier (when carrying out standardization procedure). Titration was repeated using values given below to obtain the accurate titre reading:

Fehlings’ solution = 20 mL

Sample volume = (Approximate titre reading from the first titration – 1 mL)

Distilled water = [75- (20 + sample volume)] mL

Accurate titration reading recorded.

Same procedure mentioned above was carried out with 0.25% glucose solution to obtain an accurate titre reading.

\[
\text{Reducing sugar } \% = \frac{(V_0 \times 25 \times f)}{(C \times V_1)}
\]

\(V_0\) = Volume of standard sugar solution in the accurate titration

\(V_1\) = Volume of sample in the accurate titration

\(C\) = % sample (w/v)

\(f\) = Correction factor if large amount of sucrose is present in the sample
3.2.3 Estimation of Glycaemic Indices of foods

GI of food items were determined by standard methods given in FAO/WHO, (1998) and Brouns et al., (2005). Each food was given to healthy individuals (n=10) on separate days and the study was performed in a random cross over design. A total number of 24 individuals participated in the study.

Prima crust top bread (white sliced bread) was used as the standard with all the foods. White sliced bread was given to 11 randomly selected individuals on three occasions as a pilot study to compare the CV of incremental area under curve (IAUC) of three days when standard was given with first two days. According to the results (Appendix IV) it was decided to serve standard only twice to the individuals.

Glucose was also used as a standard with randomly selected foods (n=9). This was carried out to calculate the conversion factor (ratio) that can be used to convert GI values obtained with bread as the standard to values expected with glucose.

Standards and test foods were given in random order not exceeding 6-8 weeks duration between standard and test. Subjects were restricted from having unusual vigorous activities and asked to refrain from smoking and taking alcohol on previous day. However, dinner meals on previous days were not controlled due to practical difficulties.

3.2.3.1 Calculation of 50 g available carbohydrate portions of basic foods

\[
\text{50 g available carbohydrate portion} = \frac{100}{\text{Digestible carbohydrate %}} \times 50
\]
When calculating digestible carbohydrate contents of bananas the digestible starch and all other sugars (free glucose, fructose, and sucrose) were taken into account.

3.2.3.2 Calculation of 50 g available carbohydrate portions of mixed meals

\[
50 \text{ g available carbohydrate portion} = \left( \frac{100 \times A}{1} \right) + \left( \frac{100 \times B}{2} \right) + \text{etc.}
\]

1 - Digestible carbohydrate% of one starchy food in the meal
2 - Digestible carbohydrate% of a second starchy food in the meal
A - Proportion of digestible carbohydrate from 1.
B - Proportion of digestible carbohydrate from 2.

The contribution of available carbohydrate from food 1 and 2 were taken considering a standard meal and palatability.

3.2.3.3 Foods analyzed

GI of basic foods (Plate 3.1), mixed meals and bananas listed in Table 3.2 were determined with healthy individuals. Selected mixed meals are presented in Plate 3.2.
Table 3.2: Foods given to healthy individuals for determination of GI

<table>
<thead>
<tr>
<th>Type</th>
<th>Food items</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic foods and</td>
<td>Wholemeal bread</td>
</tr>
<tr>
<td>Bananas</td>
<td>Ordinary bakery bread</td>
</tr>
<tr>
<td></td>
<td>Banana – <em>kolikuttu</em></td>
</tr>
<tr>
<td></td>
<td>Banana – <em>embul</em></td>
</tr>
<tr>
<td></td>
<td>Banana – <em>anamalu</em></td>
</tr>
<tr>
<td></td>
<td>Banana – <em>seeni kesel</em></td>
</tr>
<tr>
<td>Mixed meals</td>
<td>Wholemeal bread &amp; lentil curry</td>
</tr>
<tr>
<td></td>
<td>Red rice &amp; <em>kiri hodi</em></td>
</tr>
<tr>
<td></td>
<td>Red rice, lentil curry, egg, <em>gotukola sambol</em>, <em>kiri hodi</em></td>
</tr>
<tr>
<td></td>
<td>String hopper (wheat flour), egg, <em>kiri hodi</em>, coconut sambol</td>
</tr>
<tr>
<td></td>
<td>String hopper (red rice flour), egg, <em>kiri hodi</em>, coconut sambol</td>
</tr>
<tr>
<td></td>
<td>Manioc, coconut sambol</td>
</tr>
<tr>
<td></td>
<td>Jackfruit, jack seeds, coconut scrapings, onion sambol</td>
</tr>
</tbody>
</table>
3.2.3.4 Determination of Glycaemic Index values

In order to determine GI values of foods the serum glucose concentrations of fasting and following ingestion of foods were estimated using the enzymatic kit method (GOD-PAP). As a second measure blood glucose concentrations were recorded using a glucometer. GI values of foods were calculated with both enzymatic kit values and glucometer readings. Glucometer values were taken to compare the GI values obtained with two methods and to evaluate the feasibility of using a glucometer in determining GI values.

3.2.3.5 Estimation of serum glucose concentrations using enzymatic kit method

Volunteers were requested to undergo an overnight fast of 10–12 hours. Fasting blood samples were taken from the fingertips by pricking with Softclix lancet device. Blood samples (50 -100 µl) were collected into eppendorf tubes containing NaF (15 µl from a stock of 0.35 g/10 mL). Standard [white sliced bread/glucose (dextrose monohydrate from Glucolin, Glaxo Wellcome Ceylon Ltd, Sri Lanka)] containing 50 g available carbohydrate was given with 250 mL of water. Volunteers consumed the meals within 15 min and further blood samples were obtained at 30, 45, 60, 90 and 120 min intervals after taking the first bite. Standards and test foods were given on separate mornings. Serum was separated and glucose concentrations estimated using enzymatic kit (GOD-PAP). Blood, serum samples and needles were discarded after autoclaving (Autoclave sterilizer Sturdy SA-232, Gemmy Industrial Corporation, Taiwan).
Plate 3.1: Bread varieties given to healthy individuals

(a) White sliced bread; (b) Wholemeal bread; (c) Ordinary bakery bread
3.2.3.6 Standard curves of glucose

Standard curves of glucose were plotted with both the absorbance values obtained with the enzymatic kit and glucose concentrations obtained with the glucometer.

A stock glucose solution of 250 mg/dL was prepared with distilled water. Glucose concentrations of 50, 75, 100, 125, 150, 200 mg/dL were prepared by diluting the stock with distilled water. Six samples of each concentration were assayed for glucose using the enzymatic kit (GOD-PAP).

A stock glucose solution of 250 mg/dL was prepared with 0.1 M phosphate buffer (pH 7.4). Glucose concentrations 50, 75, 100, 125, 150, 200 mg/dL were prepared by diluting the stock with the phosphate buffer (pH 7.4). Glucose concentrations of each dilution (six samples of each) were recorded with glucometer.

3.2.3.7 Estimation of blood glucose concentrations using the glucometer.

Glucometer used for the present study was Accu-Check Active meter. To measure blood glucose using the glucometer, Accu-Check Active glucose strips were used. When volunteers reported to the lab after the overnight fast fingertips were pricked using the Softclix lancet device. A glucose strip was placed on Accu-Check Active detector. The first drop of blood was placed onto the strip and a reading was taken within 5 sec. Readings were recorded and test strips removed. Apart from giving a reading, each test strip contained a round control window which displays a colour depending on the glucose concentration. Glucose strip container displays a colour chart which shows the glucose
concentration according to the colour obtained. Therefore glucometer readings were further confirmed with the colour chart.

Test principle: Glucose dye oxidoreductase mediator reaction (PQQ dependent glucose dehydrogenase mediator reaction). Each strip contains (per cm²) - glucose dye oxidoreductase 0.7 U, bis-(2-hydroxyethyl)-(4-hydroiminocyclohexa)-2, 5-dienylidene) ammonium chloride 8.3 µg, 2, 18-phsophomolybdic acid 88 µg, stabilizer 0.18 mg.

3.2.3.8 Glucose response graphs of foods

Serum and blood glucose responses (with enzymatic kit and glucometer values) for each individual and for each food were drawn with glucose concentration values (mmol/L) for the Y axis and time intervals (fasting, 30, 45, 60, 90 and 120 min) for the X axis. Two sets of glucose response graphs (with enzymatic kit and glucometer) were plotted for each individual.

IAUC of graphs were calculated using the fasting value as baseline and ignoring any area below fasting level.

3.2.3.9 Calculation of Glycaemic Indices

\[
\text{GI of test food} = \frac{\text{IAUC of test food}}{\text{IAUC of standard}} \times 100
\]
Plate 3.2: Mixed meals given to healthy individuals

(a) Red rice mixed meal; (b) String hopper (rice flour meal); (c) Manioc meal; (d) Jackfruit meal
The present study used both white sliced bread and glucose (for selected foods) as standards. Accordingly, GI values were calculated with both and the conversion factor that can be used to convert GI values obtained with one standard to another was also calculated. As 10 individuals participated in determination of GI of each food the respective GI of the food was calculated by taking the average of 10 individual GI values.

3.2.4 Analysis of factors affecting Glycaemic Indices

3.2.4.1 Estimation of amylose and amylopectin contents of raw and processed foods

Standard curve of amylose was plotted prior to determination of amylose and amylopectin contents. Amylose stock solution of 10 mg/mL (10 mL), pH 6.9 - 7 was prepared using citric acid (1 M) and NaOH (0.1 M). 1 mL of KI/I₂ solutions (0.8 g KI and 0.08 g of I₂ dissolved in 100 mL distilled water) were added to 20, 25, 30, 40, 50 μL (six of each) volumes of amylose stock solutions and diluted to 10 mL. Samples were incubated at 4 °C for 20 min and absorbances measured at 620 nm.

Amylose contents in raw and processed foods were estimated by the method given by Mohammadkhani et al., (1990). Flour samples (1.00 g) were moistened with 2 mL ethanol (95%). NaOH (0.1 M) was added (20 mL) and vortexed. Blank was prepared with ethanol (2 mL) and 20 mL NaOH (0.1 M). Blank and test samples were incubated at 105 °C for 45 min. Once the incubation was over samples were allowed to reach room temperature and pH adjusted to 6.9 with citric acid (1 M). Samples were diluted to 50 mL with distilled
water. KI/I₂ solution (1 mL) was added to 100 µl sample, diluted to 10 mL and incubated at 4 °C for 20 min. Absorbance values were taken at 620 nm. Amylose concentration (C) was determined from the standard curve.

\[
\text{Amylose (mg)} = \frac{(C) \times 10 \times 50}{0.1 \times 1000}
\]

The difference between the digestible starch of each sample and amylose content was calculated as amylopectin content.

3.2.4.2 Estimation of Water Absorption and Water Solubility Indices

Water absorption index (WAI) is an indicator of gelling capacity of the starchy source. Water solubility index (WSI) expresses the amount of soluble substances dissolved in the medium. These fractions indicate the extent of gelatinization due to the processing methods of foods. Samples (2.50 g) were weighed into pre-weighed 50 mL centrifuge tubes and mixed with distilled water (30 mL). Suspensions were incubated at 30 °C for 30 min. Once incubations were over samples were centrifuged at 2000 g for 10 min. Volumes of clear supernatants were measured and transferred to flasks with known weights. Supernatants were dried till weights were constant. Weights of dried flasks and tubes were recorded. The WAI was calculated as the weight of gel (g) per weight of dry matter and
WSI as the percentage of soluble substances in the supernatant (g) per dry weight of sample (Ekanayake et al., 2006).

3.2.4.3 Microscopic examination of starch granules (qualitative study)
Starch granules of raw and processed flour were observed (x10x10) using Olympus System Microscope (Bx50, Japan). Microscopic slides were prepared by adding flour samples, distilled water and KI/I₂ (10:1 prepared as in 3.2.4.1) solution.

3.2.4.4 Rate of digestion of different carbohydrates
The method given by Englyst et al., (2000) was used to determine the rate of digestion of carbohydrates by estimating the rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS) and slowly digestible starch (SDS) fractions in basic foods. The data of above fractions of basic foods of which GI is already known (Table 4.5, and Widanagamae, 2007) were used to calculate the glucose and starch fractions present in mixed meals containing more than one carbohydrate source as this had not been attempted previously.

This procedure first subjects samples to digestion with pepsin (protease) to disrupt starch-protein interactions, followed by digestion with digestive enzymes with mechanical mixing to stimulate the digestion in vivo. Guar gum is added to increase the viscosity of the medium, so that the excessive breakdown of starch granules by the action of glass balls will be prevented.
According to the rate of digestion with digestive enzymes carbohydrates are categorized into RAG, SAG, RDS and SDS fractions. In order to estimate RAG and SAG fractions, glucose available at 20 min (G20) and 120 min (G120) from adding digestive enzymes respectively were taken into consideration. The analytical time point 20 min is selected for determination of RAG fraction because foods with high amount of rapidly digested starch release glucose within 20 min under standard conditions provided.

Foods with slow release carbohydrates are digested between 20-120 min. RAG includes, starch hydrolyzed to glucose and free sugar glucose (FSG) (free glucose and sucrose hydrolyzed to glucose). Thus, FSG is omitted when calculating RDS fraction.

3.2.4.4 (i) Estimation of rapidly available glucose and slowly available glucose

The method was modified following a pilot study to suit laboratory conditions and available facilities while maintaining standard conditions and starch digestion procedure. Kellogg’s corn flakes (Kellogg’s Company, USA) was used as the reference sample [G20 =79 (±1), G120=81 (±1)] to optimize the enzyme activity and stroke speed.

3.2.4.4 (ii) Preparation of enzyme mixture for the determination of rapidly and slowly available glucose fractions - (For 2 samples)

Pancreatin (4.0 g) was weighed into a centrifuge tube, distilled water (12 mL) added and vortexed. Mixture was centrifuged at 1500 g for 10 min. Supernatant (8.2 mL) was pipetted into a tube. Amyloglucosidase (1.8 mL from 14 mg/ mL stock), and invertase (80
mg) were added to the same tube and mixed. Enzyme mixture was prepared immediately before use.

3.2.4.4 (iii) Preparation of pepsin–guar gum solution for the analysis of rapidly and slowly available glucose fractions (For 2 samples)
Pepsin (0.10 g) was dissolved in 20 mL of HCl (0.05 M). Guar gum (0.10 g) was added and mixed. Pepsin–guar gum solution was also prepared each day immediately before use.

3.2.4.4 (iv) Procedure for determination of rapidly and slowly available glucose fractions
Guar gum powder (50 mg) was weighed into three beakers, i.e., blank, standard (2). Glucose standard (20 mL) was pipetted (25 mg/mL) into two of those beakers (standard) and 20 mL of acetate buffer into the third (blank) and all were mixed to disperse the gum. Samples (0.50 g) were weighed into separate beakers. A reference sample of Kellogg’s corn flakes was included with every batch of samples. The pepsin–guar gum solution (10 mL) was added to each sample, and incubated at 37 °C for 30 min. Once the incubation was over two glass balls were added to each beaker (blank, standard, sample) and 0.25 mol L⁻¹ sodium acetate (10 mL) to each sample beaker. Contents were mixed and beakers replaced in water-bath at 37 °C to equilibrate. Sodium acetate buffer (5 mL) was added to blank, replaced in water bath and vigorous shaking action (linear strokes- 150 /min) started (Time zero of incubation). Sodium acetate buffer (5 mL) was added to each standard beaker. One sample was removed from 37 °C water-bath and 5 mL of enzyme mixture was
added and kept in the water-bath. Aliquots of enzyme mixture (5 mL) were added to rest of the samples at timely intervals and placed in the shaking water-bath.

Each beaker was removed from the water bath exactly at 20 min after addition of the buffer/enzyme mixture. A 0.5 mL aliquot was transferred from each beaker into 20 mL of 66% ethanol and vortex-mixed to stop the hydrolysis (G20 portion). Beaker was returned to water-bath immediately after taking 0.5 mL aliquot. Two hours after adding enzyme (120 min) a second 0.5 mL from beakers was transferred into a tube containing 20 mL of 66% ethanol and vortex-mixed (G120 portion). Shaking action was continued till all the G120 portions were collected. RAG and SAG fractions were calculated as given in 3.2.4.4 (vi) using equations 1-3.

3.2.4.4 (v) Estimation of free sugar glucose

As mentioned earlier the FSG was omitted when calculating RDS fraction. Thus, FSG was determined by the protocol given below:

Sodium acetate buffer (25 mL from 0.1 mol/L stock) was added to one beaker (blank), 25 mL of glucose standard into two other beakers (standard). Samples (0.50 g) were weighed and two glass balls were added to each beaker. Sodium acetate buffer (25 mL) was added to each sample, contents were mixed vigorously and incubated in a boiling water-bath for 30 min. Invertase (40 mg) was added and incubated at 37 °C for 30 min while shaking. An aliquot (1 mL) of each sample/standard solution was transferred to a tube with absolute ethanol (2 mL). Contents were centrifuged at 500 g for 5 min and supernatant (1 mL) was
transferred into water (5 mL) and mixed. This is the FSG portion. Supernatant of standard (1 mL) was transferred into 20 mL of distilled water.

RDS and SDS fractions were calculated as given in 3.2.4.4 (vi) using equations 1 and 4-5.

3.2.4.4 (vi) Calculation of glucose and starch fractions

All collected samples (G20, G120 and FSG) were centrifuged at 500 g for 5 min before determination of glucose. Glucose was determined using the enzymatic kit (GOD-PAP). Glucose contents (g/100 g fresh weight) were calculated by equation (1) using the standard concentrations provided in Table 3.3.

Table 3.3: Volume (V) and concentration (C) values for the different analytes

<table>
<thead>
<tr>
<th>Analyte</th>
<th>V (mL)</th>
<th>C (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G20</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>G120</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>FSG</td>
<td>25</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Glucose (%) = \( \frac{A(0)VC}{A(3)W} \)  \hspace{1cm} (1)
\( A(t) \) - Absorbance of the test solution

\( V \) - Total volume of the test solution (mL)

\( C \) - Concentration (mg/mL) of the standard used

\( A(s) \) - Absorbance of the standard used

\( W \) - Weight (mg) of sample taken for analysis (after correcting for moisture)

RAG, SAG, RDS and SDS contents of basic foods (g/100 g fresh weight) were calculated using equations below (2-5).

\[
\text{RAG} = G_{20} \quad (2)
\]

\[
\text{SAG} = G_{120} - G_{20} \quad (3)
\]

\[
\text{RDS} = 0.9 \ (G_{20} - \text{FSG}) \quad (4)
\]

\[
\text{SDS} = 0.9 \ (G_{120} - G_{20}) \quad (5)
\]

RAG, SAG, RDS, and SDS contents of portions given in determination of GI (50 g/25 g available carbohydrate) of basic foods were calculated by multiplying said fractions (g/100 g fresh weight) with respective portion sizes given (g). Glucose and starch fractions of mixed meals were calculated by using the RAG, SAG, RDS, SDS values (g/100g fresh weight) of basic foods and the respective portion sizes of carbohydrate sources.
3.2.5 Prediction of glycaemic responses of foods with \textit{in vitro} hydrolysis of starch

The method by Granfeldt \textit{et al.}, (1992) was used to determine the \textit{in vitro} hydrolysis of starch. With this process foods are first subjected to chewing. This is carried out to disrupt food particles and initiate digestion of starch with salivary \( \alpha \)-amylase which cleaves \( \alpha \)-1, 4 linkages of starch into maltose, dextrin etc. Food samples then follow digestion with proteins in an acidic environment (pH 1.5) with enzyme pepsin. The acidic environment inactivates the salivary amylase enzyme, and pepsin will degrade the interactions between starch and proteins.

\( \alpha \)-amylase is then added to sample after adjusting the pH of the contents to 6.9 to resemble the pancreatic starch digestion. Digestion of starch with \( \alpha \)-amylase takes place inside a dialysis bag immersed in Na, K phosphate buffer. Although starch cannot cross the membrane and is retained inside the dialysis bag, the digested product, maltose (molecular weight-360), diffuses out into the dialysate. However, the volume of the solvent outside the bag (800 mL) is much greater than inside (30 mL). Thus, over time, most of maltose will appear in the dialysate. The passage of maltose out of the bag (diffusion along a concentration gradient) mimics the \textit{in vivo} absorption. Thus, the aliquots taken every half hour for three hours represent the rate of diffusion of maltose into the dialysate.

Starch hydrolysis curves of foods representing percentage hydrolysis with respect to time were drawn and area under curve (AUC) calculated. The Hydrolysis Index (HI) for each food was calculated as given below:

\[
HI = \frac{\text{AUC of test food} \times 100}{\text{AUC of standard}}
\]
3.2.5.1 Standard curve of maltose

Maltose stock solution of 0.01 M (10 mM) was prepared with 0.05 M Na, K-phosphate buffer (pH 6.9). Concentrations of 2, 3, 4, 5, 6, 7 mM were prepared by diluting the stock with phosphate buffer. Standard curve was plotted with dilutions of maltose stock using 3'5' dinitrosalicylic acid (DNS) reagent as given below:

Diluted stock (1 mL) was added to a tube followed by 1 mL of DNS solution. The mixture was incubated in a boiling water bath for 5 min. Distilled water (15 mL) was added and the absorbance measured at 530 nm. Blank was prepared by adding 1 mL distilled water to 1 mL DNS solution and following the same procedure mentioned above (Miller, 1959).

3.2.5.2 Determination of in vitro hydrolysis of starch

Individuals (n=6) participated in the study, were requested not to consume food for 1–1 ½ hours prior to the experiment and rinse mouth with water when starting the experiment. Available starch portion (1 g) of Prima crust top bread (standard) and test foods were given to individuals. Standard was given twice. Test foods included both basic foods and mixed meals. Basic foods were selected to cover cereals (wholemeal bread, ordinary bakery bread, red rice, wheat roti, atta roti) and legumes (chickpea, cowpea, mung beans, lentil curry).

The mixed meals given below were analyzed:

1. Wholemeal bread and lentil curry
2. Red rice and lentil curry
3. Red rice, lentil curry and gotukola sambol
4. Red rice, lentil curry, gotukola sambol and egg

5. Red rice, lentil curry and gotukola sambol (twice the amount as 3)

6. Jackfruit flesh, seeds and coconut scrapings

7. String hoppers (wheat flour), coconut sambol and egg

Each food was analyzed on a separate day. Individuals chewed the given food 15 times and expectorated into a beaker containing 6 mL of 0.05 M Na, K-phosphate buffer (containing 0.4 g/L NaCl) and pepsin (50 mg). Subjects rinsed their mouths with 5 mL of water 15 times and expectorated into the same beaker. pH was adjusted to 1.5, incubated at 37 °C for 30 min while shaking (linear strokes – 80 /min). pH of contents was adjusted to 6.9, α-amylase (110 units) added and volumes adjusted to 30 mL with phosphate buffer. The contents were transferred to dialysis bags (molecular weight cut off 12-14000 Daltons). Each bag was placed in a 1 L beaker [Plate 3.3 (a)] containing 0.05 M phosphate buffer (800 mL) and incubated at 37 °C for 3 hours in a stirring water bath (80 revolutions/min). Aliquots of dialysate (2 mL) were removed every half an hour and analyzed for reducing sugar content with 3'5' DNS reagent [Plate 3.3 (b)]. Proportion of the available starch hydrolyzed to maltose was taken as the percentage of hydrolysis.

The starch hydrolysis curves of the standard and test foods for each individual were plotted against time (30, 60, 90, 120, 150, 180 min) and area under hydrolysis curves (AUC) were calculated. HI was calculated as mentioned previously (3.2.5). HI for each food was calculated as an average of HI values of the six individuals.
Plate 3.3: *In vitro* hydrolysis of starch

(a) Chewed food samples in dialysis bags; (b) and (c) represents the control (blank) and test tubes after 3'5' DNS treatment (Test tubes 1-6 represent the aliquots of dilaysates taken at 30 min to 3 hours at half an hour intervals).
3.2.6 Estimation of the effect of edible portion of dietary fibre and bulk of a meal on Glycaemic Index

The rice mixed meal containing *gotukola* which was given in determination of GI with healthy individuals (see section 3.2.3, Table 3.2) yielded the lowest GI among all meals analyzed. Thus, the aim of this study was to determine the inclusion of two sources of fibre as accompaniments in a standard rice meal on the subsequent GI. The quantities of two sources of fibre were adjusted to resemble edible portions due to the practical difficulties in offering a meal with portions that are more than the actual serving sizes.

As *gotukola* was already included in the rice mixed meal (meal 1), *kohila* was selected as the other source of fibre in meal 2. Both the *gotukola* and *kohila* were given as sambols. The introduction of *kohila* increased the bulk of the meal. Thus, to exclude the effect of increase in bulk, the amount of *kohila* was replaced by snake gourd (high moisture) and served with meal 3.

Thus, meals 1-3 contained boiled red rice (185 g), lentil curry (75 g), boiled egg, *kiri hodi* (30 mL) with either one or two items as given in the Table 3.4.

Although meal 1 was given earlier when determining GI (Table 3.2), all three meals in Table 3.4 were given as a new group of healthy volunteers (n=12, age-20-30 yrs, BMI-24±2 kg/m²) participated in this study. GI of the three meals were determined as described in section 3.2.3.5 and 3.2.3.8-3.2.39.
Table 3.4: Amounts (g) of gotukola, kohila and snake gourd included in meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Gotukola</th>
<th>Kohila</th>
<th>Snake gourd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25 g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>25 g</td>
<td>25 g</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>25 g</td>
<td>-</td>
<td>25 g</td>
</tr>
</tbody>
</table>

3.2.7 Determination of glycaemic and insulinaemic responses of type 2 diabetic patients to breakfast meals

Type 2 diabetic patients on an oral drug (metformin) participated in this study. Patients were requested to refrain from taking metformin for 36 hours (the morning of the study, previous day, night before) and undergo 10-12 hours overnight fasting. The removal of metformin for the duration specified above was done with the consultation of their physician. Volunteers were requested to refrain from vigorous physical activities, smoking and drinking alcohol the previous day. The study was performed in a random cross over design. A total of 12 volunteers (11 females, 1 male) participated in the study and 11 individuals consumed each test meal. The study was carried out at the Family Practice Centre, Faculty of Medical Sciences, USJP.

GI of three selected breakfast meals including cereals (rice or whole wheat flour) and legumes were determined with diabetic patients. The test meals analyzed were chickpea, atta roti with onion sambol, red rice with lentil curry and coconut sambol (Plate 3.4).
Although a 50 g available carbohydrate portion of test food was given in determination of GI with healthy individuals, the portions given to type 2 DM patients were reduced to 25 g available carbohydrate as these patients had been advised to consume meals containing small portions. Accordingly, the standard (Prima crust top bread) containing similar carbohydrate load was also given to calculate the GI values.

A canula was inserted when the volunteers arrived after the overnight fast. Fasting blood samples were taken (0.5 mL) and one test meal was given with 250 mL of water on the first day. Volunteers consumed the meal within 15 min. Further blood samples were obtained at 30, 45, 60, 90, 120, 150 and 180 min intervals (for 3 hours) after taking the first bite. Samples were stored at 4°C till serum was separated on the same day. Blood samples were taken for 3 hours in diabetic patients unlike in the case with healthy individuals. Standard was given on two days. Test breakfast meals and standard were given on separate mornings. Each volunteer completed the study within 4-6 weeks.

Estimation of serum glucose concentrations was as described in section 3.2.3.5. GI of breakfast meals were calculated as described in section 3.2.3.9 by taking IAUC of 3 hours. The insulin concentrations were estimated with the Elecsys 1010 Analyzer. The insulin response curves for each food per individual were drawn and the area calculated by using the fasting insulin concentration as the baseline. Insulinaemic index (II) of each individual for each food was calculated as given below:

\[
\text{Insulinaemic Index (II)} = \frac{\text{IAUC of test food}}{\text{IAUC of standard}} \times 100
\]
Plate 3.4: Breakfast meals given to type 2 diabetic patients

(a) Chickpea meal (Boiled chickpea - 180 g); (b) Atta roti meal (atta roti - 76 g, onion sambol - 10 g); (c) Rice meal (red rice - 93 g, lentil curry - 38 g, coconut sambol - 25 g)
3.2.8 Determination of the effect of different breakfast meals on the glycaemic and insulinaemic responses to lunch (second meal effect) in type 2 diabetic patients

The effects of the breakfast meals (in section 3.2.7) on a standard lunch meal comprising red rice, lentil curry, gotukola sambol, kiri hodi and a boiled egg (as in Table 3.2) were analyzed as mentioned below:

Fasting blood samples (0.5 mL) were obtained after an overnight fast (10-12 hours) by inserting a canula. The breakfast meals (section 3.2.7) and the standard bread were given with 250 mL of water to volunteers on separate mornings. The standard lunch meal (containing 25 g available carbohydrate) was given at 4 hours (240 min) after taking the first bite of the breakfast meal and blood samples taken at 30, 45, 60, 90, 120, 150 and 180 min intervals (for 3 hours) after taking the lunch meal. The blood samples were stored at 4°C till serum was separated. The serum glucose and insulin concentrations were analyzed as mentioned in section 3.2.7.

Serum glucose and insulin response curves of lunch meals were plotted and the IAUC calculated. The IAUC for each food was taken as an average of 11.

3.2.9 Establishment of a data base of Glycaemic Index values of Sri Lankan foods

A database containing GI values of Sri Lankan foods is being constructed as part of an objective of the present study. It will contain the data of the present study and reported data from other researchers in Sri Lanka. This database will be linked to either the Faculty of Medical Sciences, USJP web site or the sites of the granting agencies (National Research Council/National Science Foundation) from September 2009.
3.2.10 Statistical analysis

The proximate data of foods, serum glucose concentrations, serum insulin concentrations, RAG and SAG fractions are expressed as mean ± standard deviation (SD). IAUC, GI, II, HI values are presented as mean ± standard error of mean (SEM).

The significance of difference in the parameters tested between test and the standard or between tests were analyzed by Students’ t test. Differences were considered significant if p<0.05. Data were analyzed with Microsoft Excel and Minitab version 14.
4. RESULTS

4.1 Proximate compositions of foods

Digestible carbohydrate contents (g/100 g fresh weight) used to determine Glycaemic Index (GI) values and other proximate data of foods are presented in Table 4.1 – 4.4.

4.1.1 Moisture, ash, fat and protein contents of processed foods

Fresh moisture, ash, fat and protein contents of processed foods are given in Table 4.1. Jackfruit (Artocarpus heterophyllus) had the highest moisture content (81.5%) among all foods analyzed. Other wet processed foods such as rice, manioc (Manihot esculenta) and string hoppers had significantly high moisture contents (p<0.05) compared with dry processed bread varieties.

Ash (mineral) contents of foods ranged from 0.1-1.1% with wholemeal bread and jackfruit seeds having the highest amounts.

Fat contents of foods ranged from 0.6-2.9% with bread varieties having the highest levels among the foods studied.

Protein contents of wheat flour preparations (7.0-8.9%) were significantly higher (p<0.05) than rice, manioc and jackfruit (flesh and seeds) samples (0.8-4.7%).
Table 4.1: Moisture, ash, fat and protein contents of processed foods (g/100 g fresh weight)

<table>
<thead>
<tr>
<th>Food</th>
<th>Moisture (mean ± SD)</th>
<th>Ash (mean ± SD)</th>
<th>Fat (mean ± SD)</th>
<th>Protein (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sliced bread</td>
<td>a 40.1 ± 0.9</td>
<td>0.7 ± 0.1</td>
<td>2.8 ± 0.2</td>
<td>a 7.2</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>a 39.2 ± 1.0</td>
<td>1.1 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>a 8.9</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>a 42.2 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>a 7.5</td>
</tr>
<tr>
<td>Red rice</td>
<td>b 68.1 ± 0.5</td>
<td>0.3 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>b 3.3</td>
</tr>
<tr>
<td>String hopper (wheat flour)</td>
<td>b 58.4 ± 0.7</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>a 7.0</td>
</tr>
<tr>
<td>String hopper (rice flour)</td>
<td>b 58.5 ± 0.8</td>
<td>0.6 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>b 3.5</td>
</tr>
<tr>
<td>Manioc</td>
<td>b 68.2 ± 0.5</td>
<td>0.1 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>b 0.8</td>
</tr>
<tr>
<td>Jackfruit</td>
<td>b 81.5 ± 0.9</td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>b 0.9</td>
</tr>
<tr>
<td>Jackfruit seeds</td>
<td>b 52.8 ± 0.9</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>b 4.7</td>
</tr>
</tbody>
</table>

\(^1n=6; \ ^2n=4; \text{SD- Standard deviation; Values given by}^a \text{ and}^b \text{ in the same column are significantly different (p<0.05) to each other.}\)
Figure 4.1: Standard curve of glucose (with enzymatic kit method)

Each point represents an average of 6 values.
4.1.2 Digestible, undigestible starch contents and dietary fibre of processed foods

Standard curve of glucose is presented in Figure 4.1. Digestible, undigestible starch and dietary fibre contents of processed foods are presented in table 4.2. Dry processed foods had significantly higher (p<0.05) digestible starch contents compared to wet processed foods. The highest digestible starch content (g/100 g fresh weight) was observed in white sliced bread (43.8%) while the lowest was in jackfruit (10.0%).

Undigestible starch contents of processed foods ranged from 0.3-8.0% with jackfruit seeds containing the highest levels. The insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents of foods ranged from 0.7–7.9% and 0.7–3.5% respectively. IDF content of jackfruit seeds was significantly higher (p<0.05) than the other foods studied.

4.1.3 Chemical compositions of banana (Musa spp.) varieties

Banana varieties had moisture levels in the range of 68.2–75.6% with embul having the highest level and seeni kesel the lowest level (Table 4.3). Kolikuttu contained the highest total digestible carbohydrate content (26.5%) while anamalu contained the lowest (17.9%). Fat contents of the four varieties ranged from 1.4-2.4% (Table 4.3). Total dietary fibre (TDF) contents of fruits ranged from 2.7-5.3% with IDF content of kolikuttu being significantly higher (p<0.05) than the other three and SDF content of anamalu being significantly lower than the other three varieties (p<0.05).
Table 4.2: Digestible starch, undigestible starch, IDF and SDF contents of processed foods (g/100 g fresh weight)

<table>
<thead>
<tr>
<th>Food</th>
<th>Digestible starch (mean ± SD)</th>
<th>Undigestible starch</th>
<th>IDF (mean ± SD)</th>
<th>SDF (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sliced bread</td>
<td>43.8 ± 0.6</td>
<td>0.6</td>
<td>0.7 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>38.9 ± 0.5</td>
<td>0.3</td>
<td>4.2 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>41.2 ± 0.7</td>
<td>0.5</td>
<td>1.0 ± 0.2</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Red rice</td>
<td>23.5 ± 0.5</td>
<td>0.7</td>
<td>2.2 ± 0.1</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>String hopper (wheat flour)</td>
<td>30.8 ± 0.5</td>
<td>0.4</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>String hopper (rice flour)</td>
<td>30.7 ± 0.9</td>
<td>0.4</td>
<td>2.0 ± 0.4</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>*Manioc</td>
<td>25.6 ± 0.9</td>
<td>0.6</td>
<td>1.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>*Jackfruit</td>
<td>10.0 ± 0.3</td>
<td>0.3</td>
<td>1.5 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>*Jackfruit seeds</td>
<td>21.9 ± 0.8</td>
<td>8.0</td>
<td>7.9 ± 0.5</td>
<td>3.2 ± 0.3</td>
</tr>
</tbody>
</table>

n=6; SD-Standard deviation; Undigestible starch = total starch - digestible starch; Values given by a and b in the same column are significantly different (p<0.05) to each other

*Manioc and jackfruit from three locations were analyzed for the digestible starch contents. Digestible starch contents of the three specimens were not significantly different (p<0.05). Thus, specimen closest to the average digestible starch content of the three was selected for analysis of other proximate data and determination of GI.
4.1.4 Proximate contents of meal accompaniments

Cooked staple starchy foods were served with curries i.e., lentil (*Lens culinaris*) curry, coconut (*Cocos nucifera*) sambol, *gotukola* (*Centella asiatica*) sambol, *kohila* (*Lasia spinosa*) sambol and snake gourd (*Trichosanthes cucumerina* - *pathola*) sambol. The proximate data (g/100 g fresh weight) of cooked or raw (according to the preparation method) meal accompaniments were analyzed to calculate the total nutrient contents of the meals served when determining GI (Table 4.4). Moisture contents of foods ranged from 40.7-88.3%.

Digestible starch contents of all the meal accompaniments were significantly lower (p<0.05) than the amount contributed by lentil curry. The highest fat content was observed with coconut scrapings (39.2%) followed by lentil curry (11.2%).

All meal accompaniments contained higher IDF contents (3.8-12.0%) compared with the SDF contents (0.6-2.3%). Coconut contained significantly high IDF content (p<0.05) compared with others. *Kohila* and coconut had significantly high SDF contents (p<0.05) compared with lentil curry and *gotukola*. Proximate contents of the other meal accompaniment given, i.e., snake gourd (Chuku *et al.*, 2008) are presented in Appendix VI.
Table 4.3: Chemical compositions of bananas (g/100 g fresh weight)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kolikutu (mean ± SD)</th>
<th>Embul (mean ± SD)</th>
<th>Anamalu (mean ± SD)</th>
<th>Seei kesel (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>70.9 ± 1.1</td>
<td>75.6 ± 0.5</td>
<td>74.2 ± 1.1</td>
<td>68.2 ± 0.5</td>
</tr>
<tr>
<td>Ash</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Digestible starch</td>
<td>3.7</td>
<td>NP</td>
<td>1.3</td>
<td>NP</td>
</tr>
<tr>
<td>Free glucose</td>
<td>4.6 ± 0.4</td>
<td>5.4 ± 0.6</td>
<td>4.1 ± 0.3</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>Fructose</td>
<td>11.0</td>
<td>13.4</td>
<td>4.9</td>
<td>12.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.2 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>6.8 ± 0.2</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>*Total digestible carbohydrate</td>
<td>26.5</td>
<td>23.2</td>
<td>17.9</td>
<td>22.6</td>
</tr>
<tr>
<td>Undigestible starch</td>
<td>3.8</td>
<td>4.4</td>
<td>4.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Fat</td>
<td>2.1 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>2.4 ± 0.5</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>IDF</td>
<td>a3.0 ± 0.2</td>
<td>b1.9 ± 0.1</td>
<td>b1.9 ± 0.1</td>
<td>b1.7 ± 0.2</td>
</tr>
<tr>
<td>SDF</td>
<td>a2.3 ± 0.2</td>
<td>a1.6 ± 0.5</td>
<td>a0.8 ± 0.1</td>
<td>a2.3 ± 0.5</td>
</tr>
<tr>
<td>TDF</td>
<td>5.3</td>
<td>3.5</td>
<td>2.7</td>
<td>3.0</td>
</tr>
</tbody>
</table>

SD = Standard deviation; n=6; NP= Not present; Fructose content = Total reducing sugar content (3.2.2.10) – free glucose levels (3.2.2.9); *Total digestible carbohydrate = digestible starch + free glucose + sucrose + fructose; TDF = IDF + SDF; Values given by a and b in each raw (for IDF and SDF) are significantly different (p<0.05) from each other.
Table 4.4: Proximate compositions of meal accompaniments (g/100 g fresh weight)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lentil curry (mean ± SD)</th>
<th>Coconut (raw) (mean ± SD)</th>
<th>Gotukola (raw) (mean ± SD)</th>
<th>Kohila (raw) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>69.5 ± 0.3</td>
<td>40.7 ± 0.9</td>
<td>86.8 ± 0.6</td>
<td>88.3 ± 1.1</td>
</tr>
<tr>
<td>Ash</td>
<td>0.5 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Digestible starch</td>
<td>11.8 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Undigestible starch</td>
<td>0.9</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat</td>
<td>11.2 ± 0.7</td>
<td>39.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>*Protein</td>
<td>8.0</td>
<td>3.9</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>IDF</td>
<td>4.7 ± 0.2</td>
<td>12.0 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>SDF</td>
<td>1.6 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

n= 6; *n=4; SD- Standard deviation. ND - Not detected.
4.2 Glycaemic Indices of foods

4.2.1 Portion sizes of food items

Table 4.5 represents the portions of basic starchy staples, meal accompaniments and bananas given to healthy individuals for determination of GI. The serving sizes of basic foods, mixed meals and bananas contained 50 g available carbohydrates.

4.2.2 Incremental Area Under Curves and Glycaemic Indices of foods

Prima crust top bread (white sliced bread) was used as the standard to determine GI of all the foods. White sliced bread was given to 11 randomly selected individuals on three occasions to compare the coefficient of variations (CV) of incremental area under curve (IAUC) of three days against first two days. CV of inter individual variations of three days differed from two days by 2% (Appendix IV).

Serum and blood glucose concentrations at fasting and following ingestion of foods were estimated by enzymatic kit (GOD-PAP) method and a glucometer (Accu-Check Active). Standard curves of glucose using values of enzymatic kit and glucometer are presented in Figure 4.1 and 4.2 respectively.

GI values calculated with enzymatic kit and glucometer values using white sliced bread as the standard are presented in Table 4.5. GI obtained with enzymatic kit values elicited a -8 to +1% difference compared with the glucometer values. The GI values calculated
with enzymatic kit method and glucometer were not significantly different (p>0.05) in
spite of the IAUC values (p<0.05) being significantly different.

4.2.3 Serum glucose responses to foods with enzymatic kit method

Average serum glucose responses (n=10) to foods are presented in Table 4.6. Average
fasting serum glucose values ranged from 4.3-4.8 mmol/L with their standard deviation
(SD) values ranging from 0.3-0.8 mmol/L. The highest postprandial serum glucose levels
(peak levels) ranged from 6.2–7.8 mmol/L with glucose solution yielding the highest value
and wholemeal bread & lentil curry meal eliciting the lowest level. However, SD values of
peak levels between individuals (0.7-1.1 mmol/L) were significantly higher (p<0.05)
compared with the fasting SD levels. Among the foods and glucose analyzed in the present
study, 10 foods elicited the highest serum glucose levels at 30 min following ingestion.
The three bread varieties elicited the highest serum glucose responses at 60 mins. Glucose
and wholemeal bread & lentil curry meal elicited similar blood glucose concentrations at
two adjacent time points (glucose; 30-45, wholemeal & lentil curry meal; 45-60 minutes)
indicating the highest glucose concentration to fall in between the two time points.

Average (n=10) glycaemic response curves of selected foods with enzymatic kit values are
presented in Figure 4.3 - 4.5.
Table 4.5: Portion sizes, IAUC, GI values against bread with enzymatic kit method, glucometer, GL values, categorization of GI, CV of GI values

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion size (g)</th>
<th>Enzymatic kit</th>
<th>Glucometer</th>
<th>GL</th>
<th>Categorization of GI</th>
<th>*CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. White sliced bread</td>
<td>114</td>
<td>181 ± 18</td>
<td>100</td>
<td>167 ± 14</td>
<td>100</td>
<td>39</td>
</tr>
<tr>
<td>2. Glucose</td>
<td>55</td>
<td>233 ± 29</td>
<td>141 ± 12</td>
<td>206 ± 26</td>
<td>137 ± 13</td>
<td>NA</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>128</td>
<td>179 ± 13</td>
<td>103 ± 11</td>
<td>173 ± 17</td>
<td>108 ± 10</td>
<td>38</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>121</td>
<td>189 ± 19</td>
<td>114 ± 9</td>
<td>181 ± 21</td>
<td>115 ± 9</td>
<td>40</td>
</tr>
<tr>
<td>Wholemeal read (64% starch)</td>
<td>83</td>
<td>145 ± 14</td>
<td>87 ± 6</td>
<td>146 ± 16</td>
<td>92 ± 7</td>
<td>31</td>
</tr>
<tr>
<td>Lentil curry (36% starch)</td>
<td>150</td>
<td>184 ± 29</td>
<td>99 ± 10</td>
<td>159 ± 22</td>
<td>92 ± 9</td>
<td>*40</td>
</tr>
<tr>
<td>Red rice Kiri hodi</td>
<td>203</td>
<td>184 ± 29</td>
<td>99 ± 10</td>
<td>159 ± 22</td>
<td>92 ± 9</td>
<td>*40</td>
</tr>
<tr>
<td>Red rice (82% starch)</td>
<td>176</td>
<td>97 ± 16</td>
<td>60 ± 5</td>
<td>85 ± 14</td>
<td>55 ± 8</td>
<td>24</td>
</tr>
<tr>
<td>Lentil curry (18% starch)</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gotukola sambol</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiri hodi</td>
<td>30 mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>String hopper (wheat flour)</td>
<td>162</td>
<td>168 ± 15</td>
<td>104 ± 7</td>
<td>176 ± 32</td>
<td>99 ± 12</td>
<td>36</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiri hodi</td>
<td>30 mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut sambol</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.5: Portion sizes, IAUC, GI values against bread with enzymatic kit method, glucometer, GL values, categorization of GI, CV of GI values

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion Size (g)</th>
<th>Enzymatic kit IAUC</th>
<th>Glucometer IAUC</th>
<th>GL</th>
<th>Categorization of GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>String hopper (red rice)</td>
<td>163</td>
<td>186 ± 18</td>
<td>103 ± 11</td>
<td>110 ± 10</td>
<td>40</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiri hodi</td>
<td>60 mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut sambol</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manioc</td>
<td>200</td>
<td>206 ± 21</td>
<td>120 ± 9</td>
<td>118 ± 12</td>
<td>*45</td>
</tr>
<tr>
<td>Coconut sambol</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackfruit</td>
<td>400</td>
<td>132 ± 19</td>
<td>75 ± 11</td>
<td>79 ± 12</td>
<td>27</td>
</tr>
<tr>
<td>Jackfruit seeds</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut scrapings</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion sambol</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kolikuttu</td>
<td>190</td>
<td>88 ± 11</td>
<td>61 ± 5</td>
<td>66 ± 8</td>
<td>22</td>
</tr>
<tr>
<td>Embul</td>
<td>220</td>
<td>107 ± 12</td>
<td>61 ± 5</td>
<td>67 ± 9</td>
<td>22</td>
</tr>
<tr>
<td>Anamalu</td>
<td>270</td>
<td>119 ± 16</td>
<td>67 ± 7</td>
<td>73 ± 11</td>
<td>24</td>
</tr>
<tr>
<td>Seeni kesel</td>
<td>220</td>
<td>123 ± 19</td>
<td>69 ± 9</td>
<td>67 ± 10</td>
<td>25</td>
</tr>
</tbody>
</table>

GI and IAUC values are given as mean ± SEM (Standard error of mean); *n=24; n=14; n=10 for other foods; GL = (GI against glucose x available carbohydrate in the portion)/100; GL is calculated with only enzymatic kit values; *GL – these portions represent edible portion sizes; NA-not applicable; * CV-%-given for the GI values obtained with the enzymatic kit method.
Each point represents an average of 6 values.
Table 4.6: Average serum glucose concentrations of foods determined using enzymatic kit

<table>
<thead>
<tr>
<th>Food</th>
<th>Fasting glu. conc. (mmol/L)</th>
<th>Peak glu. conc. (mmol/L)</th>
<th>Peaking time (min)</th>
<th>Glu. conc. at 120 min (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'White sliced bread</td>
<td>4.6 ± 0.3</td>
<td>6.8 ± 0.9</td>
<td>60</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>2Glucose</td>
<td>4.7 ± 0.4</td>
<td>7.8 ± 1.0</td>
<td>30-45</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>4.7 ± 0.3</td>
<td>6.8 ± 0.9</td>
<td>60</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>4.6 ± 0.4</td>
<td>7.1 ± 0.8</td>
<td>60</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Wholemeal bread &amp; lentil</td>
<td>4.6 ± 0.4</td>
<td>6.2 ± 0.9</td>
<td>45-60</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Red rice &amp; kiri hodi</td>
<td>4.5 ± 0.3</td>
<td>7.2 ± 0.8</td>
<td>30</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Red rice mixed meal</td>
<td>4.7 ± 0.4</td>
<td>6.4 ± 0.9</td>
<td>30</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>String hopper meal 1</td>
<td>4.5 ± 0.4</td>
<td>6.5 ± 0.9</td>
<td>30</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>String hopper meal 2</td>
<td>4.7 ± 0.4</td>
<td>6.6 ± 0.7</td>
<td>30</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>Manioc meal</td>
<td>4.8 ± 0.7</td>
<td>7.6 ± 1.0</td>
<td>30</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Jackfruit meal</td>
<td>4.6 ± 0.8</td>
<td>6.9 ± 1.1</td>
<td>30</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>Kolikuttu</td>
<td>4.7 ± 0.6</td>
<td>6.6 ± 0.8</td>
<td>30</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>Embul</td>
<td>4.6 ± 0.4</td>
<td>6.9 ± 0.9</td>
<td>30</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Anamalu</td>
<td>4.7 ± 0.6</td>
<td>6.8 ± 1.1</td>
<td>30</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Seeni kesel</td>
<td>4.3 ± 0.5</td>
<td>7.2 ± 0.8</td>
<td>30</td>
<td>4.9 ± 0.9</td>
</tr>
</tbody>
</table>

\(^1n=24; \(^2n=14; \)other foods n=10; Values are given as mean ± SD (Standard deviation); String hopper meal 1- wheat flour; string hopper meal 2- rice flour; Glu.- Glucose; conc. – concentration
4.2.4 Incremental Area Under Curves and Glycaemic Indices of foods with glucose as the standard using enzymatic kit method and glucometer

The GI values obtained with glucose as the standard are presented in Table 4.7. GI values obtained against glucose with enzymatic kit method yielded a +3 to -4% differences compared with GI values obtained from glucometer values. The two sets of GI values were not significantly different (p>0.05).

4.2.5 Factor to convert Glycaemic Index values obtained with one standard to another

GI values can be calculated with either local bread or glucose. The ratio of GI values obtained with bread:GI values obtained with glucose using enzymatic kit and glucometer values are presented in Table 4.7. The average ratio (conversion factor) that can be used to interconvert GI values obtained with bread to glucose (or vice versa) with enzymatic kit was 1.34 (1.24–1.44) while it was 1.29 (1.10-1.40) with glucometer values.

4.2.6 Categorization of Glycaemic Indices of foods

According to the GI values calculated with glucose as the standard, foods can be classified as low (<55), medium and high (>70) (Beals, 2005). GI values of foods that were calculated only with white sliced bread as the standard were multiplied by the conversion
factor 1.34 (4.2.5) to obtain GI values expected with glucose as the standard. When all the foods analyzed in the present study were categorized according to the classification system mentioned above, red rice mixed meal, jackfruit meal and banana varieties belong to low GI category, wholemeal bread & lentil curry meal, medium GI, and all the others high GI category (Table 4.5).

4.2.7 Energy levels of meals

Energy (KJ) levels of meals given for determination of GI were calculated according to the nutrient contents (g) present in the portions. Energy (KJ) provided by meals (containing 50 g available carbohydrate portions) ranged from 1111 – 2502 KJ. Bread varieties provided 1111-1177 KJ energy while red rice & kiri hodi meal and red rice mixed meal provided 1414 and 1940 KJ energy respectively. Two string hopper meals prepared with wheat and rice flour provided the highest energy levels with 2502 and 2419 KJ respectively.

4.2.8 Glycaemic Load values of basic foods, mixed meals and bananas

Glycaemic loads (GL) of 50 g available carbohydrate portions are presented in Table 4.5. Portions given for red rice & kiri hodi meal and manioc represented normal edible serving sizes while all other meals were more than the actual serving sizes. The GL values are categorized as low (<10), medium (10-20) and high (>20). Thus, all the portions served in determination of GI are high GL.
Table 4.7: GI values of foods against glucose with enzymatic kit method, glucometer and conversion factors (inter-convertible ratio) of the standards

<table>
<thead>
<tr>
<th>Food</th>
<th>Enzymatic kit values</th>
<th>Glucometer values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI</td>
<td>Ratio</td>
</tr>
<tr>
<td>White sliced bread</td>
<td>77 ± 6</td>
<td>1.29</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>76 ± 6</td>
<td>1.36</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>80 ± 4</td>
<td>1.43</td>
</tr>
<tr>
<td>Wholemeal bread &amp; lentil</td>
<td>61 ± 5</td>
<td>1.43</td>
</tr>
<tr>
<td>Red rice &amp; kiri hodi</td>
<td>80 ± 12</td>
<td>1.24</td>
</tr>
<tr>
<td>Red rice mixed meal</td>
<td>47 ± 6</td>
<td>1.28</td>
</tr>
<tr>
<td>String hopper (wheat) meal</td>
<td>72 ± 7</td>
<td>1.44</td>
</tr>
<tr>
<td>String hopper (rice) meal</td>
<td>79 ± 12</td>
<td>1.30</td>
</tr>
<tr>
<td>Manioc</td>
<td>90 ± 15</td>
<td>1.33</td>
</tr>
</tbody>
</table>

GI values are given as mean ± SEM (Standard error of mean); \(^1 n= 14; n=10 \) for other foods. Inter-convertible ratio = GI values obtained with bread (Table 4.5) as the standard/ GI values obtained with glucose as the standard; Range (enzymatic kit) = 1.24-1.44; (glucometer) = 1.10 -1.40; The average conversion factor with enzymatic kit values was 1.34, with glucometer values 1.29.
Figure 4.3: Glycaemic responses to wholemeal bread, wholemeal bread & lentil curry meal and standards (white sliced bread and glucose)

Figure 4.4: Glycaemic responses to red rice (AT 353) meals and standards (white sliced bread and glucose)
Figure 4.5: Glycaemic responses to manioc, jackfruit, banana (*kolikutu*) and standard (white sliced bread)
4.3 Factors affecting Glycaemic Indices

4.3.1 Amylose and amylopectin content

Amylose and amylopectin contents (g/100 g starch) of both raw and processed foods are presented in Table 4.8. Standard curve of amylose used to calculate the amylose concentrations in samples is presented in Figure 4.6. Boiled jackfruit seeds contained the highest amylose content (54%). Amylose contents of cooked flour were significantly higher (p<0.05) in wheat flour preparations, manioc and jackfruit seeds compared to raw flour.

4.3.1.1 Correlation between Glycaemic Index values and amylose contents

Among the foods studied, jackfruit meal contained the highest amylose content in the portion given for determination of GI. When GI of all basic foods and mixed meals were correlated with amylose contents of 50 g available carbohydrate portions, a non significant negative relationship (p=0.054) was obtained (Figure 4.7).

4.3.2 Water Absorption and Water Solubility Indices of raw and processed foods

Water absorption indices (WAI) and water solubility indices (WSI) of raw and processed foods are presented in Table 4.8. WAI contents of raw and cooked foods ranged from 1.9-4.2 with string hoppers prepared with wheat flour having the highest level.
WSI contents of raw and processed foods ranged from 1.5–28.7 with boiled jackfruit containing the highest levels.

All cooked foods had significantly higher (p<0.05) WAI and WSI contents than their corresponding raw flour except raw manioc flour (high WSI), jackfruit seeds (high WAI and WSI).

4.3.3 Microscopic examination of starch granules

Starch granules of raw and processed wheat based foods are presented in Plate 4.1. White sliced bread and wholemeal bread had intact slightly swollen starch granules while ordinary bakery bread had some disintegrated granules as well (Plate 4.1). String hoppers prepared with wheat flour showed disintegrated starch granules.

Starch granules of red rice (raw), red rice (boiled) and string hoppers made with rice flour are presented in Plate 4.2. Boiled rice had some intact swollen starch granules as well as disintegrated granules. However, string hopper preparation had only disintegrated granules.

Manioc flour (raw) contained starch granules which were not clumped together unlike in the cases of raw wheat or rice flour (Plate 4.3). Boiled manioc preparation had disintegrated starch granules.
Figure 4.6: Standard curve of amylose

Each value represents an average of 6 readings.
Table 4.8: Amylose, amyllopectin, WAI and WSI of processed foods and raw flour

<table>
<thead>
<tr>
<th>Processed food/raw flour</th>
<th>¹Amylose</th>
<th>¹Amylopectin</th>
<th>²WAI</th>
<th>²WSI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processed foods:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White sliced bread</td>
<td>35</td>
<td>65</td>
<td>3.0 ± 0.2</td>
<td>17.7 ± 0.3</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>28</td>
<td>72</td>
<td>2.8 ± 0.1</td>
<td>14.5 ± 0.3</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>32</td>
<td>68</td>
<td>3.4 ± 0.1</td>
<td>13.0 ± 0.3</td>
</tr>
<tr>
<td>Red rice</td>
<td>34</td>
<td>66</td>
<td>2.9 ± 0.1</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>String hopper (wheat flour)</td>
<td>24</td>
<td>76</td>
<td>4.2 ± 0.1</td>
<td>9.2 ± 0.8</td>
</tr>
<tr>
<td>String hopper (rice flour)</td>
<td>29</td>
<td>71</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Manioc</td>
<td>45</td>
<td>55</td>
<td>3.7 ± 0.1</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>Jack fruit</td>
<td>29</td>
<td>71</td>
<td>3.9 ± 0.2</td>
<td>28.7 ± 0.4</td>
</tr>
<tr>
<td>Jackfruit seeds</td>
<td>54</td>
<td>46</td>
<td>3.0 ± 0.1</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>Lentil</td>
<td>10</td>
<td>90</td>
<td>2.6 ± 0.1</td>
<td>20.9 ± 0.2</td>
</tr>
<tr>
<td><strong>Raw flour:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prima wheat flour</td>
<td>21</td>
<td>79</td>
<td>1.9 ± 0.1</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Red rice</td>
<td>30</td>
<td>70</td>
<td>2.2 ± 0.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Manioc</td>
<td>40</td>
<td>60</td>
<td>2.3 ± 0.2</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>Jackfruit</td>
<td>29</td>
<td>71</td>
<td>2.7 ± 0.3</td>
<td>19.5 ± 0.5</td>
</tr>
<tr>
<td>Jackfruit seeds</td>
<td>41</td>
<td>59</td>
<td>3.6 ± 0.4</td>
<td>13.5 ± 0.3</td>
</tr>
<tr>
<td>Lentil</td>
<td>24</td>
<td>76</td>
<td>2.4 ± 0.2</td>
<td>13.5 ± 0.4</td>
</tr>
</tbody>
</table>

Amylose values are given as g/100 g starch; ¹n=3; ²n=6; Amylose contents of banana varieties are not given due to very low amounts/negligible levels present.
Figure 4.7: Correlation between GI values and amylose contents of 50 g available carbohydrate portions.
Plate 4.1: Starch granules of raw and processed wheat based foods (10x10)

(a) Wheat flour (raw); (b) White sliced bread; (c) Wholemeal bread; (d) Ordinary bakery bread; (e) String hopper (wheat flour)
Plate 4.2: Starch granules of raw and processed rice based foods (10x10)

(a) Red rice flour (raw); (b) Red rice (boiled); (c) String hopper (red rice flour)
Boiled jackfruit contained disintegrated starch granules (Plate 4.4). Boiled jackfruit seeds contained intact swollen as well as disintegrated granules (Plate 4.5).

Starch granules of raw and cooked lentils are given in Plate 4.6. Cooked lentils contained swollen but cell enclosed starch granules.

4.3.4 Correlations between Glycaemic Indices of processed foods and nutrient contents.

Protein, fat, IDF, SDF and TDF contents of 50 g available carbohydrate portions of basic foods and mixed meals are given in Table 4.9.

The highest IDF content (13.5 g) was observed in jackfruit meal. Wholemeal bread meal (10.3 g) and red rice mixed meal (10.1 g) had IDF content >10 g, while all other foods had low IDF contents.

The highest SDF content was also observed in jackfruit meal (6.5 g), while the lowest was observed with manioc (1.9 g). Except white sliced bread and ordinary bakery bread, all other foods had higher IDF contents compared with the SDF contents.

As observed for IDF and SDF, the highest TDF content was also observed with the jackfruit meal (20.1 g), while white sliced bread (3.2 g) and ordinary bakery bread (3.7 g) contained the lowest amounts.
Significant negative correlations were observed between GI & IDF (p=0.032), GI & SDF (p=0.010) and GI & TDF (p=0.038) (Figure 4.8 - 4.10).

Wholemeal bread meal contained the highest protein content (19.4 g) followed by red rice mixed meal and string hoppers (wheat flour) meal (each 16.7 g). Manioc yielded the lowest levels (2.5 g). Protein contents and GI of foods elicited a non significant negative relationship (p=0.165).

Two string hoppers meals had the highest fat contents (36 g and 36.4 g respectively). Three bread varieties contained lowest levels of fat, while all other meals had quantities above 10 g. Fat contents and GI also did not show a significant (p=0.594) correlation.
Plate 4.3: Starch granules of raw and boiled manioc (10x10)

(a) Manioc flour (raw); (b) Manioc (boiled)

Plate 4.4: Starch granules of raw and boiled jackfruit flesh (10x10)

(a) Jackfruit (raw); (b) Jackfruit (boiled)
Plate 4.5: Starch granules of raw and boiled jackfruit seeds (10x10).
(a) Jackfruit seeds (raw); (b) Jackfruit seeds (boiled)

Plate 4.6: Starch granules of raw and cooked lentils (10x10)
(a) Lentils (raw); (b) Lentils (cooked)
Table 4.9: Nutrient contents (g) in 50 g available carbohydrate portions of foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Protein</th>
<th>Fat</th>
<th>IDF</th>
<th>SDF</th>
<th>TDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>White sliced bread</td>
<td>8.2</td>
<td>3.2</td>
<td>0.8</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>11.4</td>
<td>3.5</td>
<td>5.3</td>
<td>4.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>9.0</td>
<td>3.5</td>
<td>1.3</td>
<td>2.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Wholemeal bread &amp; lentil</td>
<td>19.4</td>
<td>15.5</td>
<td>10.3</td>
<td>5.2</td>
<td>15.5</td>
</tr>
<tr>
<td>Red rice &amp; kiri hodi</td>
<td>7.0</td>
<td>11.7</td>
<td>5.6</td>
<td>4.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Red rice mixed meal</td>
<td>16.7</td>
<td>21.2</td>
<td>10.1</td>
<td>5.1</td>
<td>15.2</td>
</tr>
<tr>
<td>String hopper (wheat) meal</td>
<td>16.7</td>
<td>36.0</td>
<td>6.7</td>
<td>4.8</td>
<td>11.5</td>
</tr>
<tr>
<td>String hopper (rice) meal</td>
<td>10.9</td>
<td>36.4</td>
<td>8.2</td>
<td>3.3</td>
<td>11.5</td>
</tr>
<tr>
<td>Manioc meal</td>
<td>2.5</td>
<td>10.7</td>
<td>6.3</td>
<td>1.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Jackfruit meal</td>
<td>6.8</td>
<td>11.5</td>
<td>13.5</td>
<td>6.5</td>
<td>20.0</td>
</tr>
</tbody>
</table>

¹Nutrient contents of meals were calculated as a cumulative value of all the components in the meal; TDF = IDF + SDF
Figure 4.8: Correlation between GI and IDF content

Figure 4.9: Correlation between GI and SDF content
Figure 4.10: Correlation between GI and TDF content
4.3.5 Rate of digestion of different carbohydrates

The rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS) and slowly digestible starch (SDS) fractions of basic foods \( (n=17) \) were analyzed and presented as g/100 g fresh weight in Table 4.10. RAG and RDS levels of cereal based foods, tuber and other vegetable fruits were significantly higher \( (p<0.05) \) than the corresponding SAG and SDS contents, except for breadfruit \( (Artocarpus altilis) \) and legumes.

The glucose and starch fractions of 50 g available carbohydrate portions of mixed meals \( (n=3) \) containing more than one carbohydrate sources are presented in Table 4.11.

4.3.5.1 Correlations between glucose or starch fractions with Glycaemic Indices or Incremental Area Under Curves

The glucose, starch fractions; RAG, RDS, SAG, SDS (present in the portion given for determination of GI) and glucose, starch ratios; RAG/SAG, SAG/RAG, RDS/SDS and SDS/RDS were correlated with GI and IAUC values of both basic foods and mixed meals (Table 4.5, Widanagamage, 2007). Significant positive correlations were observed with RAG, RDS fractions with GI, IAUC [Figure 4.11 (a), (b), (c) and Appendix VII]. The ratio SAG/RAG elicited significant negative correlations with GI and IAUC [Figure 4.11 (d), Appendix VII].
Table 4.10: RAG, SAG, RDS and SDS fractions of basic foods (g/100 g fresh weight)

<table>
<thead>
<tr>
<th>No.</th>
<th>Food item</th>
<th>$^{1}\text{RAG} \pm \text{SD}$</th>
<th>$^{1}\text{SAG} \pm \text{SD}$</th>
<th>$^{2}\text{RDS}$</th>
<th>$^{2}\text{SDS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>White sliced bread</td>
<td>38.5 ± 0.4</td>
<td>7.1 ± 0.7</td>
<td>34.6</td>
<td>6.4</td>
</tr>
<tr>
<td>2.</td>
<td>Wholemeal bread</td>
<td>38.0 ± 0.9</td>
<td>9.9 ± 0.6</td>
<td>34.2</td>
<td>8.9</td>
</tr>
<tr>
<td>3.</td>
<td>Red rice</td>
<td>18.8 ± 0.6</td>
<td>10.5 ± 0.3</td>
<td>16.9</td>
<td>9.5</td>
</tr>
<tr>
<td>4.</td>
<td>String hopper (wheat flour)</td>
<td>26.9 ± 0.8</td>
<td>9.9 ± 0.5</td>
<td>24.2</td>
<td>8.9</td>
</tr>
<tr>
<td>5.</td>
<td>String hopper (red rice flour)</td>
<td>28.1 ± 0.5</td>
<td>10.9 ± 0.2</td>
<td>25.3</td>
<td>9.8</td>
</tr>
<tr>
<td>6.</td>
<td>Pittu (wheat flour)</td>
<td>35.0 ± 0.9</td>
<td>3.8 ± 0.4</td>
<td>31.5</td>
<td>3.4</td>
</tr>
<tr>
<td>7.</td>
<td>Roti (wheat flour)</td>
<td>38.3 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>34.5</td>
<td>0.9</td>
</tr>
<tr>
<td>8.</td>
<td>Roti (atta flour)</td>
<td>37.8 ± 0.3</td>
<td>13.3 ± 0.3</td>
<td>34.0</td>
<td>12.0</td>
</tr>
<tr>
<td>9.</td>
<td>Roti (rice: wheat flour)</td>
<td>35.4 ± 0.9</td>
<td>7.5 ± 0.2</td>
<td>31.9</td>
<td>6.8</td>
</tr>
<tr>
<td>10.</td>
<td>Manioc</td>
<td>21.5 ± 0.5</td>
<td>9.8 ± 0.2</td>
<td>19.3</td>
<td>8.8</td>
</tr>
<tr>
<td>11.</td>
<td>Breadfruit</td>
<td>6.8 ± 0.3</td>
<td>18.4 ± 0.3</td>
<td>6.1</td>
<td>16.5</td>
</tr>
<tr>
<td>12.</td>
<td>Jackfruit flesh</td>
<td>13.6 ± 0.5</td>
<td>2.8 ± 0.5</td>
<td>12.2</td>
<td>2.6</td>
</tr>
<tr>
<td>13.</td>
<td>Jackfruit seeds</td>
<td>19.2 ± 0.6</td>
<td>9.6 ± 0.4</td>
<td>17.2</td>
<td>8.6</td>
</tr>
<tr>
<td>14.</td>
<td>Chickpea</td>
<td>9.5 ± 0.7</td>
<td>8.2 ± 0.4</td>
<td>8.5</td>
<td>7.4</td>
</tr>
<tr>
<td>15.</td>
<td>Mung beans</td>
<td>10.7 ± 0.3</td>
<td>7.5 ± 0.4</td>
<td>9.6</td>
<td>6.8</td>
</tr>
<tr>
<td>16.</td>
<td>Cowpea</td>
<td>7.1 ± 0.4</td>
<td>7.5 ± 0.4</td>
<td>6.4</td>
<td>6.8</td>
</tr>
<tr>
<td>17.</td>
<td>Lentil (curry)</td>
<td>8.9 ± 0.4</td>
<td>7.3 ± 0.5</td>
<td>8.0</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Values are given as $^{1}\text{mean} \pm \text{SD}$ (Standard deviation); $^{2}\text{mean}$; $n=4$
Table 4.11: RAG, SAG, RDS and SDS fractions of mixed meals containing two carbohydrate sources (g/50 g available carbohydrate portion)

<table>
<thead>
<tr>
<th>No</th>
<th>Meal</th>
<th>RAG</th>
<th>SAG</th>
<th>RDS</th>
<th>SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wholemeal bread meal</td>
<td>44.9</td>
<td>19.2</td>
<td>40.4</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>(Wholemeal bread &amp;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lentil curry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Red rice mixed meal</td>
<td>39.8</td>
<td>22.9</td>
<td>35.7</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>(Red rice &amp;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lentil curry)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Jackfruit meal</td>
<td>46.4</td>
<td>19.4</td>
<td>36.7</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>(Jackfruit flesh &amp;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>jackfruit seeds)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as fresh weight basis and calculated from the data of basic foods.
Figure 4.11: Correlations between glucose or starch fractions of basic foods and mixed meals with GI or IAUC.

(a) GI & RAG (p=0.023); (b) IAUC & RAG (P=0.002)
Figure 4.11 (cont): Correlations between glucose or starch fractions of basic foods and mixed meals with GI or IAUC.

(c) GI & RDS (p=0.011); (d) GI & SAG/RAG (p=0.036)
4.4 Prediction of glycaemic responses with *in vitro* hydrolysis of starch

*In vitro* hydrolysis of starch of basic foods (cereal based – 05, legumes - 04) and mixed meals (n= 07) were determined. Portion sizes comprising 1 g available starch and the respective hydrolysis indices (HI) of foods are presented in Table 4.12. Standard curve of maltose used to calculate the percentage of hydrolysis of starch into maltose is given in Figure 4.12.

4.4.1 Rate of hydrolysis of starch of basic foods and mixed meals

Graphs representing hydrolysis of starch over the period of 3 hours of basic foods are depicted in Figure 4.13. Bread varieties had 18-20% starch hydrolyzed to maltose within the first 30 minutes. Mung beans and lentils had 21% and 18% starch hydrolyzed while chickpea and cowpea yielded the lowest release of maltose with 14% and 15% respectively at the first time interval (30 minutes). The two *roti* varieties and red rice contained similar percentage of starch hydrolyzed (~17.5) when analyzed at 30 minutes.

Wholemeal bread showed the highest area of the hydrolysis curve and the highest HI among all the foods studied (Table 4.12).

Rate of hydrolysis of starch in mixed meals are presented in Figure 4.14.
Figure 4.12: Standard curve of maltose

Each point represents and average of 6 values

\[ y = 0.3531x \]
\[ R^2 = 0.9801 \]
4.4.2 Correlation between *in vivo* Glycaemic Indices and *in vitro* Hydrolysis Indices

The *in vivo* GI values of both basic foods and mixed meals elicited a significant positive relationship (p<0.001, r = 0.949) with *in vitro* HI values (Figure 4.15). A relationship of Y=1.1367X-12.138 was obtained for the foods analyzed in the present study.
Table 4.12: Portion sizes and HI (n=6) of basic foods and mixed meals

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion size (g)</th>
<th>HI (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic foods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>2.6</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>Ordinary bakery bread</td>
<td>2.4</td>
<td>102 ± 7</td>
</tr>
<tr>
<td>Red rice</td>
<td>4.1</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>Roti (wheat flour)</td>
<td>3.7</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>Roti (atta flour)</td>
<td>3.0</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>Lentils</td>
<td>8.5</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>Chickpea</td>
<td>7.4</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>Mung beans</td>
<td>6.9</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>Cowpea</td>
<td>6.9</td>
<td>58 ± 6</td>
</tr>
<tr>
<td><strong>Meal 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread, lentils</td>
<td>1.6, 3.1</td>
<td>81 ± 6</td>
</tr>
<tr>
<td><strong>Meal 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red rice, lentils</td>
<td>3.3, 1.5</td>
<td>84 ± 8</td>
</tr>
<tr>
<td><strong>Meal 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red rice, lentils, gotukola sambol</td>
<td>3.3, 1.5, 0.5</td>
<td>71 ± 6</td>
</tr>
<tr>
<td><strong>Meal 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red rice, lentils, gotukola sambol, egg</td>
<td>3.3,1.5,0.5, 0.4</td>
<td>65 ± 7</td>
</tr>
<tr>
<td><strong>Meal 5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red rice, lentils, gotukola sambol</td>
<td>3.3, 1.5,1.0</td>
<td>74 ± 8</td>
</tr>
<tr>
<td><strong>Meal 6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackfruit flesh, seeds, coconut scrapings</td>
<td>8.0, 0.9, 0.5</td>
<td>85 ± 8</td>
</tr>
<tr>
<td><strong>Meal 7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>String hopper (wheat), egg, coconut sambol</td>
<td>3.2, 0.4</td>
<td>98 ± 8</td>
</tr>
</tbody>
</table>

SEM-Standard error of mean
Figure 4.13: Rate of hydrolysis of starch in basic foods and standard (white sliced bread)

Each point represents an average of 6 values
Figure 4.14: Rate of hydrolysis of starch of mixed meals

Meals 1-7 (Details of meals are given in Table 4.12); Each point represents average of 6 values.

Figure 4.15: Correlation between *in vivo* GI and *in vitro* HI values
4.5 Effect of dietary fibre and bulk of a meal on Glycaemic Indices

4.5.1 Dietary fibre contents of meals

The three rice meals served to healthy individuals to determine the effect of edible portions of fibre and increase of bulk of meal on GI values are presented in Table 4.13. Inclusion of a normal serving size of kohila (in meal 2) had increased the IDF by 8.2%, SDF 3.9%, and TDF 7.2% when comparing with meal 1 (Table 4.13). Meal 3 contained similar fibre contents as meal 1 and similar total meal size as meal 2 so that any effect due to bulking will be nullified.

4.5.2 Glycaemic responses to meals

The mean incremental peak serum glucose concentrations IAUC, GI values of meals analyzed are presented in Table 4.13. The serum glucose concentrations at time intervals from fasting up to 2 hours (following ingestion of the three meals) are presented as incremental serum glucose values (taking average fasting serum glucose level as baseline) (Figure 4.16). Peak serum glucose value of meal 1 was 2.34 mmol/L while the other two meals were 2.27 mmol/L. All three meals gave rise to the highest blood glucose values (peak) at 30 minutes from ingestion of foods.

The serum glucose curve of meal 1 gradually increased for the first 30 minutes and decreased till 120 minutes (2 hours) showing a 0.07 mmol/L incremental serum glucose level (Figure 4.16) at the last time point (120 minutes). Meal 2 gradually decreased from...
30 minutes onwards till 90 minutes (1.5 hours – 0.19 mmol/L) and rose again to a level of 0.36 mmol/L at 120 minutes. However, the lowest serum glucose level the meal 3 reached was 0.33 mmol/L at 90 minutes.

GI of meal 1, 2, 3 were 63±6, 57±5, 61±5 respectively. IAUC values or GI of meals were not significantly different from each other (p>0.05).

Meal 1 was given previously (Table 4.5) using a different batch of the same red rice variety (AT 353) to a different group of individuals when determining the GI of this rice mixed meal. A GI of 60±5 was obtained for this meal at the previous occasion and 63±6 with the second group of volunteers and the two sets of data are not significantly different (p>0.05).
<table>
<thead>
<tr>
<th>Meal</th>
<th>IDF</th>
<th>SDF</th>
<th>TDF</th>
<th>GI</th>
<th>* Incremental AUC</th>
<th>peak serum glucose value (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal 1</td>
<td>10.1</td>
<td>5.1</td>
<td>15.2</td>
<td>2.34</td>
<td>63 ± 6</td>
<td>129 ± 17</td>
</tr>
<tr>
<td>Meal 2</td>
<td>11.0</td>
<td>5.3</td>
<td>16.3</td>
<td>2.27</td>
<td>57 ± 5</td>
<td>119 ± 17</td>
</tr>
<tr>
<td>Meal 3</td>
<td>10.1</td>
<td>5.1</td>
<td>15.2</td>
<td>2.27</td>
<td>61 ± 5</td>
<td>128 ± 19</td>
</tr>
</tbody>
</table>

Meal 1: Rice, lentil curry, boiled egg, *hiri bread* with gokhala; Meal 2: Components in meal 1 and kohli; Meal 3: Components in meal 1 and snake gourd; IDF, SDF, TDF values given as g/50 g available carbohydrate portion; * Incremental peak glucose values were calculated by using fasting value as zero; SEM: Standard error of mean. N=12.
Figure 4.16: Incremental serum glucose values of rice meals.

Each point represents average of 12 samples.
4.6 Glycaemic and insulinaemic responses of type 2 diabetic patients to breakfast meals

Glycaemic and insulinaemic responses to breakfast meals (n=3) were determined using white sliced bread as the standard. Proximate compositions of the three meals (containing 25 g available carbohydrate portion) are presented in Table 4.14.

Standard yielded the highest glucose (5.0 mmol/L) and insulin (36.0 microIU/mL) peak concentrations compared with the test breakfast meals (Table 4.15).

GI of chickpea, rice and roti meals were 40±7, 64±11, and 88±9 respectively (Table 4.15). Insulinaemic indices (II) of three meals were 76±13, 90±20 and 115±28 (Table 4.15) respectively.

Chickpea elicited the lowest glycaemic (Figure 4.17) and insulinaemic responses (Figure 4.18) and a serum glucose curve with a plateau.

4.6.1 The differences of glucose responses to standard with type 2 diabetic patients and healthy individuals

Standard was given twice to type 2 diabetic patients and the inter and intra individual variations of fasting serum glucose concentrations and IAUC values of the two days were calculated to compare with that of healthy individuals (Appendix IV).
CV of fasting intra individual variations of diabetic patients ranged from 1.5-29.0% while inter individual variation was 12.3%. Similarly, when CV of IAUC intra individual variations ranged from 0.3 -55%, whereas the inter individual variation was 19.5%.

When comparing the CV inter individual variations of fasting with healthy individuals and diabetic patients, a higher variation (by 7.2%) was observed with diabetic patients. However, the differences of CV of IAUC had reduced between two groups to a 2.5% increase with diabetic patients compared with healthy subjects.

4.7 Effect of different breakfast meals (4.6) on the glycaemic and insulinaemic responses of lunch (Second meal effect) in type 2 diabetic patients

Glycaemic and insulinaemic responses to a standard lunch meal (red rice mixed meal) following the consumption of above three meals (section 4.6) as the breakfasts are presented in Table 4.16. Glycaemic and insulinaemic responses to roti meal were lower than that of standard bread (Figure 4.17, 4.18). However, the decline was not significant (p>0.05).

The glycaemic and insulinaemic responses to foods of each individual (n=12) are presented in Appendix VII.
Table 4.14: Proximate compositions (g) of foods in 25 g available carbohydrate portions of
breakfast meals and lunch

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chickpea</th>
<th>Roti</th>
<th>Rice meal</th>
<th>Rice mixed meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>111.2</td>
<td>26.3</td>
<td>99.2</td>
<td>138.8</td>
</tr>
<tr>
<td>Ash</td>
<td>1.67</td>
<td>1.4</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Protein</td>
<td>16.4</td>
<td>11.4</td>
<td>9.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Fat</td>
<td>5.6</td>
<td>12.5</td>
<td>7.2</td>
<td>11.1</td>
</tr>
<tr>
<td>IDF</td>
<td>15.3</td>
<td>6.7</td>
<td>9.1</td>
<td>7.9</td>
</tr>
<tr>
<td>SDF</td>
<td>5.8</td>
<td>1.8</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>TDF</td>
<td>21.1</td>
<td>8.5</td>
<td>12.8</td>
<td>11.2</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>915</td>
<td>1070</td>
<td>918</td>
<td>963</td>
</tr>
<tr>
<td>GL</td>
<td>7.1</td>
<td>15.7</td>
<td>11.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

1Breakfast meals; 2Lunch meal; ND – Not determined; TDF=IDF+SDF

Proximate data (g/100 g edible portion) of chickpea and roti are given in Appendix VI.
Table 4.15: Glycaemic and insulinaemic responses of breakfast meals with type 2 diabetic patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>aStandard</th>
<th>bChickpea</th>
<th>bRoti</th>
<th>bRice meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose concentration</td>
<td>8.8 ± 3.1</td>
<td>8.3 ± 3.2</td>
<td>8.7 ± 3.1</td>
<td>8.6 ± 3.2</td>
</tr>
<tr>
<td>Peak glucose concentration</td>
<td>13.7 ± 3.1</td>
<td>9.9 ± 3.3</td>
<td>13.1 ± 3.8</td>
<td>11.8 ± 3.6</td>
</tr>
<tr>
<td>Peak glucose time (min)</td>
<td>90</td>
<td>120</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>Fasting insulin concentration</td>
<td>11.4 ± 5.4</td>
<td>10.4 ± 9.3</td>
<td>9.7 ± 6.6</td>
<td>13.5 ± 6.7</td>
</tr>
<tr>
<td>Peak insulin concentration</td>
<td>47.5 ± 33.9</td>
<td>27.6 ± 14.4</td>
<td>34.2 ± 25.8</td>
<td>32.5 ± 15.3</td>
</tr>
<tr>
<td>Peak insulin time (min)</td>
<td>90</td>
<td>150</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>IAUC (glucose)</td>
<td>547 ± 45</td>
<td>210 ± 33</td>
<td>478 ± 64</td>
<td>357 ± 71</td>
</tr>
<tr>
<td>GI</td>
<td>100</td>
<td>40 ± 7</td>
<td>88 ± 9</td>
<td>64 ± 11</td>
</tr>
<tr>
<td>IAUC (insulin)</td>
<td>4042 ± 1125</td>
<td>2326 ± 452</td>
<td>3142 ± 755</td>
<td>2215 ± 343</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>76 ± 13</td>
<td>115 ± 28</td>
<td>90 ± 20</td>
</tr>
<tr>
<td>GL</td>
<td>17.9</td>
<td>7.3</td>
<td>15.7</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Glucose concentrations are expressed as mmol/L; Insulin concentrations are expressed as microIU/mL; ¹Values are given as mean ± SD (Standard deviation); ²Values are given as mean ± SEM (Standard error of mean); ³n=12; ⁴n=11
Figure 4.17: Glycaemic responses to (a) breakfast meals and (b) lunch meal following the breakfast meals and standard

Each test food represents an average of 11 (standard -12)
Table 4.16: Glycaemic and insulinaemic responses of lunch meal following the breakfast meals in type 2 diabetic patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(^a)Standard</th>
<th>(^b)Chickpea</th>
<th>(^b)Roti</th>
<th>(^b)Rice meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1)Glucose concentration at 4 h (fasting)</td>
<td>8.7 ± 3.8</td>
<td>8.5 ± 3.1</td>
<td>9.7 ± 4.3</td>
<td>9.0 ± 4.2</td>
</tr>
<tr>
<td>(^1)Peak glucose concentration</td>
<td>11.2 ± 4.1</td>
<td>11.3 ± 3.5</td>
<td>12.4 ± 4.8</td>
<td>12.3 ± 4.6</td>
</tr>
<tr>
<td>Glucose peaking time (min)</td>
<td>90</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>(^1)Glucose concentration at 7 h (180 min)</td>
<td>9.5 ± 3.7</td>
<td>9.9 ± 3.8</td>
<td>10.3 ± 4.2</td>
<td>10.1 ± 4.6</td>
</tr>
<tr>
<td>(^1)Insulin concentration at 4 h (fasting)</td>
<td>16.2 ± 4.6</td>
<td>19.2 ± 11.9</td>
<td>21.8 ± 13.9</td>
<td>18.2 ± 6.5</td>
</tr>
<tr>
<td>(^1)Peak insulin concentration</td>
<td>32.1 ± 18.4</td>
<td>41.2 ± 31.5</td>
<td>36.9 ± 18.2</td>
<td>40.3 ± 17.7</td>
</tr>
<tr>
<td>Insulin peaking time (min)</td>
<td>90</td>
<td>60</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>(^1)Insulin concentration at 7 h (180 min)</td>
<td>25.3 ± 16.8</td>
<td>30.7 ± 16.8</td>
<td>22.4 ± 9.9</td>
<td>33.7 ± 15.6</td>
</tr>
<tr>
<td>(^2)IAUC (glucose)</td>
<td>320 ± 40</td>
<td>351 ± 61</td>
<td>264 ± 58</td>
<td>382 ± 51</td>
</tr>
<tr>
<td>(^2)IAUC (insulin)</td>
<td>2171 ± 462</td>
<td>3098 ± 668</td>
<td>2070 ± 339</td>
<td>3196 ± 648</td>
</tr>
</tbody>
</table>

Glucose concentrations are expressed as mmol/L; Insulin concentrations are expressed as microIU/mL; \(^1\)Values are given as mean ± SD (Standard deviation); \(^2\)Values are given as mean ± SEM (Standard error of mean); \(^a\)n=12; \(^b\)n=11: h-hour.
Figure 4.18: Insulinaemic responses to (a) breakfast meals and (b) lunch meal following the breakfast meals and standard

Each test food represents an average of 11 (standard -12)
5. DISCUSSION

The discussion of the thesis is presented under following sections; proximate composition of foods, glycaemic indices of basic foods and mixed meals, factors affecting GI, prediction of glycaemic responses with in vitro hydrolysis of starch, effects of dietary fibre and bulk of a meal on GI and glycaemic and insulinaemic responses of type 2 diabetic patients to foods.

Proximate compositions of foods

The first section of the discussion is devoted to the proximate compositions of processed foods, meal accompaniments and bananas. Proximate data of foods were expressed on the basis of g/100 g fresh weight as this will reflect on the actual amounts present in edible portions. The effects of protein, fat and dietary fibre contents on GI values (factors affecting GI) were studied by using the data of fresh weight to calculate the amounts of these nutrients present in the portions given for determination of GI.

Moisture and ash contents

Moisture contents of processed foods studied, ranged from 39.2-81.5% (Table 4.1). Dry heat processed bread varieties (n=3) had the lowest moisture levels (39.2-42.2%), while all the other wet heat processed foods contained high levels of moisture (52.8-81.5%)
stressing the influence of processing methods on moisture levels. Jackfruit (*Artocarpus heterophyllus*) contained the highest moisture content (81.5%) indicating the low percentage of dry matter in this food compared with other processed foods. String hoppers prepared in the same manner using two different kinds of flours, (wheat or rice) resulted in similar moisture contents.

Ash (mineral) contents of processed foods and meal accompaniments ranged from 0.1-1.1% with wholemeal bread having the highest level followed by jackfruit seeds (1.0%). Wheat flour used in preparation of wholemeal bread contains the outer layers (bran) of the grain which is a rich source of minerals. Jackfruit seeds are reported to be a good source of minerals such as sodium, magnesium and calcium (Ajayi, 2008).

Portions given for determination of Glycaemic Indices

Portions containing 50 g available carbohydrates were served when determining GI of foods (Table 4.5). Portion sizes were significantly influenced by the processing conditions. When considering portion sizes of all foods (g) bread varieties were the lowest with 114-128 g while the portions of all wet heat processed foods were ≥162 g.

Jackfruit meal given for determination of GI comprised of 40 g available carbohydrate from jackfruit flesh (400g) and 10 g from jackfruit seeds (46 g). The portion given for determination of GI was large due to the highest moisture content present in jackfruit flesh and according to individuals participated in the study it was the most difficult to consume.
Portion sizes of banana varieties due to the high moisture levels ranged from 190-280 g which are comparable with the reported data (Yusof et al., 2005).

Protein contents

Among all foods studied wholemeal bread contained the highest protein content (8.9%). The production of wholemeal bread using the whole grain which comprises the bran, endosperm and germ might be responsible for the higher protein content compared with other foods. The significantly higher (p<0.05) protein contents of wheat flour preparations (g/100 g fresh weight) than that of rice, manioc (Manihot esculenta) and jackfruit samples (Table 4.1) could be due to the high gluten content present in wheat flour (Jenkins et al., 2001). Though a legume, when lentils (Lens culinaris) were prepared as a curry with coconut milk the protein content of cooked lentils (8.0%) had reduced due to increased moisture content during preparation.

Fat contents

Total fat content of all processed foods (Table 4.1) were relatively low (<3.0%). Fat content of different bread varieties (2.7-2.9%) were not significantly different (p>0.05) as all were prepared with wheat flour. Foods prepared with similar sources under two processing methods had yielded significantly different (p<0.05) fat contents i.e., string hoppers prepared with red rice flour
had higher fat content (1.2%) compared with the boiled red rice (0.7±0.03%). This could be attributed to the differences in processing methods and the moisture levels.

Dietary fibre and undigestible starch contents

The insoluble and soluble dietary fibre (IDF and SDF) contents of foods analyzed ranged from 0.7-12% and 0.7-3.5% respectively.

Among the starchy staples studied, wholemeal bread which is prepared with whole wheat flour contained the highest SDF content and the lowest was seen in manioc (tuber). Soluble fibres are reported to form a viscous solution, limiting the accessibility to α-amylase and reducing the blood glucose response (Yusof et al., 2005). Studies have further shown beneficial effects of SDF on reducing weight gain (van Dam and Seidell, 2007), risk of cancers (Key and Spencer, 2007), and decreasing both total and low density cholesterol levels (Mann, 2007).

The coconut scrapings which were used as a meal accompaniment contained the highest IDF content. IDF (i.e., cellulose and hemicellulose) is important as structural materials (Hallfrisch and Behall, 2000) and increase the bulk of the portion.

Certain plant foods contain high amounts of "undigestible starch" (resistant starch) that escapes digestion in the small intestine, pass into the colon and act like dietary fibre (Sajilata et al., 2006). Resistant starch (RS) contents of jackfruit seeds and banana varieties were significantly higher (p<0.05) than the other foods analyzed (Table 4.2-4.4). RS is categorized into four types (RS1-RS4) (Nugent, 2005). Banana is reported to contain
RS₂ type. RS₁ is present as physically inaccessible starch and mainly present in whole grains and seeds. Thus, undigestible starch of jackfruit seeds might be RS₁. Although raw lentils are reported to contain high percentage of resistant starch (Garcia-Alonso et al., 1998) only 0.8% was observed in the lentil curry preparation. This would be as a result of the negative effect of cooking on resistant starch (Garcia-Alonso et al., 1998; Sajilata et al., 2006).

Digestible carbohydrates

The highest levels of digestible carbohydrate contents were observed in three dry heat processed bread varieties (38.9-43.8%) and the lowest was in jackfruit flesh (10%) with the highest moisture content. Wet heat processed foods had digestible carbohydrate contents in the range of 10.3-31.2% indicating a reduction compared to bread varieties due to the processing methods. String hoppers prepared with wheat and rice flour had the same moisture contents as well as the digestible carbohydrate contents.

Banana varieties (Musa spp.) contained free glucose, starch, sucrose and fructose as available carbohydrate sources (Table 4.3). The highest percentages of starch (14%), sucrose (38%), free glucose (29%) and fructose (58%) were observed with kolikutu, anamalu, seeni kesel, and embul respectively. Kolikutu (14%) and anamalu (7%) contained a certain percentage of available carbohydrate as starch while embul and seeni kesel had negligible amounts. Thus, the starch in the latter two varieties had been converted to sugars upon ripening. With increasing ripeness of fruits the free sugars have
been identified as the main carbohydrate fraction (Englyst and Cummings, 1986). *Kolikutu* and *embul* analyzed in the present study contained comparable available carbohydrate contents (26.5% and 23.2% respectively) with a previous study done on Sri Lankan banana varieties (*kolikutu* - 30.4%, *embul* - 21.0%) (Mahawithanage, 1998).

**Glycaemic Indices of basic foods, mixed meals and bananas**

GI values of basic foods (n=02), mixed meals (n=07) and banana varieties (n=04) were determined with healthy volunteers using white sliced bread as the standard (Table 4.5). Bread was selected as the standard as it is a true meal and follows the physiological digestion and absorption as other test foods (Wolever et al., 2003). However, to compare the GI data worldwide the values obtained with bread as the standard need to be converted to values expected with glucose as the standard. Thus, nine randomly selected foods were assayed against glucose (Brouns et al., 2005) to calculate the conversion factor that can be used to convert GI values obtained with bread to that of glucose.

When determining GI values, test foods and standards were given in a random cross over design. The standards were given on two occasions to every individual following a pilot study.

GI values of white sliced bread, wholemeal bread and ordinary bakery bread were 77±6, 76±6, 80±4 against glucose respectively and not significantly different (p<0.05). The results of the present study are in agreement with data reported for white bread from 16 studies (range; 59-89, average; 75±2) and for wholemeal bread from several countries
(74±2) (Atkinson et al., 2008). The reported differences in GI of some bread varieties may be due to different wheat origins/processing and the amount of gluten present/added during production (Crapo et al., 1981; Bornet et al., 1987). Storage is another factor that contribute to a difference in GI as bread samples after freezing & defrosting and toasting fresh had yielded lower glycaemic responses compared with consuming fresh bread (Burton and Lightowler, 2008). This could be due to the retrogradation of starch and change of crystalline structure due to the changes in moisture contents (Tovar, 1992).

Among the three bread varieties analyzed, wholemeal bread was selected to be given in a mixed meal with lentil curry as wholemeal bread is preferred in the dietary control of diabetic patients compared to other varieties. Lentil curry was included mainly because it is a commonly consumed accompaniment with bread, rice and other wheat/rice based products in Sri Lanka and legumes are reported to decrease the glycaemic responses (Hallfrisch and Behall, 2000). The wholemeal bread & lentil curry meal elicited a lower GI of 61±5 (with glucose) but was not significantly different from three bread varieties consumed alone.

GI values of six other mixed meals ranged from 60-120 (with white bread as the standard). Red rice & *kiri hodi* meal elicited a GI value of 99±10. GI of rice varieties had shown to vary widely (Brand-Miller et al., 1992; Hettiarachchi et al., 2001). When the same rice variety was given as a mixed meal (red rice mixed meal) containing typical components of a Sri Lankan rice meal (lentil curry, boiled egg, *gotukola* sambol, *kiri hodi*), the GI (60±5) declined by 39%. A rice meal served with lentil and cauliflower curry had elicited a GI
value of 86 against bread (Atkinson et al., 2008). The differences in preparation methods and nutrient levels might have contributed to the differences in GI values. String hopper meals prepared with wheat and rice flour elicited similar GI values against bread (104±7 and 103±11). Manioc being a tuber had high blood glucose raising potentials as other tubers (i.e., potato-70-130; sweet potato–63-111, steamed cassava-133±16) (Foster-Powell et al., 2002; Atkinson et al., 2008) and thus, the highest GI. Jackfruit elicited a GI of 75±11. This is the first reported data on GI of jackfruit meal in spite of including 2487 data on GI of different foods in the recent “International tables of Glycaemic Indices and Glycaemic Load values” (Atkinson et al., 2008).

Glycaemic indices of red rice mixed meal and jackfruit meal were significantly lower (p<0.05) than all other foods but not significantly different (p>0.05) from each other. Among the vast number of fruits available in Sri Lanka, bananas (kolikuttu, embul, anamalu and seeni kesel) were selected to determine GI as these are the most commonly consumed fruit in Sri Lanka as well as available throughout the year in all parts of the country. GI of kolikuttu, embul, anamalu and seeni kesel were 61±5, 61±5, 67±7, 69±9 (with bread as the standard) respectively and were not significantly different (p>0.05) from each other. The reported GI data of 10 studies involving banana, ranged from 43-100 (mean-74±5 against bread) (Foster-Powell et al., 2002). Another study reported with 3 varieties of banana from Malaysia had GI values of 59, 60 and 62 with glucose as the standard (Yusof et al., 2005).

The foods analyzed in the present study were categorized as low, medium and high GI against glucose as the standard. Red rice mixed meal, jackfruit meal and banana varieties
belonged to low GI category, wholemeal bread & lentil curry meal as medium GI while all other foods belonged to high GI category.

GI values calculated against glucose and bread

In the present study GI values of all foods (n=13) were determined with bread as the standard and randomly selected foods (n=9) from the lot were determined against glucose as well. This was carried out as there is a need to be able to convert the GI values of test foods obtained with bread as the standard to a GI value one would expect to have if glucose was used as the standard. Thus, the conversion factor was calculated with the intention of obtaining an average factor and a range to compare with the reported data. According to the results of the present study the average factor obtained was 1.34 with a range of 1.29-1.44 (Table 4.7).

A ratio of 1.4 (with a range of 1.22-1.58) was reported as the factor (Bornet et al., 1997) that inter converts GI values obtained with bread to glucose. The reported ratio was established with studies carried out using western foods and mainly Caucasians. The value obtained with the present study confirms this ratio to be within similar range in spite of physiological differences between different populations and particularly with the observation that Asian Indians appeared to be relatively insulin resistant (Dickinson et al., 2002; Chowdhury et al., 2003).
Feasibility of using a glucometer to determine Glycaemic Indices

Present study used an enzymatic kit (GOD-PAP) as the main method in estimating blood glucose concentrations following ingestion of foods to calculate GI. As a second measure the Accu-Check Active glucometer (Roche Diagnostics GmbH, Germany) was used in parallel with the enzymatic kit for the same purpose.

The percentage difference between GI of enzymatic kit and glucometer values for all foods studied was -8 to +1%. Despite using serum in the enzymatic method and whole blood with glucometer the percentage difference of GI is within the accepted range (±10%). Similarly GI values calculated using two methods were not significantly different (p>0.05).

While the percentage difference between the IAUC of two methods was -13 to +5%. As the percentage difference of IAUC is higher, it can be stated that the expected differences between two methods still exist when estimating the blood glucose concentrations and even IAUC of foods. Since GI is calculated as a ratio of IAUC of test and the standard, Accu-Check Active glucometer can be used to determine GI values of foods with healthy individuals. Thus, this particular glucometer instead of the enzymatic method can be recommended for determining GI when trained personnel and facilities are not available for the enzymatic assays.

Although many Sri Lankans are unaware of GI concept, it is increasingly in demand with visitors to Sri Lanka as the importance of GI of foods is apparent in The Western world (Kabir et al., 1998). Thus, efforts are taken to prepare meals with traditional Sri Lankan foods with novel recipes to lower GI while still maintaining the palatability. The
usefulness of a glucometer in determining GI is very practical and useful in situations of this nature.

Factors affecting GI

The variations of glycaemic responses to different foods analyzed is attributed to intrinsic factors of foods and extrinsic physiological factors (Arvidsson-Lenner et al., 2004; Vosloo, 2005).

Intrinsic factors

A variety of intrinsic factors have been reported to affect the glycaemic responses. Some of the factors that might affect the glycaemic responses of Sri Lankan meals were studied in the present study. The following chapters discusses the intrinsic effects of nutrients, different carbohydrate sources, amylose-amylopectin contents, starch granules, water absorption indices, water solubility indices and rate of digestion of different carbohydrates on the glycaemic indices of foods. However, more than one factor among these might contribute to the observed differences in GI values.
Effects of protein, fat and dietary fibre (present in 50 g available carbohydrate portions)

Significant negative correlations were observed with IDF (p=0.032, r=-0.676), SDF (p=0.010, r=-0.765) and TDF (p=0.016, r=-0.730) contents and GI values (Figure 4.8-4.10). Dietary fibre especially SDF is recognized as a main factor responsible in reducing blood glucose responses. The high viscosity of SDF is responsible in delaying the gastric emptying/intestinal absorption of glucose thus, lowering the glycaemic responses (Hallfrisch and Behall, 2000).

Among the foods studied the highest soluble and insoluble dietary fibre content in the portion given was in the jackfruit meal [TDF-20.1 (g/50 g available carbohydrate portion)]. In addition the jackfruit seeds contained the highest undigestible starch content. Both these factors must have contributed to a lower GI.

Protein contents of 50 g available carbohydrate portions and GI indicated a non significant negative relationship (p=0.165, r=-0.475), while fat contents of the foods analyzed did not show a relationship with GI (p=0.694, r=-0.143). However, fat and protein in a meal have also shown to be beneficial in reducing glycaemic response by delaying upper gastrointestinal transport (Latge et al., 1994) and increasing the secretion of insulin (Collier et al., 1984; Bornet et al., 1987) respectively. However, the actual amounts of proteins, fat and fibre necessary to produce a significant effect are reported to be high compared to the normal portions taken (Beaton et al., 1979; Jenkins et al., 1984).

The diets high in complex carbohydrate and fibre and low in fat are reported to be beneficial in improving carbohydrate metabolism in individuals with diabetes (Jenkins et
When considering the Sri Lankan meals the general consumption of protein and fat (except for coconut fat) is generally low among the majority of the population compared with that of the other nations. Thus, the significant relationship of TDF on glycaemic responses clearly indicate the beneficial effect of inclusion of dietary fibre in the diets of both urban and rural Sri Lankan population to avoid succumbing to chronic diseases.

When considering GI values of foods prepared with wheat flour (bread varieties, wholemeal bread & lentil curry meal and string hoppers meal) significant negative correlations with protein (p=0.032, r=-0.910) was observed along with IDF (p=0.028, r=-0.917) and TDF (p=0.041, r=-0.894) contents. This could be due to the lower GI and high protein content (19.4) observed with wholemeal bread & lentil curry meal compared with other wheat based foods (Table 4.9).

Effects of different sources of carbohydrates

The sources of carbohydrates available in a meal also influence plasma glucose and insulin responses (Crapo et al., 1980; Coulston, 1981). In the present study red rice mixed meal and wholemeal bread & lentil curry meal contained two carbohydrate sources from cereal (rice or wheat) and legume (lentils). This might have caused GI of these two meals to reduce from either high to low (99 to 60) or high to medium (103 to 87) compared with red rice & kiri hodi meal and wholemeal bread taken alone.
Wholemeal bread & lentil curry meal elicited the lowest incremental peak level (1.6 mmol/L) and a flat glucose response curve among all foods studied. This could be also due to the presence of 36% starch from the slow released lentil curry preparation as this meal had the highest level of starch from legumes among the meals studied. The reduction of the glucose peak and the subsequent response with a plateau will be beneficial for a diabetic patient on dietary control.

The incorporation of legumes in a meal is reported to reduce both the postprandial and 24 hour glucose profiles in individuals with diabetes (Chew et al., 1988). This is important in long term glycaemic control of diabetics as rapidly absorbed carbohydrates stimulate a considerable increase in blood insulin which is followed by a rapid decrease in blood glucose (Jenkins et al., 1982; Jenkins et al., 1988; Wolever et al., 1988). The decrease in blood glucose may in turn stimulate counter regulatory hormones and release of free fatty acid, which may be associated with insulin resistance and impaired carbohydrate tolerance (Wolever et al., 1988). This further strengthens the approach in including a curry prepared with legumes i.e., lentil, chickpea, mung bean with any standard Sri Lankan starchy staple in order to reduce the glycaemic response.

Jackfruit meal containing components of jackfruit (flesh and seeds) also contain two sources of carbohydrates i.e., vegetable and seeds. The inclusion of 10% carbohydrate from seeds or presence of other compounds in the seeds such as α-D-Galactose specific lectin which have the capacity to bind mono- and oligosaccharides as reported for another species of Moraceae family, Artocarpus integra (also called as jackfruit) (Kumar et al., 1982) might also be another factor responsible for low GI of this meal. The presence of
compounds of this nature that can bind glucose would either reduce the absorption of glucose or slow the process of digestion thereby yielding a low glycaemic response.

Effects of amylose and amylopectin contents

Amylose-amylopectin ratios present in foods also contribute to differences in GI values of foods (Brand-Miller et al., 1992), as hydrolysis of amylose results in less glucose molecules compared with hydrolysis of amylopectin and a lower GI with foods containing high amylose starch (Arvidsson-Lenner et al., 2004).

Amylose contents of foods of the present study ranged from 24–45 g/100 g starch (Table 4.8). When GI of basic foods and mixed meals were correlated with amylose contents of portions given for determination of GI, a non significant negative relationship (p=0.054) was obtained for the two parameters (Figure 4.7). The “non significance” was due to manioc and red rice mixed meal. According to the graph, manioc with high amylose should yield a lower GI, while red rice mixed meal with low amylose levels a higher GI value. This clearly indicates that in these 2 foods other factors are contributing to GI more than the amylose contents.

However, decreased amylose in wholemeal bread (28%) compared with white sliced bread (35%) may be responsible for similar GI values with the two bread varieties despite the high TDF content in wholemeal bread.
The rice variety analyzed in the present study had 35% (or 35 g/100 g starch) amylose content but still a high GI for the rice & *kiri hodi* meal (99±10). The rice varieties with similar amylose contents had shown to give different glycaemic responses (Panlasigui *et al.*, 1991) and this has been attributed to other physicochemical properties of starch.

Effects of starch granule

The starch granules of both raw and processed foods were compared under a light microscope to study the effects of processing on GI. The starch granules of bread varieties had more intact swollen granules in white sliced and wholemeal bread while ordinary bakery bread had disintegrated granules too (Plate 4.1). Thus, in spite of having similar nutrient contents among white sliced and ordinary bakery bread the slight increase in GI (4%) of the latter variety might partly have been attributed to this.

Cooked lentils had only intact highly swollen cell enclosed granules (Plate 4.6) which are characteristic of legumes (Tovar *et al.*, 1991). This could also be contributing to the slow-release of starch and hence decrease of GI in wholemeal bread & lentil curry and red rice mixed meals. The presence of disintegrated starch granules (Plate 4.2) in cooked rice in spite of having high amylose (Brand-Miller *et al.*, 1992) contents (35%) might be partly responsible for the high GI (99±10) of rice & *kiri hodi* meal (Panlasigui *et al.*, 1991).

String hopper meal (rice flour, GI-103±11) with increased protein (37%), fat (67%) and TDF (17%) contents had similar GI as the rice & *kiri hodi* meal (GI-99±10). This also
could be due to the presence of extensively degraded starch granules in string hoppers indicating the correlation between GI and subsequent effect of starch granules on processing.

Thus, inclusion of other macronutrients in the amounts consumed (in actual portion sizes) had not been effective in reducing GI in string hopper meals unlike as in the rice mixed meal. Two string hopper meals (made with rice, and wheat) contained same sources and identical amounts of fibre and fat but increased protein (35%) in wheat flour preparation. Despite differences in flour and protein contents in string hoppers, when given with same curries the blood glucose responses were similar (i.e., GI not significantly different, p=0.712). This further strengthens the hypothesis that the extensive degradation of starch granules during string hopper preparations causes the starch to be readily available following ingestion. Thus, the degree of gelatinization plays a major role in influencing the glycaemic response of a food.

Effects of Water Absorption and Water Solubility Indices

Water absorption index (WAI) indicates the gelling capacity of the starchy source while water solubility index (WSI) expresses the amount of soluble substances dissolved in the medium (Ekanayake et al., 2006). These are indicators of the extent of gelatinization due to the processing methods of foods.
The GI values of foods did not indicate a significant association (p>0.05) with neither WAI nor WSI contents of foods. This could be because a variety of foods were included in the analysis.

All cooked foods had significantly higher (p<0.05) WAI and WSI contents than their corresponding raw flour. An exception was high WSI in raw manioc flour in which cooking might have caused coagulation of starch, thus decreasing WSI. The increase in WAI and WSI of other foods could be due to gelatinization during cooking process and leaching of soluble substances.

String hoppers made with wheat flour had the highest WAI (4.2) followed by manioc (3.7) which can be attributed to the gelatinization of starch in these foods. The high WAI as well as the extensive disintegration of starch granules may contribute to the high GI of manioc in spite of having the highest amylose content.

Bread varieties, jackfruit flesh and lentils contained high WSI values (> 10) indicating hydrolysis and leaching of more soluble substances (proteins, amylose etc.) during cooking. Though the WSI of jackfruit is high, the leached out substances could be less bioavailable leading to a lower GI.

Effect of rate of digestion of different carbohydrates on their Glycaemic Indices

According to the rate of digestion of foods, glucose released were categorized into rapidly available glucose (RAG) and slowly available glucose (SAG). The RAG and SAG fractions and the corresponding starch fractions [rapidly digestible starch (RDS), slowly
digestible starch (SDS) of basic foods were determined (Table 4.10) with the intention of correlating these with known GI values. The glucose and starch fractions of basic foods were used to calculate the said fractions in mixed meals (containing more than one carbohydrate source) as this had not been attempted previously.

Significant correlations (p<0.05) were observed when glucose and starch fractions of 50 g available carbohydrate portions of basic foods and mixed meals were correlated with the observed GI values (Table 4.5 and Widanagamage, 2007). RAG and RDS contents showed significant positive correlations with GI values (r =0.546, p =0.023 and r = 0.597, p = 0.011) (Figure 4.11) and SAG/RAG ratio indicated a significant negative correlation with GI (r = -0.512, p = 0.036). Thus, RAG, RDS fractions, and SAG/RAG ratios can be taken as important food related determinants of the glycaemic response of basic foods as well as meals containing different sources of carbohydrates.

Thus the foods with high GI contain a higher percentage of carbohydrates in a form that can be rapidly hydrolyzed to yield glucose and correspondingly increased postprandial glucose levels. Legumes analyzed in the present study contained RAG and SAG contents not significantly different from each other thus contributing to a lower GI.

The high GI white sliced bread and wholemeal bread had RAG contents of 38.5 and 38 (g/100 g edible portion) respectively. Reported amounts of RAG in similar bread varieties were 42 and 36 (g/100 g edible portion) respectively (Englyst et al., 2000) as the starch in bread varieties are rapidly absorbed.

Significant relationships (p<0.05) were not observed among GI and SAG nor the SDS contents, contradicting the findings of certain other studies (Garsetti et al., 2005).
could be due to the presence of low percentage of SAG or SDS compared with the rapidly available fractions in Sri Lankan foods, except for breadfruit (*Artocarpus altilis*) and legumes. Percentage of slowly released carbohydrates are expected to increase with less gelatinization process (Garsetti *et al.*, 2005) and most Sri Lankan foods except legumes are highly gelatinized leading to starch being in physically accessible state (Sajilata *et al.*, 2006). Thus, the influence of SAG of Sri Lankan foods on the glycaemic response will be minimal. Significant correlations between both RAG and SAG contents of basic foods with GI had been reported previously from other countries (Englyst *et al.*, 1999; Garsetti *et al.*, 2005).

The present study attempted to categorize foods based on RAG, RDS contents or SAG/RAG ratio as those showed significant correlations with the GI values. However, complete ranking of foods based on the glucose and starch fractions into the 3 categories (low, medium and high GI) could not be achieved. The low GI foods i.e., legumes, bread fruit, red rice mixed meal and medium GI wholemeal bread meal could be ranked as low and medium respectively with any of these glucose, starch fractions or ratios while other foods could not be so ranked. This might be due to the other physiological factors, i.e., gastric emptying rate, viscosity of the gastric content (Vosloo, 2005) *etc*. influencing the observed glycaemic responses.
Prediction of glycaemic responses with *in vitro* hydrolysis of starch

Although the *in vivo* method is the acceptable practice for determination of GI, the *in vitro* methods are used as supportive tools due to practical difficulties experienced with the *in vivo* method (Granfeldt *et al.*, 1992). Thus, in the present study the starch hydrolysis indices (HI) of basic foods were determined using an existing *in vitro* starch hydrolysis method to study the applicability of this method to analyze Sri Lankan foods. When the *in vitro* HI data of basic foods and *in vivo* GI values were correlated, a significant positive relationship for the two parameters was observed ($p<0.0001; r=0.953$).

Wholemeal bread had the highest HI among all the basic foods. When wholemeal bread and ordinary bakery bread were considered, 18-20% starch was hydrolyzed within the first 30 min. Although the process of hydrolysis of starch of legumes is slow, (Tovar, 1992) mung beans and lentils had 21% and 18% starch hydrolyzed within 30 min which are comparable with bread varieties. Whereas, starch hydrolyzed at 30 min in chickpea and cowpea were low (14% and 15% respectively). The values of the present study are in agreement with the reported data for bread (17-24%) as well as for legumes (0-19%) for the first 30 min period (Granfeldt *et al.*, 1992).

As this *in vitro* procedure had only been used to analyse the basic foods, but not employed to determine the HI of mixed meals previously, the present study was extended to cover this inadequacy by including mixed meals with similar proportions of components of the meal as in the case of *in vivo* study.
The compositions of mixed meals analyzed are given in Table 4.12. Meal 1 (wholemeal bread & lentil curry) had an *in vivo* GI of 87±6 and a HI of 81±6 which were not significantly different from each other (p>0.05). Meals 2-5 were prepared as rice meals with different accompaniments. The rice mixed meal given for *in vivo* determination of GI included rice, lentil curry, boiled egg, *gotukola* sambol and *kiri hodi* and had a GI of 60±5 (Table 4.5). In order to study the rate of hydrolysis of this meal the present study attempted the analysis with a step by step approach to determine the manner in which the meal should be constituted when estimating the HI. Meal 2 included only the carbohydrate sources of the above meal and resulted in a HI of 84±8. As the difference between the *in vivo* and *in vitro* values was high (24 units) the sample for *in vitro* assay was adjusted to include the dietary fibre in the same proportion as in the *in vivo* meal (Meal 3). This resulted in a HI of 71±6 which was lower than in meal 2. Further to study the effect of including all accompaniments on HI, meal 4 was comprised. Thus, meal 4 contained a portion of egg with other accompaniments and resembled the rice mixed meal given for *in vivo* GI determination. This resulted in a HI of 65±7. The composite mixed meal had reduced the difference between the *in vivo* GI value and *in vitro* HI. However, the HI of the three rice meals (meal 2, 3, 4) were also not significantly different from each other (p>0.05). These observations clearly indicate that a HI which will reflect the GI closely can be obtained when composite meal is analyzed.

Meal 5 comprised double the amount of dietary fibre included in meal 3. This resulted in a HI of 74±8 which was not significantly different from meal 3. This reflected that inclusion
of additional quantity of fibre as given in this case might not have an effect on lowering of HI.

Meal 6 and 7 were given for *in vitro* assay using the same components as *in vivo* determination of GI. The GI and HI data of those two meals were also not significantly different (p<0.05).

The *in vivo* GI values of both basic foods and mixed meals were correlated with *in vitro* HI and a significant correlation (p<0.001; r=0.949) was obtained for the two parameters (Figure 4.15). A relationship of \( Y=1.1367X-12.138 \) was also obtained for all the foods analyzed in the present study.

This *in vitro* method can be applied to determine *in vitro* hydrolysis of both basic foods and mixed meals containing different sources of carbohydrate and other accompaniments. The *in vitro* data will be useful as a secondary source in determination of GI when there are practical difficulties in finding volunteers (Venn and Green, 2007).

An attempt was made to calculate the GI of the wholemeal bread & lentil curry meal (as in Table 2.1) from the individual GI values of wholemeal bread and lentil curry (Atkinson *et al.*, 2008). This was successful as only two carbohydrate sources were included in the meal. However, using a similar calculation the GI of a typical rice mixed meal which containing several meal accompaniments could not be calculated from the individual components as GI of some components (low carbohydrate) are not available. Thus this *in vitro* method is useful in predicting GI values of mixed meals.
Effect of dietary fibre and bulk of a meal on GI

As significant negative correlations were observed between in vivo GI values of foods and IDF, SDF, TDF contents, the effect of increasing dietary fibre content while maintaining the normal edible portion sizes and palatability was studied. Among the foods analyzed in the present study rice mixed meal which contained gotukola sambol (green leaf), a good source of dietary fibre (Bazzano et al., 2008) elicited the lowest glycaemic response (Table 4.5). Thus, another source of fibre in the form of kohila was included in the rice mixed meal along with gotukola (meal 2). However, with the inclusion of kohila the edible portion size (bulk) increased. Bulk of the meal also influence the glycaemic response to foods (Bornet et al., 1997). Thus, to narrow down the effect only to dietary fibre and not to bulk a third meal was prepared. Meal 3 replaced kohila with snake gourd with high moisture and meal 2 and 3 contained similar total portion sizes.

Although gotukola and kohila are good sources of fibre the moisture contents are also high (86.8±0.6 and 88.3±1.1 respectively). Thus, inclusion of 25 g portion of kohila in meal 2 increased IDF by 8.9%, SDF by 3.9% and TDF by 7.2%.

GI values of meal 1, 2 and 3 were 63±6, 57±5, 61±5 respectively and comparable. According to the results of this study increase of TDF by 7.2% or the bulk of the meal by 8% had decreased the GI by 9% without significantly reducing neither the glycaemic response nor the peak serum glucose levels. This was further observed with the in vitro study eliciting comparable HI for meal 3 (71±6) and meal 5 (74±8) with different fibre contents (Table 4.12).
Thus, increased portions of fibre sources, more than the amounts given in the present study (>25 g) might further decrease the GI. However, consumption of such a meal will only be possible when the carbohydrate load is reduced to decrease the bulk of the meal.

**Glycaemic and insulinaemic responses of type 2 diabetic patients to foods**

Glycaemic and insulinaemic responses to breakfast meals

As the GI of foods have been determined with healthy individuals a comparison of those data with diabetic patients was attempted using 3 meals, i.e., chickpea (boiled and tempered), red rice (with lentil curry & coconut sambol), atta roti (with onion sambol). This was carried out mainly to identify the foods suitable for diabetic patients as well as to study the applicability of GI obtained with healthy individuals to the diabetic patients. Apart from determining GI of the above mentioned foods insulinaemic indices (II) were also calculated to study the relationship between glycaemic and insulinaemic responses. The GI of chickpea, red rice meal and atta roti were 40±7, 64±11 and 88±9 respectively (Table 4.5). Chickpea and red rice meal can be categorized as low GI foods, while atta roti was medium. GI of atta roti (50 g available carbohydrate portion) and chickpea (25 g available carbohydrate portion) meal were analyzed previously with healthy volunteers (Widanagamage *et al.*, 2009) and observed to be low GI foods (67±9, and 29±5 respectively). The GI values of all three foods with diabetic patients were higher than the values observed with healthy individuals but not significantly different (*p* > 0.05). However, similar higher GI value for chickpea
with type 2 diabetic patients compared with healthy individuals had been reported [46 and 34 (average) respectively for the two categories of individuals] (Atkinson et al., 2008). IAUC of foods in diabetic patients were also higher than the reported values of chickpea and atta roti with healthy individuals (Widanagamage et al., 2009) in spite of sampling blood for 3 hours in diabetic patients compared with the sampling for two hours in healthy individuals. The high IAUC values with diabetic patients might be due to their different levels of insulin resistance, and longer time to clear glucose from the blood stream as the process of uptake into peripheral cells is low.

The GI of atta roti had shifted from low to medium GI category when given to diabetic patients. When giving the 25 g available carbohydrate portion of roti to diabetic patients the amount of coconut was half the amount contained in the portion given for healthy individuals. Although the GI values of foods with diabetic patients tend to be higher than the GI observed with healthy, it can be speculated that the reduction of SDF content and the lowering of bulk of roti in the present study might also have contributed to the increased GI observed for roti.

Another interesting observation with diabetic patients was the delayed peaking of test foods and standard compared with the healthy individuals. This could be due to the differences in the physiological digestion and absorption processes in diabetic patients, i.e., delayed gastric emptying (Kojecky et al., 2008) and absorption of glucose in to the blood stream etc.

When comparing the incremental blood glucose peaks of breakfast meals and the standard, lower peaks were observed for chickpea and rice with 27% and 14% reductions compared
to the standard respectively (Table 4.17). This clearly indicates the beneficial effect of inclusion of chickpea (legumes) or rice meals in diabetic diets. The available nutrient data of the three meals indicated that the chickpea meal which has the lowest GI contains the highest protein (16.4 g), IDF (15.3), SDF (5.8), and TDF (21.1) contents compared with other meals suggesting that these contribute to the significant lowering (p<0.05) of the GI. The percentages of RAG present in the meals given for GI determination (chickpea-54%, rice meal-64%, roti-74%) also indicated a linear positive relationship (r=0.999) strengthening the fact that more than one factor influences the GI of foods.

Insulin responses to three meals were also analyzed to observe the effect of meals on insulin secretion. The insulinaemic index (II) which was calculated with IAUC of test and standard determines the amount of insulin produced to counteract the effect of a given carbohydrate load (Holt et al., 1997). II of chickpea, rice and roti meals were 76±13, 90±20, and 115±28 respectively. Thus, the glycaemic and insulinaemic responses of these foods show a positive linear relationship (r=0.984). The IIs indicate that the low GI food elicit a lower insulin output and vice versa as low GI foods release glucose at slower rates than medium or high GI foods. Thus, low GI foods will be beneficial in reducing the risk of development of insulin resistance leading to metabolic syndrome (Hsu et al., 2007) and other associated complications. The intra individual variations of fasting insulin responses were 1.9-32.2% while the inter individual variations for the foods tested were 25-45%. The variations of inter and intra individual CV values could be due to the different degrees of insulin resistance of the diabetic patients participated in the study.
The glucose and insulin responses of carbohydrate foods had generally shown to elicit a linear positive relationship (Bjorck et al., 2000; Crapo et al., 1980). However, certain foods such as dairy products show insulin secretagogue properties and are reported to deviate from this relationship while eliciting higher insulin responses compared with the corresponding glycaemic response (Arvidsson-Lenner et al., 2004).

Effect of breakfast meals on the glycaemic and insulinaemic responses of lunch – Second meal effect

Certain meals consumed on the previous occasion (at dinner or breakfast) have shown to improve the glycaemic responses of the subsequent meal (second meal effect) (Jenkins et al., 1982; Liljeberg et al., 1999). Second meal effect of breakfast meals on lunch is determined by calculating the IAUCs of lunch meals following both standard and test breakfast meals. The significantly lower (p<0.05) IAUC of test meals compared with standard is responsible in producing a second meal effect.

Identification of Sri Lankan foods that extend second meal effects will be of use in dietary management of diabetic patients as these data regarding Sri Lankan or even Asian foods are not available.

Thus, second meal effects of previously mentioned breakfast meals (chickpea, rice meal and roti) were analyzed following the rice mixed meal as the lunch (Table 4.5). These three breakfast meals were selected as these were low and medium GI foods and it is not
practical to serve high GI meals to diabetic patients in order to observe any extended beneficial effects.

The IAUCs of glycaemic responses of rice mixed meals (lunch) following ingestion of test breakfasts were not significantly different from that of lunch following bread. (Table 4.16, Figure 4.17). Although the IAUCs of glucose responses of lunch following ingestion of roti breakfast (264±58) was lower (-18%) than the standard (320±40), the IAUCs of the other two meals were higher than bread.

IAUCs of insulin responses of lunch meal also showed similar observations as glucose responses (Table 4.16, Figure 4.18).

As such, it is clear that the tested breakfast meals had no effect on the glycaemic response nor insulin responses of the subsequent lunch. Thus, it is advisable for each meal of a diabetic patient to be carefully planned in order to reduce blood glucose peaks as well as being hyperglycaemic.

However, when considering the differences of serum glucose and insulin responses from 180 min (3 hours of breakfast) to 240 min (4 hour - start of the lunch), standard bread resulted in the highest decline. The glycaemic response of bread at 4 hour was even below fasting blood glucose concentrations. This could be due to the high percentage of RAG (85%) present in bread enabling rapid digestion, absorption of glucose as well as uptake into peripheral cells.

Highest incremental glucose and insulin peaks were observed with post rice breakfast. Bread resulted in the lowest incremental glucose peak and the second lowest insulin peak. This could be a reason for the test breakfast meals not to exhibit any second meal effect as
the rise of glucose and insulin after lunch following bread (breakfast) was less than other meals.

Second meal effect of mainly low GI cereals with normal fibre (Wolever et al., 1988) or increased added fibre contents (barley) are reported (Clark et al., 2006; Nilsson et al., 2008c). These studies have been mainly carried out with healthy individuals. However, the present study analyzed the second meal effect with only diabetic patients as the data can be directly applied to their dietary therapy.

Although the slow release (lente) carbohydrates, and foods with high dietary fibre which can undergo colonic fermentation have been recognized as main factors responsible in extending a second meal effect, the present study did not observe such an effect in spite of presence of lente carbohydrates in legumes (chickpea, lentils) and high dietary fibre in chickpea. Thus, the 54% of RAG content (Table 4.10) and presence of increased levels of low molecular weight carbohydrates in chickpea as observed with gel filtration chromatography (Widanagamage, 2007) might be responsible for not extending any second meal effect. However, it is not possible to come to a definite conclusion as to why the low GI meals analyzed in the present study did not show any second meal effect and the data of the present study agree with the hypothesis that all low GI foods do not extend second meal effects.
Glycaemic Loads of basic foods and mixed meals

The "Glycaemic load" concept (GL) introduced later in 1997 make use of the glycaemic index values (Barclay et al., 2005) in determining the actual glycaemic load of a normal serving size (NSS). According to GL values, foods are categorized as low <10, medium 10-20, high >20 against glucose as the standard (Barclay et al., 2005).

In the present study when considering the portions given for determination of GI and NSS only red rice & kiri hodi and manioc meal contained edible portions. Bread varieties when given for determination of GI comprised of 8-9 slices (GL-38-40, Table 4.5). However, normal edible portions of white sliced bread and ordinary bakery bread would comprise 50% of the portions given (~4 slices) for determination of GI. Thus, GL would be less (~20). The NSS of wholemeal bread is even less (~3 slices) due to its high satiety score and less palatability due to high fibre content compared with other two types and correspondingly low GL (16). Although the GI values of wholemeal and white sliced bread were similar, GL of wholemeal bread is low indicating the suitability of inclusion of wholemeal bread in diabetic food regime.

When considering the actual consumable portion sizes of wholemeal & lentil curry meal, red rice mixed meal and the two string hopper varieties (NSS - 2/3 of the portion given for GI determination) the GL would reduce to 19, 16, 24 (wheat flour), 26 (rice flour) respectively. Thus, categorizing wholemeal bread & lentil curry meal and red rice mixed meal as medium GL foods while the two string hopper meals as high GL.
Likewise the portion given for jackfruit was large and difficult for most of the individuals (80%) to consume. The edible portion would be half the portion given for GI determination (GL - 13) thus, further decreasing the glycaemic response and eliciting a medium GL.

Glycaemic loads of foods can be reduced by adjusting the contributions of main starchy staple and other accompaniments, i.e. by inclusion of accompaniments prepared with lentils, chickpea, mung bean, green leafy salads or mallung, other protein sources, i.e., GL of string hopper meals can be reduced by including a lentil or a chickpea curry. Manioc although is a high GI/GL food, can be consumed as a curry with rice or even as a breakfast menu with a reduced portion size. High GL foods can also be included in the meals of high risk groups by carefully planning the diets thus, highlighting the importance of knowledge of GI. These data can be useful in formulating food based dietary guidelines for diabetic patients and healthy individuals.

A single banana considered as the NSS had GL values 6, 4, 6.0, and 5 respectively for kolikuttu, embul, anamalu and seeni kesel. Thus, both GI and GL of banana varieties are low indicating the beneficial effects of including bananas as part of a meal.

When considering the portions of foods given for type 2 diabetic patients only rice meal represented a normal edible portion while the portion of chickpea was more and roti was less. According to the patients the NSS of chickpea would be 2/3 of the portion given and roti, twice the amount. Thus, the GL of NSS of chickpea, rice and roti are 5, 11, and 31 respectively making chickpea a low GL meal, rice a medium and roti a high GL meal. Thus, it will be beneficial to include the two low GI meals (chickpea and rice) in diabetic diet.
These data represent on the glycaemic responses of commonly consumed foods in Sri Lanka. The relationship between consumption of high GL meals for a longer period and the increased incidence of diabetes is evident (Bornet et al., 1997). According to the results of the present study impaired glucose tolerant (IGT) and diabetic patients who are on dietary control can be advised to reduce the portion sizes of basic Sri Lankan starchy staples (cereals, tubers) while increasing consumption of legumes and non starchy accompaniments to reduce GL as well as to reduce glycaemic response. This practice will be beneficial not only for the diabetic but also for the non diabetic individuals to avoid an excessive insulin response and hypoglycaemia (Kabir et al., 1998). However, this approach should be introduced at a young age to reduce the epidemic of diabetes in Sri Lanka.
Recommendations from the study:

- Use of glycaemic index data obtained with diabetic patients when formulating diets for such patients whenever possible.
- In instances where glycaemic control is desired low or medium GI and GL foods are recommended.
- If high GI/GL meals are consumed it is recommended to reduce the portion sizes and have combinations with low or medium GI/GL foods to reduce the glycaemic load.
- Control each meal of a diabetic or impaired glucose tolerant (IGT) patients' diet regime to observe maximum beneficial effects.
- Consumption of a mixed rice meal at least twice a day especially for diabetics is recommended to improve the glycaemic response.
- Inclusion of different sources of carbohydrates in a meal (cereals, legumes, vegetables, tubers, seeds etc.) and dietary fibre even from widely available sources (green leaves, high fibre vegetables) are recommended.

Limitations of the thesis

1. The previous day dinner meals of healthy volunteers and type 2 diabetic patients could not be controlled due to practical difficulties.
2. Glucose was not given as the standard when analyzing all foods (instead only with nine foods) due to the cost involved and the practical problems experienced with the volunteers.
3. For analysis of RAG, RDS, SAG, SDS fractions (3.2.4.4) and prediction of glycaemic responses with *in vitro* hydrolysis of starch (3.2.5) some basic food data (GI values and compositions) were taken from previous work done in the same laboratory as the present study was focused mainly on mixed meals.

4. When determining the effect of dietary fibre on GI it would have been ideal to reduce the carbohydrate load (to ~ 40 g) while increasing the fibre component to keep the portion size same. However, this could not be achieved as determination of GI requires a standard amount of carbohydrate load.

**Recommendations for future studies**

1. Study the long term effect of typical Sri Lankan low and medium GI foods with IGT and type 2 DM patients.

2. Study the second meal effect of low, medium GI foods with IGT patients who are on diet control.

3. Determination of GI of other commonly consumed foods with IGT and type 2 diabetic patients to study the differences between this group and healthy volunteers to decide on better foods in the dietary management for diabetic patients.

4. Development of commercial functional foods with added dietary fibre to suit the life styles of urban population.

5. Study the effect of dietary fibre with IGT, type 2 DM patients using a variety of starchy staples.
6. CONCLUSIONS

- Bread varieties, mixed meals and bananas analyzed in the present study can be categorized as low GI (red rice mixed meal, jack fruit meal, bananas), medium GI (wholemeal & lentil curry meal) and high GI (bread varieties, string hopper meals, red rice & kiri hodi meal, manioc) foods.

- GI values obtained with white bread of Sri Lankan origin can be converted to GI values expected with glucose by using a conversion factor of 1.34.

- GI values calculated with enzymatic kit method did not differ significantly (p>0.05) from GI values calculated with a glucometer (Accu-Check Active).

- GI indicated significant negative correlations with IDF (p=0.032), SDF (p=0.010) and TDF (p=0.038) contents of 50 g available carbohydrate portions of foods while a non significant negative relationship was obtained with the protein contents. Thus, the data of the present study reinforces the beneficial effect of inclusion of dietary fibre in reducing GI of a meal with less costly accompaniments (green leaves, high fibre vegetables) which are suitable for both urban and rural populations in Sri Lanka.
• Inclusion of different sources of carbohydrates in a meal (cereals, legumes, seeds) and the extent of gelatinization following processing of foods had also influenced the GI of meals.

• The rapidly and slowly available glucose (RAG, SAG) and starch fractions (RDS, SDS) present in basic foods and mixed meals were correlated with GI values. Significant positive correlations were observed with GI and RAG (p=0.023), RDS (p = 0.011). This clearly indicates the positive contribution of rapidly available carbohydrates on GI.

• The GI of a rice mixed meal was reduced by 9% when total dietary fibre content of the actual meal was increased by 7.2%.

• In vivo GI values were successfully predicted with in vitro hydrolysis index (HI) values of basic foods and mixed meals (p<0.001). Data obtained clearly showed that this method could be applied to determine GI of mixed meals by including all the components of the composite mixed meal. Thus, this in vitro method will be useful as a supportive tool in determining GI values when there are practical difficulties.

• The three breakfast meals given to type 2 diabetic patients belonged to low GI [chickpea (40±7), rice meal containing lentil curry & coconut sambol (64±11)] and
medium GI [atta roti (88±9) categories. The insulinaemic indices (II) of the meals were 76±13, 90±20 and 115±28 respectively. Glycaemic and insulinaemic responses indicated a positive linear relationship (r=0.987). The GI values obtained with diabetic patients were higher than the values reported with healthy individuals.

- None of the breakfast meals (chickpea, rice meal or roti) elicited a second meal effect on the subsequent lunch meal. Thus, indicating the need to carefully plan each meal of the diabetic patients in terms of the portion sizes, nutrient content and the components.

- When studying the glycaemic responses of all the mixed meals analyzed in the present study it was observed that balanced rice mixed meal is better in terms of GI compared to other meals. Thus, the data of the present study will assist dieticians and medical practitioners in planning meals for high risk groups.
7. REFERENCES


markers, and increases satiety after a subsequent standardized breakfast. *Journal of Nutrition.* **138**: 732-739.


APPENDIX I

Publications and Communications

Publications


5. **Hettiaratchi UPK**, Ekanayake S and Welihinda J. (Submitted). Do different fractions of digestible carbohydrates in foods influence the glycaemic responses?" *Journal of National Science Foundation.*


**Communications**


   This was awarded the best presentation at the Scientific Sessions of the Nutrition Society of Sri Lanka 2010 held on 23rd January 2010.
APPENDIX II

Letters of Ethical clearance

Letter 1

Ethical Review Committee
Faculty of Medical Sciences,
University of Sri Jayewardenepura
Gangodawila, Nugegoda, Sri Lanka

25th January 2005

Ms. U.P.K. Hettiaratchi
Department of Biochemistry
Faculty of Medical Sciences
University of Sri Jayewardenepura

Dear Ms. Hettiaratchi

Application No: A 224

Study of the glycaemic indices of pure standard foods and mixed Sri Lankan meals

We are pleased to inform you that ethical clearance was granted for your proposal at the ethical review committee meeting held on the 19th January 2005.

Dr. S.D. Jayaratne
Chairman/Ethical Committee.

Dr. Renu Wickremasinghe
Secretary/Ethical Committee.
05th February

Dear Ms. U.P.K. Hettiarachi,

Department of Biochemistry,
Faculty of Medical Sciences,
USJP,

Application /Approval No 365/7

Second meal effect of certain breakfast meals with different glycaemic indices

I am pleased to inform you that provisional ethical clearance was granted. Definitive clearance will be given once the study is completed and a report submitted to the ethical review committee.

Chairman

Dr. S.D. Jayaratne

Secretary

Dr. Neluka Fernando
23rd January 2009

Mrs.U.P.K.Hettiarachchi
Lecturer
Department of Biochemistry
Faculty of Medical Sciences
Nugegoda.

Request for Ethical Clearance (Application No:104)

A Study on “Analysis of Glyceamic indices of Sri Lankan pure foods and mixed foods”

This is to inform you that ethical clearance was granted for the above proposal at the Hospital Ethical Review Committee meeting held on 23rd January. The Committee also observed that you have obtained approval from the University Ethical Committee for the study.

You are kindly requested to obtain permission from the relevant consultants to use their patients or data for the study.

Jayawardana
Chairman, Hospital Ethical Review Committee

Cc: Director/CSTH
APPENDIX III

Consent form 1- Given to healthy individuals

RESEARCH CONSENT FORM

Project: Study of Glycaemic Indices of pure standard foods and mixed Sri Lankan meals.
Name of investigator: Ms. U.P.K. Hettiarchi

You are required to undergo an overnight fast of ~10-12 hours. When you arrive at the Research Laboratory/Dept. of Biochemistry/FMS a fasting blood sample will be taken (~0.1 ml) from your fingertip. Then a meal containing 50 g starch will be given to be taken over a course of 10-15 minutes. Further capillary blood samples will be taken at 30, 45, 60, 90, 120 minutes after taking the first bite. Refreshment will be provided after taking the final blood sample.

The risks of donating blood in this manner (fainting, infection at the puncture site) are very few. You will experience a slight pain when pricking the finger. A doctor will be at hand to deal with any eventuality. There will be no direct benefit to you during the study period but once the study is over you will be educated about GI of foods. It is also my understanding that he/she can withdraw from the study whenever he/she wishes without giving any reason.

I have read and understood the contents in this form and hereby I give my consent to withdraw capillary blood to be used for this study.

……………………………………….. …………………………………………..
Signature of person giving consent Date

Address:……………………………………………………………………………………

I have been present while the above went through the information sheet and have witnessed his/her consent.

……………………………………….. …………………………………………..
Witness’s signature Date

Full name: ……………………………………………………………………………
Address: ……………………………………………………………………………
Consent for 2- Given to type 2 diabetic patients

RESEARCH CONSENT FORM

Project: Second meal effect of certain breakfast meals with different glycaemic indices
Name of investigator: Ms. U.P.K. Hettiaratchi

You are required to undergo an overnight fast of ~10-12 hours. A canula will be inserted when you arrive at the Family Practice Centre/FMS. A fasting blood sample will be taken (~0.5 ml). Then a meal containing 25/50 g starch will be given to be taken over a course of 10-15 minutes. Further venous blood samples will be taken at 30, 45, 60, 90, 120, 150, 180 and 240 minutes after taking the first bite. Then a standard rice meal will be given soon after taking the 240 minutes blood sample and further blood samples at 30, 45, 60, 90, 120, 150 and 180 minutes will be taken after taking the first bite of the rice meal. Refreshment will be provided after taking the final blood sample.

The risks of donating blood in this manner (fainting, infection at the puncture site) are very few. You will experience a slight pain when inserting the canula. A doctor will be at hand to deal with any eventuality.

There will be no direct benefit to you during the study period but once the study is over you will be educated about a better dietary management. It is also my understanding that he/she can withdraw from the study whenever he/she wishes without giving any reason.

I have read and understood the contents in this form and hereby I ....................................................
give my consent to withdraw venous blood to be used for this study.

Signature of person giving consent Date

Address...................................................................................................

I have been present while the above went through the information sheet and have witnessed his/her consent.

Witness’s signature Date

Full name: ..............................................................................................
Address: ..............................................................................................
APPENDIX IV

Table 1: Fasting serum glucose concentrations and IAUC values of healthy individuals on different days when standard (white sliced bread) was given

<table>
<thead>
<tr>
<th>No.</th>
<th>Fasting 1st day (mmol/L)</th>
<th>Fasting 2nd day (mmol/L)</th>
<th>Fasting 3rd day (mmol/L)</th>
<th>IAUC 1st day (mmol/L)</th>
<th>IAUC 2nd day (mmol/L)</th>
<th>IAUC 3rd day (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4.3</td>
<td>4.1</td>
<td>4.1</td>
<td>150.7</td>
<td>123.2</td>
<td>138.7</td>
</tr>
<tr>
<td>02</td>
<td>5.1</td>
<td>4.6</td>
<td>4.9</td>
<td>80.9</td>
<td>94.8</td>
<td>113.7</td>
</tr>
<tr>
<td>03</td>
<td>4.6</td>
<td>4.7</td>
<td>4.9</td>
<td>367.5</td>
<td>283.4</td>
<td>279.3</td>
</tr>
<tr>
<td>04</td>
<td>4.4</td>
<td>4.2</td>
<td>4.3</td>
<td>87.9</td>
<td>99.2</td>
<td>107.9</td>
</tr>
<tr>
<td>05</td>
<td>4.7</td>
<td>4.3</td>
<td>4.5</td>
<td>171.1</td>
<td>135.6</td>
<td>151.0</td>
</tr>
<tr>
<td>06</td>
<td>3.8</td>
<td>4.5</td>
<td>4.4</td>
<td>168.7</td>
<td>186.7</td>
<td>135.2</td>
</tr>
<tr>
<td>07</td>
<td>4.5</td>
<td>4.6</td>
<td>4.2</td>
<td>80.1</td>
<td>110.2</td>
<td>121.8</td>
</tr>
<tr>
<td>08</td>
<td>5.1</td>
<td>5.3</td>
<td>5.1</td>
<td>128.7</td>
<td>145.6</td>
<td>154.3</td>
</tr>
<tr>
<td>09</td>
<td>5.1</td>
<td>4.7</td>
<td>4.9</td>
<td>126.5</td>
<td>108.5</td>
<td>118.5</td>
</tr>
<tr>
<td>10</td>
<td>4.7</td>
<td>4.3</td>
<td>4.2</td>
<td>189.3</td>
<td>167.2</td>
<td>176.2</td>
</tr>
<tr>
<td>11</td>
<td>4.8</td>
<td>4.4</td>
<td>4.6</td>
<td>209.2</td>
<td>234.4</td>
<td>219.3</td>
</tr>
</tbody>
</table>

\(^a,b\) Values of columns were not significantly different (p>0.05); CV of fasting values (1st, 2nd and 3rd day) - 4.6%; CV of fasting values (1st and 2nd day) - 5.1%; CV of IAUC (1st, 2nd, and 3rd day) - 15%; CV of IAUC (1st and 2nd day) - 17%.
APPENDIX V

Glycaemic responses to foods with healthy individuals

Figure 1: Glycaemic responses to string hoppers (wheat and rice flour), ordinary bakery bread and standards (glucose and white sliced bread)

Figure 2: Glycaemic responses to bananas and standard (white sliced bread)
APPENDIX VI

Table 1: Proximate data of wheat and rice flour (g/100 g fresh weight) used in string hopper and roti preparations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wheat flour</th>
<th>Rice flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>75.6</td>
<td>79.0</td>
</tr>
<tr>
<td>Fat</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Protein</td>
<td>11.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>1.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>


Table 2: Proximate data of snake gourd, chickpea and atta roti (g / 100 g fresh weight) used as meal accompaniments and meals for type 2 diabetic patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 Snake gourd</th>
<th>2 Chickpea (mean ± SD)</th>
<th>2 Atta roti (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>90.5</td>
<td>59.8 ± 1.2</td>
<td>23.0 ± 2.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.7</td>
<td>13.4 ±0.4</td>
<td>33.4 ± 0.6</td>
</tr>
<tr>
<td>Protein</td>
<td>2.5</td>
<td>8.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Fat</td>
<td>0.9</td>
<td>3.0 ± 0.2</td>
<td>16.6 ±0.5</td>
</tr>
<tr>
<td>IDF</td>
<td>0.9</td>
<td>8.2 ±0.8</td>
<td>8.9 ± 0.3</td>
</tr>
<tr>
<td>SDF</td>
<td>NA</td>
<td>3.1± 0.5</td>
<td>2.4 ±0.5</td>
</tr>
<tr>
<td>Ash</td>
<td>2.5</td>
<td>0.9 ± 0.0</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Values given as average or mean ± SD (SD-Standard deviation); NA-not available

Sources: 1(Chuku et al., 2008); 2(Widanagamage et al., 2009)
APPENDIX VII

Correlations between IAUC with RDS and SAG/RAG ratio

Figure 1: (a) IAUC & RDS (p=0.001); (b) IAUC & SAG/RAG (p=0.007)
APPENDIX VII

Glycaemic and insulinaemic responses of breakfast and lunch meals with type 2 diabetic individuals

No 1:

![Graph 1](image1)

No 2:

![Graph 2](image2)

No 3:

![Graph 3](image3)
No 7:

(a) 

(b) 

No 8:

(a) 

(b) 

No 9:

(a) 

(b)
Figure 1: Glycaemic and insulinaemic responses to breakfast and lunch meals with type 2 diabetic patients. (a) Glycaemic responses of individuals (b) insulinaemic responses of individuals.