In vitro carbohydrate hydrolysis indices and their relationship to glycaemic and insulinaemic indices of selected UK and Saudi Arabian foods

by

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To my mum, Nora Mssallem Al-Mssallem for being the best mum I could have wished for, for always believing in my dreams and supporting me throughout a journey that was unknown to herself.
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Abstract

A high prevalence of diabetes exists globally and in particular in Saudi Arabia. Several dietary and lifestyle factors are implicated although dietary carbohydrates (CHOs) have a key role in influencing diabetes risk. The physiological impact of CHO on postprandial blood profiles can be measured in vivo or predicted from in vitro digestion rates. This thesis examined the relationship between CHO digestibility in vitro and CHO bioavailability in vivo by assessing a variety of UK and Saudi Arabian foods. Our hypothesis was that traditional Saudi foods could have better health impacts on blood glucose and insulin levels.

The CHO digestibility method of Englyst was established to determine rapidly available glucose (RAG) and slowly available glucose (SAG) parameters. RAG and SAG were determined for some food standards, and some Saudi and UK foods. Also, glycaemic and insulinaemic indices (GI and II) of Saudi Arabian Hassawi Rice and dates were tested using FAO/WHO protocols and compared with Uncle Ben’s rice and dates with Arabic coffee respectively. Once the RAG, SAG and GI values for some Saudi and UK foods were determined, the role of the SAG index was assessed in terms of its ability to differentiate between two meals with essentially identical macronutrient contents and GI values.

Our results demonstrated the method for RAG and SAG measurements to be reproducible and to show good agreement with the observations in the literature. In terms of GI and II values for Hassawi rice and Uncle Ben’s rice, no significant differences were observed between the two types of rice in terms of plasma glucose responses, however, a lower insulin
response were noted for Hassawi rice ($p<0.007$). For Arabic coffee consumed with dates, there was an increase in glucose response at 45 and 60 min ($p<0.05$) although the insulin response was not significantly affected. For the high and low SAG meals, despite the similarity between the two meals it was evident that the high SAG meal resulted in a modestly lower incremental area under the glucose curve ($p=0.21$) and a lower insulin response, particularly at 45 min ($p=0.053$).

In conclusion, these studies showed that some traditional Saudi foods could have beneficial effects in terms of plasma glucose and insulin responses. Furthermore, RAG and SAG values can be used as important predictors of GI. Nevertheless, the SAG value can show important metabolic differences between the impact of foods with the same GI. The observations for staple foods may be important for people with diabetes in Saudi Arabia.
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LIST OF ABBREVIATIONS

ACS  American Chemical Society
ADP  Adenosine-5'-diphosphate
ARIC  Atherosclerosis Risk in Communities
ATP  Adenosine-5'-triphosphate
AUC  Area Under the Curve
CHO  Carbohydrate
CHD  Coronary Heart Disease
Con A  Concanavalin A
CVD  Cardiovascular Disease
DM  Diabetes Mellitus
DMSO  Dimethyl sulphoxide
eV  electron Volt
FAQ  Food and Agriculture Organization
FSG  Free sugar glucose
GI  Glycaemic Index
GIP  Glucose-dependent insulintropic polypeptide
GL  Glycaemic Load
GLP-1  Glucose-like peptide
GOD  Glucose Oxidase
GOD/PAP  Glucose Oxidase/Phenol and 4-amino-antipyrine
GOPOD  Glucose oxidase peroxidase
GPO  Glycerol-3-phosphate oxidase
HDL  High Density Lipoprotein
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental Area Under the Curve</td>
</tr>
<tr>
<td>II</td>
<td>Insulinaemic Index</td>
</tr>
<tr>
<td>ILab</td>
<td>Instrumentation Laboratory</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acid</td>
</tr>
<tr>
<td>NSP</td>
<td>Non-starch polysaccharides</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>RAG</td>
<td>Rapidly Available Glucose</td>
</tr>
<tr>
<td>RDS</td>
<td>Rapidly Digestible Starch</td>
</tr>
<tr>
<td>RS</td>
<td>Resistant Starch</td>
</tr>
<tr>
<td>SAG</td>
<td>Slowly Available Glucose</td>
</tr>
<tr>
<td>SDS</td>
<td>Slowly Digestible Starch</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerides</td>
</tr>
<tr>
<td>TG</td>
<td>Total Glucose</td>
</tr>
<tr>
<td>TS</td>
<td>Total Starch</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>YSI</td>
<td>Yellow Spring Instrument</td>
</tr>
</tbody>
</table>
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**CHAPTER 1**

**1.0 INTRODUCTION AND LITERATURE REVIEW**

**1.1 General introduction**

Diet and lifestyle play a crucial role in the development or prevention of chronic conditions such as obesity, coronary heart disease (CHD) and diabetes mellitus. It is well documented that the prevalence of diabetes is increasing dramatically worldwide at an alarming rate and the number of people with diabetes is projected to grow from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). Diabetes and in particular type 2 diabetes mellitus (T2DM) has below shown to be associated with obesity and an inactive lifestyle. Obese people have been shown to have a higher risk to develop T2DM (Colditz et al., 1990). In Saudi Arabia, there is a particularly high rate of prevalence of diabetes (24 %) and this may be due to the rapid socioeconomic changes which has led to increase rate of obesity over the last 2 decades (Alhadd et al., 2007).

Dietary carbohydrates (CHOs) have an important role in the management of diabetes. They are digested and absorbed at different rates and to different extents in human small intestine. Methods exist to study the rate of CHO hydrolysis in vitro to facilitate our understanding of their impact in vivo. In fact, Englyst (1992, 1999) developed a CHO digestion model and created two terms related to glucose release, namely, slowly available glucose (SAG) and rapidly available glucose (RAG) with the latter showing a good correlation ($P < 0.001$) with glycaemic index (GI) for a series of foods. Indeed, GI (Jenkins et al., 1981) is also a useful parameter which can be used to aid our understanding of the metabolic impact of different types of CHO containing foods (Zhang & Hamaker, 2009).
The effect of consumption of high GI foods and its associations with the progression to T2DM has been studied in large prospective cohort studies (Schulze et al., 2004; Salmeron et al., 1997; Meyer et al., 2000; Stevens et al., 2002). Reducing glucose response and subsequently insulin demand is the one target for the prevention and treatment of T2DM. It has been suggested that the high intake of low GI foods and non-starch polysaccharides (NSP) are associated with a lower risk of T2DM (Willett et al., 2002). However, the great health benefit of low GI foods remains to be fully elucidated. High SAG foods have been found to be equivalent to the low GI diet as it has been found that GI was negatively correlated to the SAG value (Englyst et al., 2003). Therefore, in vitro measurements of CHO hydrolysis have been considered useful in terms of classifying starchy foods according to their RAG and SAG values (Englyst et al., 1992).

The following review of the literature provides the general background of the series of studies that have been conducted. Initially, it will summarise the prevalence of diabetes in Saudi Arabia and the changes in Saudi eating patterns. Following this, a classification of CHOs according to their bioavailability will be briefly reviewed, with particular focus on the in vitro measurement of CHO. The GI and glycaemic load (GL) concepts and the associated factors affecting GI values will be also reviewed. Finally, the aims, hypothesis and objectives of these series of studies will be detailed.
1.2 Diabetes mellitus

1.2.1 Definition and prevalence

Diabetes mellitus (DM) is a metabolic disorder characterised by hyperglycaemia and caused primarily by a defect in insulin secretion from the islet cells of the pancreas resulting in an inability of peripheral cells to use glucose (American Diabetes Association (ADA), 2009).

The number of people with DM in the world is expected to rise from 2.8 % in 2000 to 4.4 % in 2030 due to population growth, aging, effects of modernisation, increase prevalence of obesity and decrease physical activity. It has been estimated that the greatest relative increases in the number of people with diabetes (163 %) will occur in the Middle Eastern region by the year 2030 (Wild et al., 2004).

DM is thought to develop for a variety of reasons. Indeed, several pathogenic processes ranging from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in the resistance to insulin action (ADA, 2009). The majority of cases of diabetes fall into two categories: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM results from β-cell destruction leading to absolute insulin deficiency. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase and autoantibodies to tyrosine phosphatases. Some patients with this type of diabetes may present with ketoacidosis as the first manifestation of the disease. This type of diabetes commonly occurs in childhood and adolescence and accounts for only 5-10 % of those with
diabetes. People with T1DM present with acute symptoms and markedly elevated blood glucose levels and they need insulin for survival (ADA, 2009). T2DM accounts for 90-95% of those with diabetes and results from progressive insulin secretory defect (ADA, 2009). The cause of T2DM is thought to be due to a combination of environmental and genetic factors. Most of individuals with T2DM are obese and they do not usually need insulin treatment to survive. The risk of developing this form of diabetes increases with age, lack of physical activity, and obesity. Obesity itself or having an increased percentage of body fat distributed predominantly in the abdominal region causes some degree of insulin resistance. Insulin resistance may improve with weight reduction and/or hypoglycaemic treatment (ADA, 2009).

1.2.2 Diagnosis of diabetes

Both type 1 and type 2 diabetes share similar symptoms including: weight loss, excessive thirst, frequent urination and increased appetite. Diabetes can be diagnosed based on these symptoms and confirmed by a blood glucose test that is recommended by the American Diabetes Association (ADA, 2009). The recommended blood test is a casual plasma glucose, fasting plasma glucose (FPG), or oral glucose tolerance test (OGTT). A fasting plasma glucose level of 7.0 mmol/l (126 mg/dl) or higher indicates diabetes, while this should be 11.1 mmol/l (200 mg/dl) or higher in a casual plasma glucose concentration or in an oral glucose tolerance test. FPG has been the preferred diagnostic test due to ease of use, acceptability to patients and lower cost (ADA, 2009).
1.3 Saudi Arabia: background and DM

Saudi Arabia is a country that occupies four-fifths of the Arabian Peninsula and stretches over 2,149,690 km² with a population of 20,800,000 in which about 5,000,000 are non-Saudi residents (Ministry of Planning, 2001). After the discovery of oil in 1936, Saudi Arabia has undergone very rapid improvements in all aspects of lifestyle and standards of living (Alzaid, 1997; Al-Nozha et al., 2004). The high level of food availability for consumption and the diversification of diet as well as sedentary lifestyles have led to an increase in diet-related diseases such as obesity, diabetes and coronary heart disease (Alissa et al., 2005; Al-Nozha et al., 2004; Al-Nuaim et al., 1996). It is a fact that the prevalence of diabetes is increasing at an alarming rate in the whole world. In Saudi Arabia, this disease is present in epidemic proportions (a rapid and extensive spread). A few epidemiological studies have been carried out and these have shown that the prevalence of DM was high relative to other countries (El-Hazmi et al., 1998; Fatani et al., 1987).

The overall prevalence of DM in Saudi adults was 4.3 % in 1987 (Fatani et al., 1987) but it is apparent that this number has increased about 6 times by 2004 (Al-Nozha et al., 2004). It has been found that the highest percentage with diabetes was observed in the northern region of the country (28 %), but the southern region had the lowest prevalence with a percentage of 18 % (Al-Nozha et al., 2004). DM is significantly more prevalent among Saudis living in urban areas (25 %) compared to those living in rural areas (19 %). Diabetes has also been shown to increase with age (El-Hazmi et al., 1998; Al-Nozha et al., 2004) with a prevalence of 12 % and 36.5 % at age ranges of 30-39 years and 60-70 years, respectively (Al-Nozha et al., 2004).
A large number of Saudi patients with diabetes are at a high risk for diabetic complications (nephropathy, neuropathy and retinopathy). Risk factors include their blood glucose, blood pressure and lipid are not achieving recommended levels. Also about a third of patients had undiagnosed hypertension and 17.6% of patients having low density lipoprotein (LDL) cholesterol values of more than 3.36 mmol/l, a level that suggests the need for lipid-lowering therapy (Eledrisi et al., 2007; ADA, 2008).

Many factors are involved in influencing the prevalence of DM such as gender, age, socioeconomic status, genetic susceptibility and lifestyle. In Saudi Arabia, two of the main reasons for the increase in DM may be the increase in obesity and a major change in habitual eating patterns, including modifications in the quality and quantity of dietary carbohydrates (Musaiger, 1987; Alhadd et al., 2007).

1.4 Saudi habitual consuming pattern

Saudi habitual eating patterns have altered recently due to rapid socioeconomic developments (Musaiger, 1987). As a result, there was a decrease in the CHO intake and an increase in the fat intake (Stephen et al., 1995). This trend has changed recently and there is a reduction of the intake of fat which tends to cause a compensatory increase in dietary sugar and starch (Collison et al., 2010).

The main source of CHO's in the Saudi diet comes mainly from rice (Duwais, 1983) and wheat flour (Alissa et al., 2005). It is obvious that most of Saudi traditional foods are based on whole grain wheat (e.g. Hareece; whole grain
wheat cooked with meat, Matazeez and Marqooq; whole wheat dough with vegetables and meat, Jareesh; cracked wheat cooked with vegetables and meat) and dates (Musaiger, 1987). In addition, the main dish that is served at lunch time consists of rice with vegetables and meat, the so called Kabsa. Despite these traditional meals are still consumed, finely milled cereal and grain products are replaced by whole grains wheat and flour. Moreover, these healthy traditional starchy foods become less consumed.

Table 1.1 The GI value for some Saudi traditional foods (Ba-Jaber, 1997; Nasib, 2003).

<table>
<thead>
<tr>
<th>Food</th>
<th>GI (Glucose=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabsa</td>
<td>60</td>
</tr>
<tr>
<td>Fried Motabbaq</td>
<td>52</td>
</tr>
<tr>
<td>Baked Motabbaq</td>
<td>56</td>
</tr>
<tr>
<td>Korsan</td>
<td>61</td>
</tr>
<tr>
<td>Kelija eneazaal</td>
<td>58</td>
</tr>
<tr>
<td>Kelija malakee</td>
<td>51</td>
</tr>
<tr>
<td>Hareeece (whole)</td>
<td>52</td>
</tr>
<tr>
<td>Marsa</td>
<td>51</td>
</tr>
<tr>
<td>Jareesh</td>
<td>89</td>
</tr>
<tr>
<td>Foul (mashed)</td>
<td>55</td>
</tr>
<tr>
<td>Foul (whole)</td>
<td>45</td>
</tr>
</tbody>
</table>

The GI of some of these traditional foods has been tested and has been shown to range between 45 and 89 (Table 1.1, Ba-Jaber, 1997). However, the overall GI of the most of traditional Saudis' meals is considered to be medium (> 55 and < 70).
One traditional food which is important in Saudi culture is palm dates. Daily consumption per capita of dates has been estimated to be about 100 g (Ahmed & Ahmed, 1995). Dates are mostly consumed in the Rutab or Tamer stage of development. In the Rutab stage, dates turn yellow or red depending on the variety and are then sold as fresh fruit. The Tamer stage is the dried stage of date production which is characterised by a decrease in weight due to moisture loss and a browning of the skin (Al-Shahib & Marshall, 2002; Ali et al., 2008). The most expensive variety of dates is called Ajwa from Al-Maddina which is cultivated in the North West of the Kingdom of Saudi Arabia. However, the most famous date variety is called Khulas which is grown in Al-Hassa in the Eastern Province of Saudi Arabia (Al-Jaber & Al-Jaber, 2006). Dates are usually consumed with sour milk (plain yoghurt drink) at lunch time or with Arabic coffee as a snack during the day (Miller et al., 2003; El Shabrawy & Felimban, 1993).

1.5 Dietary carbohydrates

1.5.1 Definition

In general terms, carbohydrates (CHOs) are simple organic compounds that are comprised of three elements: carbon, hydrogen and oxygen. CHOs can be synthesized by all organisms, but most CHOs are formed by photosynthetic organisms, such as the cyano-bacteria, algae and plants. CHOs have the empirical formula $C_n(H_2O)_n$ although this formula really only applies to monosaccharides; however, other types of carbohydrates, oligosaccharides and polysaccharides, differ only slightly from this formula. When more than two monosaccharides are condensed together,
oligosaccharides are formed (n = 3-10); while polysaccharides consist of a large number of monosaccharides (>10) linked together by a covalent chemical bond between each two monosaccharide units called glycosidic bond. This bond results from the condensation of the OH group at C4 with the α-OH group at C1, involving the loss of one H₂O molecule for each new link formed (Horton et al., 2006; Campbell & Farrell, 2006).

1.5.2 Chemical structure
All CHOs are built from the basic unit called a monosaccharide, such as glucose, galactose and fructose. Monosaccharides can either be in an aldose form in which the monosaccharide contains an aldehydic group as its terminal functional group or a ketose form in which the monosaccharide contains a keto group as its carbonyl terminal functional group. These monosaccharides can be tetroses, pentoses, hexoses or heptoses depending on the number of carbon atoms present within the molecule (4, 5, 6 and 7 carbon atoms, respectively). The most common aldohexose is glucose which can exist in two isomeric forms, namely the D and L configurations (Figure 1.1(a) and (b)); however, fructose is the most abundant ketoheptose which can also be found in D and L configuration (c).
As mentioned above polysaccharides consist of a large number of monosaccharides which are joined together by a series of glycosidic bonds. The polysaccharides can either contain one type of monosaccharide in which case they are called homopolysaccharides such as starch and glycogen; however, heteropolysaccharides contain more than one type of monosaccharide unit. Polysaccharides can also be divided, on the basis of their biological roles, into the storage polysaccharides (e.g. starch and glycogen) and the structural polysaccharides (e.g. cellulose) which is considered to be the most abundant CHO.
The majority of CHO's found in the daily diet are consumed in the form of starch. Starch is present as a mixture of amylose and amylopectin. Amylose is composed of linear chains of glucose with α(1→4) glycosidic linkages (Figure 1.2), whereas amylopectin has a branched structure with both α(1→4) and α(1→6) linkages (Figure 1.3, Horton et al., 2006). 

Figure 1.2 Structure of amylose (Horton et al., 2006).

Figure 1.3 Structure of amylopectin (Horton et al., 2006).
1.5.3 Classification of carbohydrate

Carbohydrates can be traditionally classified into four groups according to the number of individual simple sugar units present. These are termed monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Cummings and Stephen (2007) have suggested that CHOs should be classified, on the basis of their molecular size, into three groupings (monosaccharides and disaccharides), oligosaccharides and polysaccharides. This classification takes into account the chemical and physical properties of CHOs (Englyst & Englyst, 2005; Cummings et al., 1997; Cummings & Stephen, 2007).

However, classifying CHOs based simply on their chemical structure is not a reliable indicator of their physiological effects (Englyst et al., 1999). As such, this approach of classifying foods according to their physiological effects is more useful in understanding the health effects of diets containing CHOs. A new approach for classifying foods according to their physiological impact on blood glucose levels was proposed by Jenkins and his co-workers (1981). However, in practice GI value is not directly related to the actual amount of CHO content presented in the food as eaten and further detail in GI limitations will be discussed later in Section 1.6.7.

For nutritional purposes, starch can be classified according to their digestibility in human and divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RS is defined as a fraction of starch that escapes digestion in small intestine and reaches the large intestine where it may be fermented (Nugent, 2005; Englyst et al., 2007; Haub et al., 2010).
It has also been suggested that CHO{s could be classified on the basis of their bioavailability, which is determined by both the chemical properties and the physical structure of CHO{s (Englyst & Englyst, 2005). This classification includes rapidly available glucose (RAG) and slowly available glucose (SAG) based on their availability for absorption in the human small intestine (Englyst et al., 1999). Such classification is useful in predicting the likely glycaemic response to different foods (Englyst et al., 1996a). The RAG includes RDS, free glucose and the glucose moiety of sucrose (after hydrolysis) (Englyst et al., 1996a; 1999).

1.5.4 Digestion of carbohydrate

Carbohydrates are one of the most important sources of energy in most human diets providing 40-45 % of the energy in the western diet, whereas it may provide up to 85 % of the energy in the diets of poor people (Garrow et al., 2000). Digestion of CHO{s commences in the mouth where they are digested mechanically by chewing and chemically by salivary α-amylase, which is secreted from the parotid glands. Salivary amylase converts starch and dextrins into a mixture of maltose, glucose and limit dextrin (Garrow et al., 2000). However, in the stomach the activity of α-amylase is destroyed by low pH and thus enzyme hydrolysis of the CHO{s is substituted by acid hydrolysis. When the partly digested CHO{s reach the small intestine, pancreatic amylase, which is secreted by the pancreas, converts any remaining dextrins and starch to disaccharides (maltose). The maltose and other disaccharides (sucrose and lactose) are too large to cross the mucosal cell membrane and they must be converted to their constituent
monosaccharides by disaccharidases. The disaccharidases (maltase, sucrase, and lactase) are expressed on the brush border of the small intestine cells. Maltose is converted by maltase into two glucose molecules, sucrose is converted to glucose and fructose by sucrase, and lactose is converted by lactase into glucose and galactose (FAO/WHO, 1998; Southgare, 1995; Lichtenstein & Wu, 2004).

1.5.5 Hydrolysis of carbohydrate

Most procedures for determining starch fractions rely on the measurement of the liberated glucose after the hydrolysis of starch with either acids or enzymes (Englyst et al., 2000; Bergmeyer, 1981). However, the enzymatic approach is preferable as it is more specific and does not cause destruction of sugars or polysaccharides.

1.5.6 Measurement of carbohydrates

The total amount of carbohydrate in a food is traditionally measured by difference, and this is calculated by subtracting from 100 (i.e. average quantity expressed as a percentage after taking into account the content of moisture, protein, fat, ash and dietary fibre). However, this is prone to accumulative errors arising from the other individual analyses (Stephen et al., 1995). Direct measurement of CHO is therefore more appropriate and is a more accurate and rapid procedure for CHO measurement. Thus, CHO can be directly measured through determination of released glucose after the hydrolysis of food CHO with either acids or enzymes (Southgate, 1991). However, the enzymatic approach is favourable due to its rapid, high
specificity and greater sensitivity. In addition, the enzymatic procedure does not cause the destruction of sugars or polysaccharides (Southgate, 1991). GI is as a physiological response measurement of CHO containing foods that helps to rank these foods according to their digestibility in the human small intestine (Jenkins et al., 1981); however, it is not related directly to the actual amount of CHOs usually presented in foods as eaten but it compares the blood glucose response to the specific amount of available CHO (usually 50 g) presented in a food. RAG and SAG values are therefore used as direct measurement of absolute CHO which is available for absorption in the human small intestine (Englyst & Englyst, 2005; Englyst et al., 1999; 2003). The RAG and SAG measurements are actually based on the release of glucose solely as a result of enzyme hydrolysis. Measurement of RAG in vitro gives values for glucose that are likely to be absorbed in the human small intestine and, thus likely to influence blood glucose and insulin responses (Englyst et al., 1996a). It is evident that using the RAG and SAG measurements of foods containing CHO as a supplement to the GI approach may provide further data which could be useful in the understanding of the impact of CHO on blood glucose and other associated metabolic features (e.g. lipid levels). A list of common foods with RAG and SAG values is demonstrated in Table 1.2.
Table 1.2 The RAG and SAG values of some foods per 100 g as eaten
(Englyst, 1996a; 2003)

<table>
<thead>
<tr>
<th>Food</th>
<th>RAG (g)</th>
<th>SAG (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All bran</td>
<td>33</td>
<td>0.5</td>
</tr>
<tr>
<td>Beans in tomato sauce</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Brown rice</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Chickpea (canned)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>78</td>
<td>3</td>
</tr>
<tr>
<td>Cracker</td>
<td>59</td>
<td>7</td>
</tr>
<tr>
<td>Digestive biscuit</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>French baguette with chocolate spread</td>
<td>52</td>
<td>2</td>
</tr>
<tr>
<td>Haricot bean</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Instant potato</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Kidney bean</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Macaroni</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Oatmeal biscuits</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>Parboiled rice</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Pearled barley</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Porridge oats</td>
<td>42</td>
<td>3</td>
</tr>
<tr>
<td>Potato crisps</td>
<td>48</td>
<td>3</td>
</tr>
<tr>
<td>Puffed wheat</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Red lentil</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Rye wholemeal bread</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Sweet corn</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Water biscuit</td>
<td>66</td>
<td>4</td>
</tr>
<tr>
<td>Weetabix</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>White bread</td>
<td>38</td>
<td>4</td>
</tr>
<tr>
<td>White rice</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>White spaghetti</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>33</td>
<td>2</td>
</tr>
</tbody>
</table>
1.6 Glycaemic Index

1.6.1 Glycaemic index concept

There is increasing interest in the effects of the amount and type of dietary CHO on the metabolic profile in health and disease. However, the chemical nature of CHO is not a reliable indicator of their physiological effects. Classifying foods according to their physiological effects may therefore be more useful in understanding the health effects of different carbohydrate containing diets (Jenkins et al., 1981). The GI index of foods was initially developed and published in 1981 by Jenkins and colleagues in order to help demonstrate the physiological impact of CHOs on postprandial blood glucose rather than their chemical features (Jenkins et al., 1981). This classification added an important dimension to nutrition and dietotherapy. Therefore, the GI concept is used to rank CHO containing foods according to their immediate impact on blood glucose levels. It is more accurately defined as the incremental area under the glucose response curve (AUC) of a 50 g available CHO-containing food expressed as a percentage of the response to the same amount of CHO from standard food (pure glucose or white bread) taken by the same subject (Wolever & Jenkins, 1986; Wolever et al., 1991, 2004; Hallfrisch & Behall, 2000). However, GI per se takes only the type of CHO (i.e. CHO that can be readily hydrolysed to produce glucose) into account, ignoring the total amount of CHO in a typical portion. It is important to note that both the quality and quantity of CHO influences the postprandial glycaemic and insulinaemic responses to a food (Willett et al., 2002; Sheard et al., 2004). In order to assess simultaneously the type and amount of CHO consumed, the concept of glycaemic load (GL) was introduced (Salmeron et
al., 1997; Liu & Willett, 2002). More details about the GL are mentioned below.

1.6.2 Glycaemic load concept

The amount of dietary CHO in the diet and the rate and extent of digestion of this carbohydrate are the principal determinants of postprandial glucose and insulin responses (Liu et al., 2000; Willett et al., 2002; Englyst et al., 1999; 2003). Modifying the amount or source of dietary CHO is an important way to manipulate plasma glucose and insulin responses (Wolever, 2004). GL is a qualitative and quantitative indicator used to estimate the overall impact of CHOs on the blood glucose response (Largua & Claudio, 2004) and it was first proposed in 1997 by Salmeron and his co-workers. GL was defined as the quantity GI of a specified serving size multiplied by the weight of CHO contained in that serving size (Liu et al., 2000). A large number of GL values of foods have been published (Brand-Miller et al., 2007; Foster-Powell et al., 2002). An example for GL values of some common foods are demonstrated in Table 1.3. The importance of the GL in preventing of T2DM and reducing risk of CVD was reported in prospective cohort studies (Salmeron et al., 1997; Slujs et al., 2010; Beulens et al., 2007). This will be discussed further in section 1.6.8.2 and section 1.6.8.3.
Table 1.3 The GI and GL values for some common foods (Brand-Miller et al., 2007).

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>GL</th>
<th>GI level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>38</td>
<td>6</td>
<td>low</td>
</tr>
<tr>
<td>Banana</td>
<td>52</td>
<td>13</td>
<td>low</td>
</tr>
<tr>
<td>Chickpea (canned)</td>
<td>40</td>
<td>9</td>
<td>low</td>
</tr>
<tr>
<td>Cornflakes, Kellogg's</td>
<td>77</td>
<td>20</td>
<td>high</td>
</tr>
<tr>
<td>Dates, arabic, vacuum-packed</td>
<td>39</td>
<td>16</td>
<td>low</td>
</tr>
<tr>
<td>Long Grain Rice</td>
<td>50</td>
<td>23</td>
<td>low</td>
</tr>
<tr>
<td>Potato, mashed</td>
<td>88</td>
<td>18</td>
<td>high</td>
</tr>
<tr>
<td>Uncle Ben's Rice</td>
<td>50</td>
<td>18</td>
<td>low</td>
</tr>
<tr>
<td>Weetabix, biscuit</td>
<td>69</td>
<td>12</td>
<td>medium</td>
</tr>
<tr>
<td>White bread</td>
<td>71</td>
<td>10</td>
<td>high</td>
</tr>
<tr>
<td>Yogurt, plain</td>
<td>36</td>
<td>3</td>
<td>low</td>
</tr>
</tbody>
</table>

1.6.3 Measuring GI of food

The GI of different foods cannot be assumed by examining the composition of the food, instead it has to be measured. Jenkins' initial studies compared 50 g portions of digestible CHOs with 50 g glucose. Samples of blood were taken fasting and then every 15 minutes during the first hour and thereafter every 30 minutes through the second hour after the ingestion of the CHO-containing food. The area above the fasting glucose concentration was calculated and expressed as a percentage of the area obtained after the ingestion of 50 g glucose; the higher the area under the curve (AUC), the higher the GI of a food (Jenkins et al., 1981). As such differences in the glycaemic response to various CHO-rich foods is related to differences in the rate at which the CHO is digested and absorbed (Jarvi et al., 1999).
To measure the GI of a food, a typical sample size of between 10 to 12 subjects is required (FAO/WHO, 1998). Subjects must be matched for glucose tolerance and they are asked to drink no alcohol and not to smoke for 24 h prior to the test day. Capillary finger-prick blood samples are taken from normal subjects in the fasted state and then every 15 minutes during the first hour and thereafter every 30 minutes during the second hour after the start of consuming the 50 g of available CHO of the test or reference food (in random order). GI can be also measured in diabetic subjects although the sampling occurs every 30 minutes for 3 h. The standard food should be repeated at least three times on separate occasions by each subject in order to reduce variability. Blood glucose is measured with an automatic analyser and plotted on a graph against sampling time. The incremental area under the blood glucose response curve is calculated geometrically. It is the sum of the areas of the triangles and rectangles using the trapezoid rule (Figure 1.4) and it was first computed by hand but now it is usually calculated using a computer programme (Wolever et al., 1991, 2004). The area under the curve for each food is expressed as a percent of the mean response to the standard food taken by the same subject, and then the resulting values are averaged to obtain the GI for a particular food (Wolever et al., 1991; 1994).
The equation for calculating the AUC is:

\[
\text{Area} = \frac{(A + B + C + D/2)t + D^2/2}{t} (D + |E|)
\]

Where A, B, C, D, and E represent the blood glucose increments, t represent different time intervals between blood samples (Wolever et al., 1991).

Incremental area under the curve for 50 g available CHO from test food

\[
\text{GI} = \frac{\text{Incremental area under the curve for 50 g CHO from reference food}}{\times 100}
\]

Subjects' characteristics do not appear to have a major effect on the mean GI values determined; although the variation of the values has been shown to be highest in individuals suffering from T1DM (Wolever et al., 1987). Otherwise, the GI determined between normal vs. diabetic subjects (Wolever et al., 1988a) and children vs. adults (Wolever et al., 1988a; 1991) have
shown no significant difference. For routine GI testing, it has been recommended that healthy human subjects should be used (Brouns et al., 2005).

1.6.4 Classification of the GI of foods

GI is divided into three categories: high, medium and low. These will be described below and a list of most common foods with high, medium and low GI is shown in Table 1.4.

1.6.4.1 High GI food

High GI foods are those foods which have a high rate of conversion of available CHO into glucose with subsequent absorption by the small intestine. High GI foods have, by definition, an index of greater than or equal 70 in the case of using glucose as a standard reference or greater than or equal 100 when white bread is used as a reference (Brand-Miller et al., 2009; Wolever et al., 1991). High GI foods cause a rapid elevation in blood glucose. In response to this the body attempts to balance the rise in blood glucose levels by secreting a large amount of insulin. It is considered that repeated overproduction of insulin may lead to insulin resistance in which cells that normally respond to insulin become less sensitive to its effects (Frost et al., 1998). Indeed, excessive intakes of high GI foods over a long period are associated with high insulin levels, insulin resistance, a lower concentration of HDL and hypertriglyceridaemia (Brand-Miller, 1994; Frost et al., 1998; 1999; Jenkins et al., 1987). Research on the health effects of different GI of foods have indicated that a high GI diet and other factors associated with
increasing affluence may have assisted in creating and developing chronic disease states (Liu et al., 2000; Jenkins et al., 2004). It has been considered that refined grains may be associated with increased diabetes risk because these foods tend to have a high GI compared to whole grain containing foods (Schulze et al., 2005).

1.6.4.2 Medium GI food

Medium GI foods are those foods which have an index of greater than 55 and less than 70 in the case of using glucose as a standard reference (Brand-Miller et al., 2009; 2007). Some types of rice have a medium GI ranged from 56 to 69 (Foster-Powell et al., 2002), however; high GI rice such as waxy rice with GI value of 88 can be combined with lentils (GI value of 26) to moderate the overall value (Brand-Miller et al., 1992; 2007).

1.6.4.3 Low GI food

Low GI foods are those foods which have an index of less than or equal 55 in the case of using glucose as a standard reference or less than or equal 78 if white bread is used as a reference (Brand-Miller et al., 2009; Wolever et al., 1991). Low GI foods such as beans, oats and lentils are digested more slowly and the CHO present is converted into glucose at a slower rate, producing a more gradual rise in blood glucose and insulin responses (Kalergis et al., 2005; Granfeldt et al., 2006). Intervention and epidemiological studies showed that the consumption of low GI food was associated with the reduction of the risk of CVD and T2DM (Thomas & Elliott, 2010; Heilbronn et al., 2002; Schulze et al., 2004; Salmeron et al., 1997).
Table 1.4 List of common UK foods with high, medium, and low GI values (Foster-Powell et al., 2002; Henry et al., 2007; 2008).

<table>
<thead>
<tr>
<th>High GI (GI ≥ 70)</th>
<th>Chocolate soya drink, Cornflakes (Kellogg’s), Mashed potato, Pancakes, White and plain Baguette</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium GI (70 &gt; GI &lt; 55)</td>
<td>Malted wheat cereal, Cereal flakes with fruit, Croissant, Cranberry juice cocktail, Muesli bar containing dried fruit, Rye crispbread</td>
</tr>
<tr>
<td>Low GI (GI ≤ 55)</td>
<td>Sweet biscuit, Whole-wheat crackers with pumpkin and thyme, Hot oat cereal, Apricot and almond bar, Oat biscuits, Chocolate drink, Smoothie drink (fruit), Chicken and mushroom soup</td>
</tr>
</tbody>
</table>

1.6.5 Dietary GI

The GI of a mixed meal can be predicted from the GI values of individual component foods (Collier et al., 1986; Wolever & Jenkins, 1986; Chew et al., 1988). A high correlation \((r = 0.88; p < 0.01)\) was found between the observed GI values of mixed meals and the calculated values based on individual components foods (Chew et al., 1988). The GI value for a mixed meal can be calculated by summing the products of the proportion of available CHO content in each food eaten per meal and its GI value (Wolever & Jenkins, 1986). By following the same principle, the dietary GI of a diet can be estimated by summing the products of the absolute available CHO content in each food eaten per day and the GI value for each component.
food, divided by the total amount of available CHO eaten. The recommended formula for calculating the overall dietary GI is the following:

\[
\text{Overall dietary glycaemic index} = \frac{\sum_{i=1}^{n} \text{GI}_i \times \text{CHO}_i}{\sum_{i=1}^{n} \text{CHO}_i},
\]

where \(\text{GI}_i\) is the glycaemic index for food \(i\), \(\text{CHO}_i\) is the CHO content in food \(i\) (g/day), and \(n\) is the number of foods eaten per day (Liu et al., 2001; Salmeron et al., 1997; Mosdol et al., 2007).

### 1.6.6 Factors influence GI value of food

There are a number of factors that have important effects on the physiological properties of foods. Indeed, all the factors that influence the digestibility of starch in a given food will also apply to the GI (Englyst et al., 1996a). The differences in the physical and chemical characteristics of specific foods have been reported previously to influence the GI value of foods (Hallfrisch & Behall, 2000). These will be described in more detail below.

#### 1.6.6.1 Combinations of macronutrients

Mixtures of CHO, protein and fat have both different and variable effects on glucose response depending on the proportions of each macro-nutrient (Pi-Sunyer, 2002). Nevertheless, the presence of both fat and protein can modify the glycaemic response to a CHO meal (Beebe, 1999). The effect of adding this macronutrient to CHO may depend upon the type and amount added (Wolever et al., 1987; Henry et al., 2007). It has been found that the GI response was reduced significantly after the addition of 50 g protein (Wolever
et al., 1987). Adding protein influences glycaemic response by enhancing the plasma insulin response (Gannon et al., 2001), but fat affects GI by delaying gastric emptying. However, the amounts of fat and protein consumed in the normal diet are usually less than those required to have significant effects on glycaemic response in experimental situations (Wolever et al., 1991).

1.6.6.2 Ratio of amylose to amylopectin

The GI of foods is also affected by the ratios of amylose to amylopectin in the starch. Amylose is composed of long chains of glucose with \( \alpha(1 \rightarrow 4) \) linkages, whereas amylopectin has a branched structure with both \( \alpha(1 \rightarrow 4) \) and \( \alpha(1 \rightarrow 6) \) linkages (Horton et al., 2006). Amylose molecules tend to form tight, compact units, which break down more slowly than amylopectin which is made up of branched chains which are more easily hydrolyzed in the gut compared to the single-chain amylose (Hallfrisch & Behall, 2000; Pi-Sunyer, 2002). Furthermore, the molecular weight of amylopectin is larger than amylose, resulting in a larger surface area per molecule compared with amylose. This makes the digestion rate and subsequent absorption of the glucose monomers from amylopectin higher than that for amylose (Thorne et al., 1983). Thus, the higher the amylopectin proportion in a starchy food, the higher the GI. Indeed, it has been found that the rice varieties with the highest amylose content (23-25%) produced significantly lower glucose responses and insulin levels compared to lower amylose-containing rice varieties (Goddard et al., 1984).
1.6.6.3 Processing, preparation and cooking method

Cooking and food preparation can also modify the GI of food by changing the physical form and particle size within the food. Grinding, rolling or pressing, which is done in the processing of many grains, disrupts the outer germ layer and affects the starch granules causing an increase in the GI (Pi-Sunyer, 2002). The GI of foods eaten whole differs from that consumed as a juice or when it is mashed (GI of baked potato = 77 and mashed potato = 88; Brand-Miller et al., 2007). In addition, the cooking method disrupts the cell wall and splits the starch granules, leading to a greater amylase digestion making it more available to amylase and subsequently increasing the GI (Franz, 2001; Pi-Sunyer, 2002). It is evident that foods processed by boiling or frying may result in lower GI value compared with baking or roasting. For example, the GI value for boiled Sweet potato was 46 and this value jumped to 94 when it was baked (Bahado-Singh et al., 2006).

1.6.6.4 Physical form of starch

The CHO can be present in a form that restricts access of hydrolytic enzymes, leading to lower the rate of digestion and absorption of starch. Large differences in glycaemic and insulinaemic responses in some cereal/bean meals were observed due to differences in the botanical and physical structure of the starchy foods (Jarvi et al., 1995). Altering the physical structure of food, for example by mechanical blending, can result in faster absorption and more rapid rise in blood glucose compared with blending by hand (Franz, 2001). Therefore, food form is one major factor that appears to
affect the rate of absorption of CHO and it has a role to play in influencing the glycaemic response (Cummings & Stephen, 2007).

1.6.6.5 Non-starch polysaccharides (NSP) content

Dietary non-starch polysaccharides (NSP), either soluble or insoluble (Frost & Dornhorst, 2000), play an important role in improving postprandial glycaemic response. This improvement has been found to be due to the effect of NSP on slowing down the digestion and absorption of food (Meyer et al., 2000). In spite of this, the impact of NSP on the GI of a food is still controversial, some researchers have shown that soluble NSPs, such as guar gum, pectin, and beta-galactomannan, slow digestion and effectively slow the availability of the starch to digestion which usually lowers a food’s GI. This occurs due to the fact that soluble NSP form gelatinous gels within the stomach which delay gastric emptying as well as subsequent enzymatic digestion. Gelatinous gels therefore work as a barrier around the CHO to be digested (Frost & Dornhorst, 2000). On the other hand, it has been observed that there was no significant relation between soluble NSP content and GI ($r = 0.308$), but there was a significant relationship between insoluble NSP and GI ($r = 0.584, p < 0.01$). This might be due to the effect of uronic acids in the insoluble NSP (Wolever, 1990). However, Jenkins et al. (1981) found that no relationship existed between total NSP content of foods and the GI value. It appears that the type of NSP (soluble or insoluble) and the nature of the food being studied (e.g. particle size) may be the most important influences on the rate of digestion and absorption of foods and hence their glycaemic response compared to the amount of total NSP (Jenkins et al., 1987). Indeed, it has
been recognized that low GI foods seem to be rich in soluble NSP such as, oats, barley, and legumes.

1.6.6.6 Presence of sugars

Most foods rich in sucrose, lactose or fructose do not raise blood glucose values any more than most complex starchy foods like bread which are composed of polymers of glucose. This is in part due to a delayed stomach emptying which increases the concentration of these simple sugars, and also the restriction of gelatinisation of the starch by binding water and slowing down access of hydrolytic enzymes (Brand-Miller et al., 2007). It has been found that dietary GI was inversely associated with the intake of fructose (Ali et al., 2008; Wolever et al., 1994). Typically, the liver converts all monosaccharides (added, or presented naturally in foods) to glucose which are then stored in the liver as glycogen. In return, they are slowly converted into glucose when they are needed to enter the general circulation (Franz, 2001).

1.6.6.7 Digestibility of starches

The process of the swelling and bursting of starch granules during cooking makes them more available to digestive enzymes in the small intestine (Englyst et al., 1996b; Brand-Miller et al., 2007). In addition, foods with a dense matrix (e.g. pasta) which hinders enzymatic hydrolysis or those that contain resistant starch are digested less rapidly than other starchy foods (Franz, 2001). Resistant starch (RS) has received an interest for its physiological benefits. Indeed, it is divided into three categories: physical
inaccessible starch (RS\textsubscript{1}) such as partly milled grains and seeds. The second type is untreated starch granules (RS\textsubscript{2}) and this form is found in raw potato and banana. The third form is called retrograded starch (RS\textsubscript{3}) such as that found in cooled, cooked potato and bread (Englyst \textit{et al.}, 1992; Englyst \textit{et al.}, 2007; Haub \textit{et al.}, 2010). Recently, another type of resistant starch which is known as structurally modified starch (RS\textsubscript{4}) was introduced. Consumption of foods that rich in RS, such as legumes and barley, was associated with favourable lower glucose and insulin responses (Nugent, 2005; Haub \textit{et al.}, 2010).

**1.6.6.8 Presence of organic acids**

It has been found that increasing the acidity of a meal can influence the GI (Liljeberg & Bjorck, 1996; Pi-Sunyer, 2002). This is included lactic acid that produced during fermentation or added corresponding salts such as calcium lactate or sodium propionate (Liljeberg \textit{et al.}, 1995; Liljeberg & Bjorck, 1996). For example, the GI and II were significantly reduced by consumption of sourdough bread containing lactic acid produced during fermentation or added compared to consumption of bread without adding these acids (Östman \textit{et al.}, 2002; Liljeberg \textit{et al.}, 1995). The effect of these organic acids or salts on lowering postprandial blood glucose and insulin responses was found to be due to their influence on slowing the rate of gastric emptying in human study or due to a reduced rate of starch hydrolysis (Liljeberg & Bjorck, 1996; Liljeberg \textit{et al.}, 1995; Östman \textit{et al.}, 2002).
1.6.6.9 Presence of anti-nutrients

There are other components that may contribute to the lowering of the GI of foods such as phytates, lectins and tannins. For example, the phytic acid was found to be correlated negatively with the GI of food. The presence of phytate was associated with significant reduction (50%) in the digestibility of starch (Yoon et al., 1983). These anti-nutrients can interact with digestive enzymes and reduce their bioavailability leading to lower the digestion and subsequently the absorption of CHO. It has been observed that many low GI foods such as legumes are rich in the anti-nutrients (Messina, 1999; Jenkins et al., 1987; Panlasigui & Thompson, 2006).

1.6.7 Variability of GI

It is well documented that similar foods have been shown to have a wide range of GI values. The variations in GI values might be due to methodological factors or physicochemical characteristics of foods. These will be discussed further in the following section.

1.6.7.1 Methodological factors

1.6.7.1.1 Variability within and between subjects

It is not unusual for GI values to vary within the same subject and between different individuals (Pi-Sunyer, 2002). It has been found that considerable difference in GI values observed between subjects is due to within-individual variability of glycaemic response, when the subject consumed a test food only once (Wolever, 1990; Wolever et al., 1991). As a result it has been suggested that in order to reduce the variability in GI values between
subjects, each subject needs to carry out the test on each food repeatedly. There has been no significant difference in glycaemic response between subjects observed when three test foods were repeated four times (Wolever, 1990). In addition, within subject variation can be reduced through the method of blood sampling by using capillary instead of venous blood samples. This variability was twice in the venous blood samples compared to that obtained from capillary blood samples (Wolever et al., 2003). Furthermore, the GI value of a food is usually the average value calculated in eight to ten subjects to account for the variations that exist in the blood glucose response. It has been recommended that the inclusion of ten subjects gives a reasonable degree of precision for most purposes of measuring GI and this number of subjects can be increased if the aim of the study requires greater precision and small differences in GI (Brouns et al., 2005).

1.6.7.1.2 Food portion size

The measurement of GI requires a portion size of test food that is ingested on a 50 g available CHO basis (Jenkins et al., 1981). This is because the blood glucose response appears to be linear up to 50 g available CHO and shows a trend to plateau between 50 and 100 g (Wolever et al., 1991). The available CHO, by definition, is usually calculated by excluding dietary fibre (DF) and resistant starch (RS) from total CHO of a food (Monro, 2003; Englyst et al., 1996b). As all foods do not have the same CHO content, portion sizes of these foods vary. This variation would have an impact on the ingestion time during the GI testing which affected the glycaemic response
(Brouns et al., 2005). Also, food portion sizes are often calculated from food tables or food analysis to get a serving size containing 50 g available CHO. Dietary fibre in these tables is usually estimated using the classical method which did not measure all indigestible CHO such as RS, leading to an overestimation of the content of available CHO in test food (Brouns et al., 2005). This may result in an underestimation of the GI value due to using a portion sizes contains less than 50 g available CHO (Wolever et al., 1991), but this underestimation did not significantly affect the GI. Granfeldt and his colleagues (2006) have observed that increasing portion size in a food that contains high levels of RS and dietary fibre to adjust for its RS had no significant impact on GI or insulinaemic index (II). For the GI testing; however, it is advised to estimate available and indigestible CHO (DF and RS) using specific analyses and the portion size of test food should be restricted to 50 g available CHO basis (Granfeldt et al., 2006; Brouns et al., 2005).

In addition, the volume of a drink that is given to the subjects during GI testing should be standardised. It is recommended to supply an amount of 250 ml water with the test food and if the reference food is glucose, the 50 g glucose should be diluted into 250 ml of water (Brouns et al., 2005).

1.6.7.1.3 Choice of standard food

The original standard food was glucose (Jenkins et al., 1981) and it is still used by many investigators. However, more recently the standard food chosen has been white bread (Wolever et al., 1991). Some researchers continue to use white bread as a reference food because of the excessive
sweetness of a glucose drink which may cause nausea to some subjects (Frost et al., 1993). Nevertheless, glucose is easier to standardise and is a more logical choice for international use than white bread. As the white bread is more typical of what humans generally consume (Wolever et al., 2003; Kalergis et al., 2005), the use of white bread may stimulate more insulin relative to the blood glucose response than does a CHO matched glucose solution (Wolever et al., 1991). Also, variation in the composition and digestibility characteristics of white bread reduce its usefulness as the reference food (Wolever et al., 2003). It is recommended to use glucose as a reference, however, it is acceptable to use white bread as long as it has been calibrated against glucose and preparation condition is standardised (Brouns et al., 2005).

When glucose is used as a reference food, the GI value for glucose and white bread is 100 and 70, respectively (Wolever et al., 1991; Foster-Powell et al., 2002). Thus, the GI values obtained when white bread is used are about 1.43 times (factor of 100/70) those obtained if glucose is used as standard (Brouns et al., 2005).

1.6.7.1.4 Repeat testing of the standard and test foods

It has been found that there is a large variation in GI values if the subject is tested with a food only once (Wolever, 1990). It has been suggested that each food should be repeated at least three times on separate occasions by each subject in order to reduce variability (Wolever et al., 1991).
1.6.7.1.5 Method of area calculation

There can be a difference in GI values calculated according to the calculation methods used (Wolever, 2004). The GI value is based on the area under the blood glucose response curve AUC and above the fasting concentration, and any area beneath fasting is ignored (Wolever & Jenkins, 1986). However, several experts prefer the use of the whole AUC instead of the area above the fasting glucose as the real measure of glucose availability (Pi-Sunyer, 2002). The recommended method by the Food and Agriculture Organization (1998) for subjects without diabetes is measured by collecting seven blood samples over a 2 h period and calculating incremental area under glucose curve (iAUC). The iAUC includes all area below the blood glucose curve and above the baseline only (Wolever, 2004). A recent study compared the suitability for GI calculations of using different blood sampling schedules and other methods of calculating area under the curve, and it suggested that the recommended calculation by the Food and Agriculture Organization (1998) is the best method for calculating GI (Wolever, 2004).

1.6.7.1.6 The second meal effect

The GI of a food can be influenced by the composition of the evening meal consumed before a test (Robertson et al., 2002; Stevenson et al., 2008). Measuring GI in the morning would be lower after a low GI compared with a high GI dinner (Frost & Dornhorst, 2000; Wolever et al., 1988b). Such this effect has been seen after consuming a low GI evening meal containing high level of indigestible CHO (RS and DF). The blood glucose and insulin responses of standardised white wheat bread has been studied after
consuming pasta and barley in the evening prior to GI testing the following morning. A substantial reduction has been seen in the GI and II (23 % and 29 % respectively) of white wheat bread after a barley evening meal which contains high levels of RS and DF (Granfeldt et al., 2006). Thus, it is recommended providing a dinner or restricting it the night before the test meal in order to reduce the variability in the GI values of foods (Wolever et al., 2008). However, providing a standardised meal to subjects increases the cost of doing GI testing. It has been suggested that advising subjects to avoid certain types of foods may be more cost-effective (Wolever et al., 2008). On the evening prior to a test, therefore; subjects should consume a meal of choice and repeat that meal before each test (Brouns et al., 2005).

1.6.7.1.7 Restrictions of alcohol consumption and activity
Replacing restrictions on exercise and alcohol consumption 24 h prior to the study day on which GI testing occurs has been shown to be associated with a slight lowering of the mean GI obtained (Wolever et al., 2008). An improvement in the insulin sensitivity (Englert et al., 2006) and an increase in muscle glucose uptake (Malkova et al., 2000) were observed on the following day after an acute physical exercise. Prior physical activity or alcohol consumption may result in reducing glycaemic response and subsequently lowering GI due to improving insulin sensitivity or reducing hepatic glucose output (Wolever et al., 2008). Therefore, avoiding unusual vigorous activity and abstaining from alcohol on the previous day of GI testing was recommended (Brouns et al., 2005).
1.6.7.1.8 Ingestion time of test food

The volume and weight of the test portions varies due to the fact that all foods do not have the same CHO content. These differences in the portion size between test foods have an impact on the time required for consumption and subsequently could affect the glycaemic response to test foods (Brouns et al., 2005). Therefore, ingestion time should be standardised. Ingestion of fluid was recommended to be within 5 – 10 min; while solid and semi-solids should be consumed within 10-15 min depending on the type of foods. The first bite of the food or sip of the drink was set as time 0 and the first blood sample should be taken exactly 15 min afterwards (Brouns et al., 2005).

1.6.7.1.9 Length of time and duration for blood sampling

For GI testing, it is recommended collecting seven blood samples over 2 h in individuals without diabetes (FAO/WHO, 1998). Taking blood samples less frequently or in less than 2 h may increase the mean and variation of the GI values. The frequency and length of time is likely to be important for obtaining valid and consistent GI values (Brouns et al., 2005).

1.6.7.1.10 Method of blood sampling

The capillary blood is similar in composition to arterial blood. The glucose concentrations in capillary blood samples were consistently greater than those in venous blood samples where the tissues consume the glucose as the blood flows from arterial to the venous circulation (Granfeldt et al., 1995; Hätönen et al., 2006). The use of venous blood samples resulted in a high within-subject variation, while a greatest sensitivity and lower variability was
associated with using capillary blood samples (Wolever et al., 2003; Brouns et al., 2005). It has been observed that within subject variation was twice as high when using venous blood samples compared to capillary blood samples. Moreover, capillary fingertip blood has an ability to detect rapid changes in blood glucose after consumption of foods (Louie et al., 2008). Therefore, it is preferable for GI testing to apply fingertip capillary blood to get the greatest sensitivity with more precise GI values (Wolever et al., 2003; Brouns et al., 2005).

1.6.7.1.11 Fasting blood sample

It has been shown that analysing glucose twice in a single fasting blood sample reduces the standard deviation (SD) of the GI values more than using the average of two different fasting samples. In the case of using a less precise glucose analytical method, however; it has recommended taking 2 fasting blood samples and doing duplicate measurements of the glucose level. In addition, the length of fasting time (8-12 h) did not appear to have any significant effect on the GI value (Wolever et al., 2008).

1.6.7.1.12 Mixed meals

It has been argued that the glucose response to a single food is not the same as the glucose response to that food in mixed meal due to the fact that the presence of fat and protein may have an influence in the postprandial glucose and insulin responses (Collier et al., 1984; Beebe, 1999). However, it has been shown that the variation in the fat and protein content of meals had a negligible effect on glycaemic responses (Wolever et al., 2006). The
applicability of the GI approach to mixed meals by using the GI values of individual foods has been studied and there was a strong correlation ($r = 0.88 - 0.98$, $p < 0.05$) for the observed GI versus the predicted GI of mixed meals (Collier et al., 1986; Wolever & Jenkins, 1986; Chew et al., 1988; Wolever et al., 2006). In contrast, recent published data has not found an association between the calculated GI of individual foods and the measured GI of mixed meals (Flint et al., 2004; Alfenas & Mattes, 2005). There was a concern about the validity of the methods used to determine meal GI in those studies (Wolever et al., 2006). In Flint’s study, the GI of individual foods was based on the data obtained from international GI tables (Foster-Powell et al., 2002) and it was not clear whether the selected GI values were accurately represented the foods used (Wolever et al., 2006). It is advised that the GI values of the single foods should be measured rather than estimated from GI tables in order to get most accurate prediction for meal GI (Brouns et al., 2005). Moreover, the methods used in Alfenas’s study did not conform to standard procedure of GI testing (Wolever et al., 2006). Accordingly, available evidence indicates that the GI concept applies well to mixed meals and GI of different mixed meal can be predicted from the GI of single foods (Wolever et al., 2006; Brouns et al., 2005).

1.6.7.2 Differences in the physical and chemical characteristics

It is apparent that similar foods may look and taste almost the same, but differences in their ingredients or the processing method used can result in differences in the degree of starch gelatinisation and hence the GI values (This has been already covered in section 1.6.6).
1.6.8 GI and non-communicable diseases

The purpose of the GI classification of foods was generated to assist in physiological response classification of CHO foods that would be of relevance in the prevention and treatment of chronic diseases (Jenkins et al., 2004). GI and GL are two methods that have been investigated as potential tools for meal planning and/or assessing disease risk associated with dietary carbohydrate intake (Sheard et al., 2004). Nevertheless, it is evident that although an enormous amount of effort has been applied to understanding the role of dietary CHO in human health and disease risk, there is no definitive proof that reducing glycaemic impact will prevent disease in individuals (Seal et al., 2003). As such relationship between the GI of foods and its association with the risk factors of obesity, diabetes, and heart diseases is still to be fully explained.

1.6.8.1 GI and obesity

The relationship between GI and GL of diets and the development of obesity has been considered. The rates of obesity and overweight continue to increase in most Western countries despite the efforts of governments and health care providers to prevent and reduce this trend (Brand-Miller et al., 2002). Western diets in general produce a high glycaemic response, promoting postprandial CHO oxidation at the expense of fat oxidation, thus altering fuel partitioning in a way that may be conducive to body fat gain (Brand-Miller et al., 2007). It has been reported that obesity is associated with changes in CHO and fat metabolism and the most concentrated source...
of energy is efficiently stored as body fat (Frost & Dornhorst, 2000; Brand-Miller et al., 2002). Subjects on a low GI diet have been demonstrated to have significant reductions in energy intake and body weight (Dumesnil et al., 2001). This could be due to the fact that lowering the glycaemic index and load of the first meal leads to consumption of less food in the subsequent meal (Jenkins et al., 2002). It has been observed that total fat mass decreased by 500 g after only 5 weeks consumption of a low GI diet compared with a high GI diet of equal energy and macronutrient content (Brand-Miller et al., 2002). A systematic review of six relevant randomised trials indicated that weight loss was greater in overweight and obese people who consumed low GI or GL diets compared to people who consumed high GI or GL foods. Similarly, there was a significant decrease in body mass index in people receiving low GI or GL diets (Thomas et al., 2009).

1.6.8.2 GI and heart disease

High GI diets have also been shown to have an association with the development of CHD in women in large prospective intervention studies (Jarvi et al., 1999; Beulens et al., 2007). A low GI diet may have a positive effect on those with heart disease with favourable effects on serum cholesterol concentration and low density lipoprotein cholesterol (Jarvi et al., 1999). Jenkins et al. (1987) found that the total serum cholesterol levels were significantly lower in individuals consuming a low GI diet as compared to the high GI group. The serum cholesterol reduction was 15 % (p<0.01) on the low GI diet. Furthermore, the low GI diet has been associated with higher levels of high density lipoprotein (HDL) cholesterol (>60 mg/dl) and a
decreased risk of developing cardiovascular disease (Jenkins et al., 2004). Frost et al. (1994) observed a significant fall in serum triglycerides and total cholesterol level in type 2 diabetic patients after consuming a low GI diet for 3 months, but the reduction in low density lipoprotein (LDL) cholesterol was not significant. However, there was no evidence of a difference between the effect of a low GI and a high GI on HDL or LDL cholesterol but there was a reduction in total cholesterol with the low GI compared to high GI diet (Kelly et al., 2004). In addition, a study of 45 healthy overweight adults demonstrated a 10% decrease in LDL cholesterol and a tendency to reduce total cholesterol with the consumption of a low GI diet compared with a high GI diet (Sloth et al., 2004). Also, the association between serum lipoprotein levels, dietary GI, and fat intake has been examined and showed that the GI of the diet is a stronger predictor than dietary fat intake on serum HDL cholesterol concentration (Frost et al., 1999; Ford & Liu, 2001; Brand-Miller, 2003; Ebbeling et al., 2005; Livesey et al., 2008). It has been found that cardiovascular morbidity decreased by 2 and 3% in men and women respectively with each a 0.026 mmol/l increase in HDL cholesterol concentration. There was an improvement in CHD morbidity with low GI diets due to improve insulin sensitivity by suppression of release of non esterified fatty acids (Frost et al., 1999; Brand-Miller, 2003; Livesey et al., 2008). A systematic review for 21 randomised controlled trials in the effect of low GI diets on risk factors for CHD detected that the low GI diets have an effect on LDL, HDL and triglycerides, however; there was slight reduction of total cholesterol with low GI diets (Kelly et al., 2008).
1.6.8.3 GI and diabetes mellitus (DM)

The prevalence of diabetes in all age groups has continued to rise over the past decades (Wild et al., 2004). The major objective of the clinical management of diabetes is to control metabolic abnormalities in order to prevent acute hyperglycaemia (the presence of an abnormally high concentration of glucose in the blood) and hypoglycaemia (abnormally low blood glucose usually resulting from excessive insulin or a poor diet) as well as long term complications of diabetes including cardiovascular disease (CVD), retinopathy, neuropathy and nephropathy (ADA, 2008).

It has been observed that a strong positive association between the consumption of high GI of foods and developing risk of T2DM (Schulze et al., 2004; Salmeron et al., 1997; Sluijs et al., 2010). These findings have supported the importance of the quality and quantity of CHO consumed in preventing T2DM (Schulze et al., 2004; Sluijs et al., 2010). However, this association has not been observed in the Iowa Women’s Health Study (Meyer et al., 2000), the Atherosclerosis Risk in Communities (ARIC) study (Stevens et al., 2002) and Whitehall II study (Mosdol et al., 2007). The failure to detect an association might have been due to the limitations of the dietary assessments and the diagnosis of diabetes (Schulze et al., 2004; Meyer et al., 2000; Stevens et al., 2002). Nevertheless, all these studies have shown a significant association between cereal fibre and decreased risk of diabetes and supported the protective role for dietary fibre in the development of diabetes. It has been suggested that grains should be consumed in minimally
refined form to reduce the incidence of T2DM (Meyer et al., 2000; Stevens et al., 2002; Sluijs et al., 2010).

High GI foods may alter the risk of T2DM owing to the production of higher blood postprandial glucose concentrations and a greater insulin demand than do low GI foods. It is possible that chronically increased insulin demand may directly increase insulin resistance (an inability of a cell to respond to insulin) (Wilkin et al., 2002; Schulze et al., 2004). Indeed, it is now widely appreciated that insulin resistance precedes the development of T2DM (Yip et al., 1998). In contrast, low GI diets have been linked with improvement in metabolic control and decreased risk of development of T2DM due to the fact that they are slowly digested and absorbed, producing a gradual rise in blood glucose and insulin levels (Jenkins et al., 2004; Kalergis et al., 2005). Low GI diet may also improve insulin sensitivity (the ability of insulin to help move glucose from the bloodstream into the cell) by minimising fluctuation in blood glucose levels and reducing the secretion of insulin over the day (Thomas & Elliott, 2010). Replacing a high GI diet with a low GI diet might reduce frequent and rapid rise in blood glucose levels and also, an increase the body's sensitivity to insulin. Studies on the postprandial glucose response to CHO-containing foods have demonstrated that the low GI foods decrease the insulin and glucose response compared with high GI foods, suggesting an increase in insulin sensitivity in normal volunteers and in obese insulin resistant subjects (Frost et al., 1996). It has been found that a reduction of the fasting plasma glucose concentration in subjects on low GI diets was significantly more pronounced than in those on the high GI diet (Jarvi et al.,
1999). Also, Jenkins et al. (1987) found that the final values of glycosylated serum proteins (fructosamine) has shown a 7 % fall (p<0.01) on the low GI diet and a non significant fall of 2.2 % on the high GI diet. Similarly, Frost et al. (1994) have found that a significant fall in fasting blood glucose and fructosamine occurred in type 2 diabetic patients treated only by giving advice to lower the GI of the CHO in their diet for 3 months. These results demonstrated greater improvements in glucose control with a low GI diet as compared to a high GI diet occurred. In addition, the effect of consumption of low GI diet was also shown an improvement in glycated haemoglobin (HbA1c). A study with 104 children over a 12-month period in Australia, used either a traditional measured CHO exchange diet or a low GI diet, found that significantly better HbA1c levels in the low GI diet group (Gilbertson et al., 2001). Similar results were found in a study on subjects with diabetes and the reduction varied between 8 to 9 % in subjects consuming a low GI diet (Buyken et al., 2001; Heilbronn et al., 2002) compared to 4.6 % reduction on the high GI diet (Heilbronn et al., 2002). A systematic review indicates that there was a significant improvement in glycaemic control in people with DM receiving low GI diets in comparison to high GI diets (Thomas et al., 2009). Low GI diets reduce HbA1c level by 0.4 % which is clinically significant and comparable to the reduction achieved by medication for T2DM. It is evident that each 1 % decrease in HbA1c was associated with 21 % reduction in the risk of diabetic complications (Thomas & Elliott, 2010).

1 Serum albumin that is used to identify the fluctuation in plasma glucose concentration over the preceding 2 weeks.
2 Serum haemoglobin is reversibly glycated depending on prevailing glycaemia and thus can be used as an index of plasma glucose concentration in the preceding 6-10 weeks.
Indeed, the joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Consultation Committee advocated the use of low GI diets in the management of individuals at risk of developing diabetes and diabetes-related complications, including CVD (FAO/WHO, 1998). However, the great health benefit of the low GI foods remains to be fully elucidated.

1.6.9 GI and insulinaemic index

Insulinaemic index (II) is defined as the area under the curve of the insulin responses to a CHO-containing food compared to a reference food (either a specific glucose dose or a specific amount of white bread). The correlation between glycaemic and insulinaemic responses has been studied in several intervention trials (Bornet et al., 1987; Chew et al., 1988; Mehió et al., 1997; Liu, 2002; Ciok et al., 2004) and it has been found that the II was significantly ($r = 0.94; p = 0.005$) related to the GI (Wolever et al., 2006). It is evident that low GI diet requires lower insulin demand which plays an important role in the treatment of diseases related to insulin resistance (Frost et al., 1999; Bjorck et al., 2000). However, the ranking order of the II did not predict the ranking order for the GI (Mehio et al., 1997). Moreover, the linear correlation between GI and II does not apply to all foods. It has been observed that the II value of milk and meals with added milk was 3-6 folds higher than expected from their corresponding GI (Ostman et al., 2001). Indeed, certain foods produce higher II values than that expected from their GI, however; there is exception with some foods depending on the capacity of food proteins to stimulate insulin
1.7 Rice

1.7.1 Definition

Rice is an annual herbaceous plant. When rice grain is covered with lemma (outer envelopes), it is called rough rice. In the processing of rough rice, the lemma is husked and the product is called brown rice or hulled rice. However, the milled rice (white rice) is a result of further processing by bleaching and removing husk, bran, and germ of rice. The bleaching process causes a deficiency and (or) loss of some of the essential nutritional elements e.g. vitamins and minerals. It has been shown that brown rice contains more fat, fibre and minerals compared with milled rice (Juliano, 1985; 2005).

1.7.2 Diversity of rice

Rice is from the genus *Oryza* and there are two species of which are cultivated, *Oryza sativa* and *Oryza glaberrima* (Juliano, 1985). The original place of growth of *Oryza sativa* was believed to be in Southeast Asia (India, Vietnam, Burma) then the cultivation moved into North Africa and Southern Europe and America. *Oryza glaberrima* originated in West Africa. *Oryza sativa* can be divided into three subspecies, *indica* which is characterized with non sticky and long-grains variety. The second subspecies is known as *japonica* which was originally grown in Japan, North Korea and China, but its cultivation has also moved to the United States, Italy and Spain. It is a sticky,
short grain variety. A third subspecies was identified as a broad grain variety and is called javanica (Juliano, 1985; Kennedy & Burlingame, 2003). Countries in Asia are heavily reliant upon rice and the three top producing countries in 2008 were China, India, and Indonesia (Kennedy & Burlingame, 2003; FAOSTAT, 2009).

In Saudi Arabia, there is a type of rice called Hassawi rice from the _indica_ variety grown and consumed traditionally in Al-Hassa area of Saudi Arabia. More details on this type of rice are mentioned in Chapter 04.

**1.7.3 GI of rice varieties**

Rice is an important staple food in many parts of the world and accounts for 21% of global energy supply (Kennedy & Burlingame, 2003). Varieties of rice are commercially and locally available for consumption in different ways of preparation and cooking. The differences in the varieties of rice may be accounted to be the reason behind the discrepancies in the glycaemic and insulinaemic responses. Therefore, it is difficult to classify rice as a high or low GI food. However, the GI of most of the rice varieties has fallen within low to medium values (Ranawana et al., 2009; Foster-Powell et al., 2002).

The Bangladeshi rice (_indica_ variety) was found to have a low GI with value of 37 (Foster-Powell et al., 2002). Recent study has found that the GI values for three varieties of Bangladeshi rice ranged from 43 to 54 (Fatema et al., 2010). However, the GI value for eleven Japanese processed rice ranged from 46 to 89 and the lowest GI was found for Glutinous rice ball and the highest was for Rice cracker. Sushi was found to be low GI with GI value of 53 (Sugiyama et al., 2003).
The variability in the GI values of rice could be attributed to many factors including amylose content and processing of rice. Most rice starches are composed of about 15-20 % amylose and 80-85 % amylopectin. Rice varieties that contain a higher proportion of amylose (25-33 %) have been shown to have a slower rate of digestion and produce lower glycaemic and insulinaemic responses than those with a low (< 20 %) proportion of amylose (Foster-Powell et al., 2002; Venn & Mann, 2004; Juliano, 2005; Benmoussa et al., 2007). However, amylose content alone is not a good predictor of glycaemic response to rice starch. Other factor that can influence the glycaemic response to rice is parboiling process. Parboiled rice has shown to elicit lower plasma glucose and insulin responses compared with non-parboiled counterpart (Larsen et al., 2000; Venn & Mann, 2004; Ranawana et al., 2009). However, the effect of parboiling on the glycaemic response to rice depends on the severity of parboiling process. It has observed that the GI value for pressure parboiled rice was significantly lower ($p < 0.05$) that the GI of non-parboiled rice (Larsen et al., 2000).

The GI value of rice has shown to be correlated with II value, but the latter was usually lower on the relative scale than was the GI value (Brand-Miller et al., 1992).
1.8 Research aims

Most of the published data on GI are based upon analyses carried out in Australia, Europe, Canada and the USA. As such there is a lack of knowledge about the GI of some traditional Saudi foods. Therefore, our first aim was to understand more about the traditional foods grown in Saudi Arabia (rice and dates) and to investigate their GI.

In addition, although there is a reasonable amount of data on the RAG and SAG values of UK foods, no data is available about these indices of Saudi rice (Hassawi rice), dates and other Saudi foods. It was thus important to generate information for these foods in terms of their CHO bioavailability in vitro. This led us to ask the second question: What are the RAG and SAG values for these foods?

To follow on from this we were interested to determine whether the SAG values can help to explain the metabolic difference between foods with similar RAG and GI values. Therefore, it was essential to establish the methodology of RAG and SAG measurements during the preliminary stages of this project. Then, the GI and II of some of Saudi foods were measured using established FAO/WHO protocols. Once the GI, RAG and SAG of some Saudi and UK foods were determined, those foods with high and low SAG values were selected to create meals to be tested in terms of their metabolic effects.

As traditional practice formed a central theme of this thesis we also aimed to assess the effects of traditional Arabic coffee consumption with dates on glucose and insulin profiles when compared to consuming dates with water.
The hypotheses of this thesis were that:

1) Traditional Saudi Arabian foods (e.g. Hassawi rice) produce more favourable metabolic profiles (i.e. glucose & insulin) compared to commonly consumed Western variants (e.g. Uncle Ben’s rice).

2) The metabolic effects of different dietary CHO rich meals can be further understood via their RAG and SAG values.

3) RAG and SAG values can be used as predictors of GI for both UK and Saudi foods.

4) Traditional Saudi practices, such as the consumption of coffee with dates can be viewed as healthy from a metabolic viewpoint.

The general objectives of the studies were:

1) To measure the nutritional composition, GI and II for Hassawi rice and Uncle Ben’s rice.

2) To establish the methodology of rapidly available glucose (RAG) and slowly available glucose (SAG) measurement in a system that simulates CHO digestion and to measure RAG and SAG for some Saudi and UK foods.

3) To study the metabolic impact of two GI and macronutrient identical meals, with different SAG content on the metabolic profile (glucose, insulin and lipids levels).

4) To determine the effect of Arabic coffee consumption with dates on blood glucose and insulin levels versus dates with water.
CHAPTER 2
2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Apparatus and Equipment

1. Accu-Check, Brighton, East Sussex, UK:
   Softclix Pro Lancets.

2. Alpha Laboratories, Eastleigh, UK:
   Apex Plus Screw Cap Lilac; Apex Plus Screw Cap yellow; Microtube (0.5 ml);
   plastic pipette (5 ml).

3. Becton Dickinson and Company (BD), Oxford, UK:
   Syringes (1 ml, 5 ml, and 20 ml).

4. Carbolite Furnaces & Oven, Parsons Lane, Hope, England, UK:
   Muffle Furnace, 525 ± 5 °C.

5. Dionex UK Ltd, Camberley, UK:
   Dionex HPLC system, fitted with PA100nCarbopack ion-exchange column
   and ED40 amperometric detector.

6. Direct Medical Supplies Ltd, Blacknest Business Park, UK:
   SST vacutainer tubes (6 ml); Cannula (Y-CAN 19 G).

7. Fisher Scientific Ltd, Loughborough, Leicestershire, UK:
   Pipette tips (200 µl, 1 ml, and 5 ml); plastic tubes (15 ml); Falcon™ tubes (50
   ml); conical flask (250 ml); pyrex beakers (10 ml, 25 ml, 400 ml, 500 ml, 1000
   ml, 2000 ml); 1.5 ml plastic cuvettes; filtering flask, heavy-walled, with 1-l side
   arm; fritted crucible (60 ml), fritted disk, Pyrex, pore size (coarse), ASTM 40-
   60 µm; extraction thimbles (cellulose, size 33 x 80 mm).

8. FOSS UK Ltd, Warrington, UK:
FOSS Soxtec® 2055 Manual Fat Extraction System; FOSS Kjeltec™ 2200 Systems.

9. Grant Instruments, Cambridge, Ltd, UK:
Boiling water-bath (Grant Y22); Shaking water-bath, with maximum shaking capacity of not less than 160 strokes per minute and the stroke length of 35 mm, temperature range 35-70°C. (Grant SS40-D); Water-bath, static, with temperature range 35-90°C (Grant JB Aqua 18).

10. Instrumentation Laboratory, Birchwood Science Park, Warrington, UK:
ILab 650 clinical chemistry system.

11. Invitron Limited, Monmouth NP25 3SR, UK:
Immuno-chemiluminometric insulin kit (Lyophilised recombinant insulin in a serum matrix; Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide; Microtitre plate coated with a specific monoclonal antibody; phosphate buffered saline containing a detergent and 0.09% sodium azide).

12. Stuart Scientific Ltd, Staffordshire, UK:
Magnetic Stirrer (SMS)

13. Mettler Toledo Ltd, Leicestershire, UK:
Digital scale, sensitivity ± 0.1 mg (AL 204); pH meter (SevenEasy).

14. Konton Ltd, UK:
Uvikon 860 Spectrophotometer.

15. SARSTEDT, Leicester, UK:
300 µl fluoride oxalate microvette tubes; 300 µl plasma plain microvette tubes

16. Sigma Chemical Company Ltd, Poole, UK:
Centrifuge 6K10;
17. Teklab Ltd, Durham, UK:
Fluoride oxalate polystyrene tubes (2 ml); Dipotassium EDTA polystyrene tubes (5 ml).
18. Vacuum pump, LABOPORT.
19. Fisons Ltd, Leicestershire, UK:
Vortex-mixer (WhirliMixer)
20. YSI Life Sciences, Hampshire, UK:
YSI 2300 STAT Plus glucose analyzer; YSI analyzer printer paper.

2.1.2 Chemicals and Reagents
1. Englyst Carbohydrate Services Ltd, Southampton, UK:
Amyloglucosidase (from Aspergillus niger, 10000 U/ml in glycerol; 1 unit releases 1 µmol glucose pH 4.5, 55 °C).
2. Fisher Scientific, Loughborough, Leicestershire, UK (all chemicals were analytical grade unless stated otherwise):
Acetone (Laboratory grade); calcium chloride dihydrate (CaCl₂·2H₂O); D-glucose anhydrous; dimethyl sulphoxide (DMSO); disodium hydrogen orthophosphate anhydrous (Na₂HPO₄); ethanol (99 %, 95 %, and 78 %); glacial acetic acid; methyl red 0.01 % (laboratory grade); petroleum ether 60-80 °C (laboratory grade); potassium chloride (KCl); potassium hydroxide; hydrochloric acid (laboratory grade); invertase (general purpose grade, from yeast); sodium chloride (NaCl); sodium dihydrogen orthophosphate (NaH₂PO₄·2H₂O); sodium hydroxide (NaOH); sodium acetate (trihydrate).
3. Instrumentation Laboratory Company – Lexington, USA:
Glucose oxidase (GOD)/ Peroxidase (POD), 4-aminoantipyrine, phenol, buffer and sodium azide.

4. Megazyme International Ireland Ltd, Bray Business Park, Ireland, UK:
\( \alpha \)-amylase (from *Bacillus amyloliquefaciens*, 3000 U/ml on Ceralpha reagent at pH 5.0; amyloglucosidase (from *Aspergillus niger*, 200 U/ml on \( p \)-nitrophenyl \( \beta \)-maltoside; protease (50 mg/ml); concanavalin A; starch reference sample from maize; D-glucose standard solution (1.0 mg/ml) in 0.2% (w/v) benzoic acid; glucose oxidase, peroxidase (GOPOD) and 4-aminoantipyrine.

5. Randox Laboratories Ltd, County Antrim, UK:
Glucose GOD/PAP (glucose oxidase/phenol and 4-amino-antipyrine) test kit; non-esterified fatty acid (NEFA) kit; triacylglycerides (TAG) kit; chemistry control level 1 and 2.

6. Sigma Chemical Company Ltd, Poole, UK:
\( \alpha \)-amylase, heat-stable (from *Bacillus amyloliquefaciens*); arabinose (≥98 %); benzoic acid (≥99.5 %, ACS (American Chemical Society) grade; fructose; glucose; guar gum powder; kjeldahl catalyst tablets selenium; pancreatin (from porcine pancreas); pepsin powder (from porcine gastric mucosa, 800-2500 U/mg); glucose oxidase diagnostic kit; potato starch; anhydrous sodium acetate (≥99 %); calcium chloride (CaCl\(_2\).6H\(_2\)O); magnesium chloride (MgCl\(_2\).6H\(_2\)O); manganese chloride (MnCl\(_2\).4H\(_2\)O); folin-ciocalteu’s reagent.

### 2.1.3 Solvents and Buffers

1. Carrez A reagent:
Zinc acetate.\( _2\) \( H_2\)O (21.9 g) was dissolved in 100 ml deionised water.
2. Carrez B reagent:
Potassium ferrocyanide trihydrate (10.6 g) was dissolved in 100 ml deionised water.

3. Concentrated Concanavalin A (Con A) Solvent (600 mM, pH 6.4 sodium acetate buffer). This solution was prepared by dissolving 49.2 g of anhydrous sodium acetate, 175.5 g of sodium chloride, 0.5 g of CaCl₂·6H₂O, 0.7 g of MgCl₂·6H₂O and 0.7 g of MnCl₂·4H₂O (Sigma cat. no. M 3634) in 900 ml of distilled water. The pH of the solution was then adjusted to pH 6.4 by dropwise addition of glacial acetic acid and then the volume was adjusted to 1 l with distilled water. This solution was stable for 2 weeks at 4 °C.

4. Enzyme preparation mixture (prepared immediately prior to use). Three g of pancreatin was added to each centrifuge tube (n=6) followed by 20 ml of distilled water and suspended using a vortex-mixer. A magnetic stirring bar was then added and the suspensions were mixed for 10 min. The suspensions were then centrifuged at 1500 × g for 10 min and 12.5 ml of the cloudy supernatant was removed from each centrifuge tube and these supernatants were combined in a beaker (75 ml total). Amyloglucosidase solution (3 ml) and invertase (5 ml) were then added and the solution was mixed well by inversion and used within 3 h.

5. Glucose standard solution. Five g of glucose was weighed to the nearest 0.1 mg. Sodium acetate buffer (200 ml) was added to dissolve the glucose to give a 25 mg/ml glucose solution.

6. Hydrochloric acid solution, 0.325 N. 325 ml of HCl (1.0 N) was added to a 1 volumetric flask and water was added to the 1 l mark with thorough mixing.
7. Internal standard (arabinose) solution. Arabinose (40 g) was weighed out accurately and dissolved in water (200 ml). Then 500 ml of a saturated benzoic acid solution was added and the solution was made up to 1 l with water.

8. Pepsin-guar gum solution. One g of pepsin powder was added to 200 ml of 0.05 mol/l HCl solution and mixed in a beaker using a magnetic stirring bar. Just before use, 1 g of guar gum was added and the solution was mixed well. The pepsin-guar gum solution was always prepared immediately before use.

9. Phosphate buffer, 0.08 M, pH 6.0. Sodium phosphate (dihydrate) (12.693 g) was weighed out accurately and added to approximately 700 ml distilled water. The pH of the solution was checked and adjusted if necessary and then the solution was made to 1 l with distilled water.

10. Sodium acetate buffer, 0.1 mol/l, pH 5.2. Sodium acetate trihydrate (13.6 g) was dissolved in 500 ml of water, then 250 ml of a saturated benzoic acid solution was added and the solution was made up to 1 l with water after adjustment to pH 5.2 with 0.1 mol/l acetic acid. To stabilize and activate the hydrolytic enzymes, 4 ml of 1 mol/l calcium chloride was added to 1 l of this buffer.

11. Sodium Acetate Buffer (100 mM, pH 4.5). 5.9 ml of glacial acetic acid (density, 1.05 g/ml) was added to 900 ml of distilled water. The pH was adjusted to pH 4.5 by the addition of 1 M sodium hydroxide solution (approx. 30 ml was normally required). Sodium azide (0.2 g) was added and the volume was adjusted to 1 l. This solution was stable for > 2 years at room temperature.
12. Sodium hydroxide solution, 0.275 N. NaOH (11.0 g) was dissolved in approximately 700 ml distilled water, using appropriate handling precautions, in a 1 l volumetric flask. The solution was allowed to cool and then water was added to bring the volume up to 1 l.

13. Stock sugar mixture. Glucose (50 g) and fructose (25 g) were dissolved in water, then 500 ml of saturated benzoic acid was added and the solution was made up to 1 l with distilled water.

14. YSI 2357 buffer concentrate kit (phosphate buffer). It was made by dissolving the package content into 500 ml distilled water.

2.2 Methods

See section 1.5.3 for background on RAG and SAG.

2.2.1 Procedures for RAG and SAG measurement of reference standard foods

All procedures concerning the measurement of the CHO fractions were carried out using Englyst’s technique (Englyst et al., 2000). Three reference standard foods (wheat flour, potato starch and corn flakes) were included in every batch of samples. The CVs $G_{20}$ and $G_{120}$ were displayed in Table 2.1.
Table 2.1 The mean, standard deviation and CVs for three standard reference foods used to determine the inter-assay variation of RAG and SAG measurements.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mean (g/100 g)</th>
<th>SD</th>
<th>% CVs inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>G20</td>
<td>G120</td>
<td>G20</td>
<td>G120</td>
</tr>
<tr>
<td>Reference 1</td>
<td>38</td>
<td>80</td>
<td>0.68</td>
</tr>
<tr>
<td>Reference 2</td>
<td>8</td>
<td>25</td>
<td>0.99</td>
</tr>
<tr>
<td>Reference 3</td>
<td>79</td>
<td>83</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Reference 1 = Wheat flour; Reference 2 = Potato starch and Reference 3 = Corn flakes.

2.2.1.1 The colorimetric approach

Guar gum powder (50 mg) was weighed out into each of three 50 ml polypropylene centrifuge tubes and five glass balls were added to each tube. Glucose standard (20 ml) was added to two of these tubes and acetate buffer (20 ml) was added to the third tube which was treated as a blank. These three tubes were treated exactly the same as the samples.

An appropriate amount of sample (0.6–3 g)\(^1\) was weighed in duplicate (n=6) into 50 ml polypropylene centrifuge tubes. Ten ml of the freshly prepared pepsin-guar gum solution was added into each sample tube. The tubes were vortex-mixed and placed in a static water-bath at 37 °C for 30 min. After the incubation, the samples were removed from the water-bath and

\(^1\) See Appendix 1.
five glass balls and 10 ml of 0.25 mol l\(^{-1}\) sodium acetate were added to each tube.

*Figure 2.1 In vitro hydrolysis of starch.*

The sample tubes, standards and blank were placed in the water-bath at 37 °C to equilibrate for 10 min. Then one sample tube was removed from the 37 °C water-bath and 5 ml of the enzyme mixture (prepared as described in section 2.1.3) was added. The contents were capped and mixed gently by
inversion. The tube was secured horizontally in the 37 °C shaking water-bath and the shaking action of the water-bath was started, this was time zero for the incubation.

The addition of the enzyme mixture was repeated for the rest of the sample tubes, at exactly 1 min intervals, and the samples were placed sequentially into the shaking water-bath. At exactly 20 min and 120 min of the incubation, 0.5 ml of each sample was transferred into 20 ml of 66 % ethanol and vortex-mixed; these samples related to the $G_{20}$ and $G_{120}$ portions, respectively. Then the glucose released from the starch in these samples was analyzed colorimetrically after appropriate treatment using a spectrophotometer (Manual method) and using a glucose assay system on the Instrumentation Laboratory analyser (auto-analyser method).

2.2.1.1.1 Determination of glucose spectrophotometrically using glucose oxidase

The samples were centrifuged at $1000 \times g$ for 10 min before the determination of the glucose content. One hundred $\mu$l of the enzyme blank, samples ($G_{20}$ and $G_{120}$) and standards were added to test tubes containing 2 ml of the glucose oxidase reagent and the absorbance of the standards and samples was measured at 510 nm against the reagent blank after 20 min incubation at 37 °C.

The assay used was based on a colorimetric enzymatic procedure. In this method the glucose present in the samples is oxidized by glucose oxidase to produce gluconic acid and hydrogen peroxide. The hydrogen peroxide then reacts with o-dianisidine in the presence of peroxidase to form
a coloured product. The quantity of this product is proportional to the original glucose concentration. The assay principle is demonstrated by the following reactions:

\[
\text{Glucose} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{reduced o-dianisidine (colourless)} \xrightarrow{\text{Peroxidase}} \text{oxidized o-dianisidine (colour)}
\]

The glucose concentration (grams per 100 g of sample) is given by following equation:

\[
\text{Glucose} (\%) = \frac{A(t) \times V \times C \times 100}{A(s) \times W}
\]

where \(A(t)\) is the absorbance of the test solution, \(V\) is the total volume of the test solution\(^2\) (milliliters), \(C\) is the concentration\(^3\) (milligrams per milliliter) of the standard used, \(A(s)\) is the absorbance of the standard used and \(W\) is the weight (milligrams) of sample taken for analysis.

2.2.1.1. 2 Glucose determination using Auto-Analyser (ILab 650)

A 1 ml aliquot of the enzyme blank, samples and standards were added into skirted tubes and the absorbance of the blank, standards and samples was measured at 510 nm. The assay was based on a colorimetric enzymatic procedure in which the sample glucose is oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide produced reacts with 4-aminoantipyrine in the presence of peroxidase to form a red

\(^2\) \(V = 20\) ml (Englyst et al., 2000)  
\(^3\) \(C = 25\) mg (Englyst et al., 2000)
quinoneimine dye, which is proportional to the original glucose concentration in the sample. The assay principle is demonstrated in the following reactions:

\[
\text{Glucose} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{red quinoneimine} + 4\text{H}_2\text{O}
\]

Specific quality controls (QCs) were measured at the beginning and at the end of each run. The assay results were only deemed acceptable if the QCs were within the accepted limits as determined by the QC provided by the manufacturer (See Section 2.2.3.3.2). The glucose value was given in mmol/l and then converted into mg/100 by multiplying by 18.

### 2.2.1.2 The HPLC approach

Two tubes, one containing 4 ml of stock sugar solution added, were included as enzyme blanks (the other tube contained nothing). Then 5 ml of internal standard were added to both the sample and blank tubes.

An appropriate amount of sample (0.6–3 g)\(^4\) was weighed in duplicate (n=2) into 50 ml polypropylene centrifuge tubes. Ten ml of the freshly prepared pepsin-guar gum solution was added to each tube, vortex-mixed and placed in a water-bath at 37 °C for 30 min. After 30 min the tubes were removed from the water-bath. Five glass balls and 5 ml of 0.5 mol l\(^{-1}\) sodium acetate were added to each tube and the tubes were placed back in the water-bath at 37 °C to equilibrate for 10 min. Then one sample tube was

\(^4\) See Appendix 1.
removed from the 37 °C water-bath and 5 ml of the enzyme mixture was added. The contents were capped and mixed gently by inversion. The tube was secured horizontally in the 37 °C shaking water-bath and the shaking action of the water-bath was started. This was time zero for the incubation.

The addition of enzyme mixture was repeated for the rest of the sample tubes, at 1-min intervals to aid in the accurate timing of the procedure, and they were placed into the shaking water-bath. At exactly 20 min and 120 min after the start of the incubation, a 0.2 ml aliquot of each sample was transferred into 4 ml of absolute ethanol and vortex-mixed; these were the G\textsubscript{20} and G\textsubscript{120} portions, respectively. Glucose release was measured using the HPLC system described below.

2.2.1.2.1 Determination of glucose using HPLC-ECD

Two sugar standards were prepared: Standard 1, 1 ml of stock sugar mixture, 19 ml of water and 5 ml of internal standard; Standard 2, 10 ml of stock sugar mixture, 10 ml of water and 5 ml of internal standard. A 0.2 ml from each standard was transferred into a tube containing 4 ml of absolute ethanol. Before HPLC analysis, standards and samples were centrifuged for 10 min at 1000 \( \times \) g and diluted with Milli-Q water, 10-fold.

A Dionex DX100 HPLC system, fitted with PA100nCarbopack ion-exchange column and ED40 amperometric detector was used for the analysis of glucose. Samples were injected through a 25 \( \mu \)l loop, and eluted at a flow rate of 1 ml/min using 100 mM NaOH as mobile phase. The peak areas for glucose (G) and arabinose (A) were used to get peak area ratio (PAR) which was used to calculate the glucose concentration in the samples.
2.2.1.3 Total glucose (TG) measurement

The remainder of the samples' solution after collection of the \( G_{120} \) portion (as described in Section 2.2.1.1) was vortex-mixed vigorously and placed in a test tube rack in a boiling water-bath for 30 min. Then the tubes were removed from the bath and vortex-mixed and placed in ice-water until thoroughly chilled (for 45 min). Ten ml of 7 mol l\(^{-1}\) KOH were added and the tubes were immersed horizontally in a shaking water-bath containing ice-water for 30 min.

After 30 min, the samples were removed from the water-bath and 1 ml of the contents was immediately transferred into a 50-ml tube containing 10 ml of 0.5-mol l\(^{-1}\) acetic acid and mixed well. Amyloglucosidase solution (0.2 ml) was added to each sample, and then each of the tubes was placed in a water-bath at 70 °C.

After 30 min, the tubes were transferred to a boiling water-bath for 10 min. The tubes were cooled to room temperature and 40 ml of Milli-Q water was added; these samples contained the TG portion which was measured using the glucose oxidase method mentioned in section 2.2.1.1.1.

2.2.1.4 Free sugar glucose (FSG) measurement

Glucose standard (25 ml) was added to two 50 ml tubes and sodium acetate buffer (25 ml) was added into a third tube (blank). An appropriate amount of each sample was weighed into 50 ml centrifuge tubes and five glass balls were added to each. 25 ml of 0.1 mol l\(^{-1}\) sodium acetate buffer were added into each tube, and vortex-mixed vigorously. The tubes were then placed into a boiling water-bath for 30 min.
After 30 min the tubes were removed from the boiling water-bath, vortex-mixed vigorously and cooled to 37 °C. Then 0.2 ml of invertase solution was added to each tube and each tube was immersed horizontally in the shaking water-bath at 37°C and incubated for 30 min. After incubation, the tubes were vortex-mixed vigorously and 1 ml of each sample, standard solution and blank were transferred into separate test tubes containing 2 ml of absolute ethanol and vortex-mixed.

The tubes were centrifuged at 1000 × g for 10 min, then 1 ml of the supernatant was transferred into 5 ml of Milli-Q water and mixed well by inversion. (For the standard, 1 ml of supernatant was transferred into 20 ml of water). These samples contained the FSG portion and were measured using the glucose oxidase method mentioned in section 2.2.1.1.1.

2.2.1.5 Calculations

2.2.1.5.1 RAG and SAG values

Values for RAG and SAG were calculated from the measured $G_{20}$ and $G_{120}$ as the following:

$$\text{RAG (g)} = G_{20}$$

$$\text{SAG (g)} = G_{120} - G_{20}$$

2.2.1.5.2 Total starch (TS)

Values for TS were calculated from the measured TG and FSG as the following:

$$\text{TS (g)} = (TG - FSG) \times 0.9$$
CHAPTER 2

2.2.2 Food composition analysis

All procedures of the proximate composition for Hassawi rice, Uncle Ben’s rice and Dates were determined according to the AOAC (1995) methods. These procedures are described below.

2.2.2.1 Moisture content

An appropriate amount of sample (5 g) was weighed in duplicate (n=4) into a moisture dish and placed in an oven set at 100 °C for 24 h. After that, the dishes were moved to the desiccator to cool down and then re-weighed. The percentage moisture content of the samples was calculated as following:

\[ \text{Moisture} \% = \frac{\text{moisture weight}}{\text{sample weight}} \times 100 \]

2.2.2.2 Ash content

An appropriate amount of sample (2 g) was weighed in duplicate (n=4) into a silica dish and placed on the hot plate in the fume cupboard in order for the sample to be charred. After that, the dishes were placed into a muffle furnace at 550 °C and left for 8 h. The silica dishes weight were reported and the percentage of ash content of samples was calculated as following:

\[ \text{Ash} \% = \frac{\text{ash weight}}{\text{sample weight}} \times 100 \]

2.2.2.3 Protein content

2.2.2.3.1 Digestion of samples

An appropriate amount of sample (2 g) was weighed in duplicate (n=4) on filter paper and placed in a Kjeldahl test tube. Two Kjeldahl selenium tablets and 20 ml of sulphuric acid were added. The Kjeldahl test tubes were
loaded on to the digestion block, which was pre-heated to 450 °C and the sample was left to be digested for 3 h. After that, the samples were cooled down to room temperature.

2.2.2.3.2 Distillation of samples

A few drops of 0.4 % (v/v) screened methyl red were added into each of the flasks. Then 70 ml of water and 20 ml of 40 % NaOH were added into digest sample tubes and distilled for 3 min. The ammonia formed was collected in 30 ml of 4 % boric acid in the receiver flask.

2.2.2.3.3 Titration of samples

The receiver solution was titrated with 0.1 M hydrochloric acid until colourless.

2.2.2.3.4 Calculation of nitrogen and protein in the sample

The nitrogen percentage in the sample was calculated as follows:

\[ \text{N \%} = 100 \times \left( \frac{\text{ml of titrant of sample}}{\text{sample weight in gram}} \right) \times 0.0014 \]

Protein \% = N \times \text{factor specific for different products (5.95 for rice and 5.7 for dates (Partindol et al., 2010))}

2.2.2.4 Fat content

The metal collection cup was weighed and marked. An appropriate amount of dried sample (2 g) was weighed in duplicate (n=4) into a marked thimble. A metal ring was placed on the thimble which held the thimble in the condensing chamber. Then 100 ml of petroleum ether (60-80 °C) was added
to the collection cup. The thimble and metal collection cup were then placed into the condensers. The lipids were extracted for 60 min and then the thimble was raised and the solvents were evaporated for 20 min. After evaporation, the thimble and cup were weighed. The lipids percentage in the sample was calculated as follows:

\[
\text{Fat} \% = \left( \frac{\text{fat weight}}{\text{sample weight in g}} \right) \times 100
\]

2.2.2.5 Amylose content

A modified method of Gibson et al. (1997) was followed to determine amylose using a Megazyme kit (Megazyme International Ireland Ltd, Ireland, UK).

2.2.2.5.1 Procedure for amylose determination

Samples (20 ± 0.1 mg) were weighed into a 10 ml screw capped Kimax® sample tube. DMSO (1 ml) was added to the tube while gently stirring it at low speed on a vortex-mixer and the tube contents was heated in a boiling water bath until the sample was completely dispersed. The tube was stored at room temperature for approximately 5 min and 2 ml of 95 % (v/v) ethanol was added. A further 4 ml of ethanol was added and the tube was capped and mixed. The tube was allowed to stand for 15 min.

The tubes were centrifuged at 2000 \( \times g \) for 5 min, the supernatant was discarded and the tubes were drained on tissue paper for 10 min. The pellet was used for determination amylose and starch.

DMSO (2 ml) was added to the starch pellet. The tube was placed in a boiling water bath for 15 min and mixed occasionally. The tubes were
removed from the boiling water bath, and Con A solvent (4 ml) was immediately added and then the tubes' contents were quantitatively transferred (by repeated washing with Con A solvent) to a 25 ml volumetric flask and then diluted to volume with Con A solvent (this was Solution A).

One ml of Solution A was transferred to a 2.0 ml Eppendorf® microfuge tube and a 0.50 ml of Con A solution was added. The tube was capped and gently mixed by repeated inversion. The tube was allowed to stand for 1 h at room temperature and centrifuged at 14000 \( \times g \) for 10 min in a microfuge at room temperature. Then 1 ml of the supernatant was transferred to a 15 ml centrifuge tube and 3 ml of 100 mM sodium acetate buffer (pH 4.5) was added. The contents were mixed and then heated in a boiling water bath for 5 min to denature the Con A.

The tube was placed in a water bath at 40 °C and allowed to equilibrate for 5 min. Then 0.1 ml of amyloglucosidase/\( \alpha \)-amylase enzyme mixture (Megazyme International Ltd, Ireland, UK) was added and incubated at 40 °C for 30 min and then the tube and contents were centrifuged at 2000 \( \times g \) for 5 min. Four ml of GOPOD Reagent was added to 1.0 ml aliquots of the supernatant and incubated at 40 °C for 20 min. The reagent blank and the D-Glucose controls were incubated concurrently. The absorbance of each sample and the D-glucose controls were read at 510 nm against the reagent blank.

For total starch determination, a 0.5 ml aliquot of Solution A was mixed with 4 ml of 100 mM sodium acetate buffer, pH 4.5. Amyloglucosidase/\( \alpha \)-amylase solution (0.1 ml) was added and the mixture was incubated at 40 °C for 10 min. 1.0 ml aliquots (in duplicate) of this
solution were transferred to glass test tubes, 4 ml of GOPOD reagent was added and the mixture was incubated at 40 °C for 20 min.

2.2.2.5.2 Calculation of amylose content (%)

For the calculation of percentage amylose content the equation below was used:

\[
\text{Amylose \%} = \frac{A_{510\text{nm}} (\text{Con A Supernatant}) \times 6.15 \times 100}{A_{510\text{nm}} (\text{TS Aliquot}) \times 9.2} = \frac{\text{Absorbance (Con A Supernatant) \times 66.8}}{\text{Absorbance (Total Starch Aliquot)}}
\]

Where 6.15 and 9.2 are dilution factors for the Con A and Total Starch extracts, respectively.

2.2.2.6 Non-starch polysaccharides content

2.2.2.6.1 Preparation of sample

Samples (1 g ± 0.1 mg) were weighed in duplicate (n=4) into 400 ml tall-form beakers. A 50 ml phosphate buffer (pH 6.0) and 50 μl heat-stable α-amylase solution were added to each beaker. The beaker was covered with aluminium foil and placed in boiling water bath for 15 min. After 15 min the beakers were removed and the solutions were cooled to room temperature. The solutions were adjusted to pH 7.5 ± 0.1 by adding 10 ml 0.275 N NaOH solution.

One hundred μl of protease solution was added to the incubation and the beaker was covered with aluminium foil and incubated at 60 °C with continuous agitation for 30 min. The solution was then cooled to room
temperature and 10 ml of HCl solution (0.325 N) was added to adjust pH to 4.5±0.2. Amyloglucosidase (200 µl) was added, and then each sample was covered with aluminium foil, and incubated 20 min at 60 °C with continuous agitation. Then 280 ml of 95 % (v/v) ethanol was added (preheated to 60 °C) and the solution was allowed to form a precipitate at room temperature for 60 min.

A crucible containing Celite was weighed to nearest 0.1 mg, then wetted and distributed bed of Celite in crucible by using stream of 78 % (v/v) ethanol. Suction was applied to draw Celite onto fritted glass as an even mat. The suction was maintained and the precipitate was quantitatively transferred from the enzyme digest to the crucible. The residue was washed with three 20 ml portions of 78 % (v/v) ethanol, two 10 ml portions of 95 % ethanol, and two 10 ml portions of acetone.

The crucible containing the residue was dried overnight (10 - 12 h) in air oven (105 °C), cooled in a desiccator and weighed to nearest 0.1 mg. The crucible and Celite weights were subtracted to determine the weight of the residue. The residue was analysed from one sample of a set of duplicates for protein, and the second residue sample of duplicate was incinerated for 5 h at 525 °C.

2.2.2.6.2 Calculations
A series of calculations were performed to determine the NSP content.

Uncorrected average blank residue (UABR) = Average blank residue of duplicate blanks in mg

Blank protein residue (BPR) = (UABR x % protein in blank) /100
Blank ash residue (BAR) = UABR x % ash in blank / 100
Corrected blank (CB) = UABR - BPR - BAR
Uncorrected average sample residue (USAR) = Average sample residue of duplicate samples in mg
Sample protein residue (SPR) = USAR x % protein in sample / 100
Sample ash residue (SAR) = USAR x % ash in sample / 100
Corrected sample residue (CSR) = USAR-SPR-SAR-CB
Total dietary fibre (TDF) % = 100 x CSR/mg sample

2.2.3 The clinical studies

2.2.3.1 Participant recruitment

All subjects for the clinical studies were recruited from the postgraduate student and staff population at the University of Surrey by the distribution of both e-mails and posters. The studies design received ethical approval from the University of Surrey Ethics Committee (EC/2004/37/SBMS and EC/2007/78/FHMS).

2.2.3.2 Screening

All subjects were screened prior to each study in order to check that they met the specific study inclusion criteria. The screening involved participant attending one morning, having fasted overnight, and the following procedure was taken:
22.3.2.1 Health and lifestyle questionnaire

This questionnaire included questions regarding the subjects' past and current medical history and their lifestyle (see Appendix 2). Subjects with current or previous medical conditions (for example diabetes, cardiovascular disease) were excluded.

22.3.2.2 Blood glucose and haemoglobin concentration

Fasting blood glucose levels were checked on capillary blood and analysed on a HemoCue® glucose 201+ analyser (HemoCue, Sweden). Haemoglobin concentrations were also checked and analysed on Haemoglobin HemoCue® 201+ (HemoCue, Sweden). Subjects with concentrations outside of standard normal range (> 6 mmol/l for fasting blood glucose and < 13 g/dl for men and < 12 g/dl for women for haemoglobin concentrations) were excluded from the studies.

22.3.2.3 Anthropometric measurements

Height, weight, body mass index (BMI), body fat and blood pressure were taken at the baseline for each study using the standardised procedures as mentioned below. All these measurements were taken by the same investigator within a study in order to reduce inter-operator variation.

22.3.2.3.1 Height

A standard stadiometer (Seca Ltd., Birmingham, UK) was used to measure the height in centimetres. The subjects were asked to remove their
shoes; they then stood straight with their heels together and their back against the stadiometer. Measurements were made to the nearest 0.1 cm.

2.2.3.2.3.2 Weight, body mass index and % body fat

Weight, BMI and % body fat were measured on Tanita scales (Tanita TBF-300, Tanita UK Ltd., Middx, UK) with shoes and socks removal and pockets emptied.

2.2.3.2.3.3 Blood pressure

A diastolic and systolic blood pressure was taken using an automatic blood pressure cuff (Omron MX3 Plus, Omron Healthcare Europe, UK). Subjects were asked to sit and relax for 5 min prior to the measurement. Three readings were taken on a relaxed non-dominant arm. The three readings were then averaged.

2.2.3.3 Biochemical analysis

2.2.3.3.1 Blood samples collection

Two methods of blood sampling were applied, capillary and venous blood sampling. Capillary finger prick blood samples were collected into 300 µl plastic microvette tubes coated with fluoride oxalate, and were immediately centrifuged at 3000 × g for 10 min. The centrifuged plasma was transferred into separate 300 µl plastic plain microvette tubes. The tubes were then frozen and kept in the freezer at -20 °C until analysis within 4 weeks.

Venous blood samples were collected into 5 ml dipotassium EDTA polystyrene tubes for insulin, NEFA and TAG and 2 ml fluoride oxalate
polystyrene tubes for glucose, and were immediately centrifuged at 3000 × g for 10 min. The centrifuged plasma was transferred into separate 500 μl plastic plain microvette tubes. The tubes were then frozen and kept in the freezer at -20 °C until analysis within 4 weeks.

2.2.3.3.2 Analyses of plasma glucose

Two different automated colorimetric methods (YSI 2300 STAT plus analyser, Yellow Springs, UK; ILab analyser, Instrumentation Laboratory, UK) were applied to determine plasma glucose concentrations. In the YSI analyser method, an enzyme specific for glucose is immobilised between two membrane layers; one of polycarbonate and the other of cellulose acetate. Glucose is oxidised with the production of hydrogen peroxide, as glucose oxidase enters the enzyme layer. The hydrogen peroxide produced passes through the membrane of cellulose acetate into a platinum electrode where the hydrogen peroxide is oxidised. The current produced is proportional to the concentration of the glucose.

\[
\text{D-glucose} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{D-glucono-δ-lactone} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 \xrightarrow{\text{Platinum anode}} 2\text{H}^+ + \text{O}_2 + 2\text{e}^-
\]

Twenty four samples were analysed in each run and three quality controls were used in within each run. The mean and standard deviation of the quality controls was used to determine the intra- and inter-assay coefficient of variation (See Tables 2.2).
Table 2.2 The three quality controls (QCs) used to determine the automatic analyzer (YSI 2300 STAT plus) intra- and inter-assay variation.

<table>
<thead>
<tr>
<th>QCs</th>
<th>Mean (mmol/l)</th>
<th>SD</th>
<th>% CV</th>
<th>Mean (mmol/l)</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intra assay</td>
<td></td>
<td></td>
<td>inter assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hassawi rice study</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4.56</td>
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<td>0.22</td>
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<td>0.63</td>
<td>6.33</td>
<td>0.24</td>
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<tr>
<td>High</td>
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<td>0.11</td>
<td>0.44</td>
<td>24.65</td>
<td>0.18</td>
<td>0.73</td>
</tr>
<tr>
<td>Uncle Ben's rice study</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4.37</td>
<td>0.01</td>
<td>0.22</td>
<td>4.40</td>
<td>0.13</td>
<td>2.95</td>
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<tr>
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<tr>
<td>Low</td>
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<td>0.11</td>
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<tr>
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<td>1.12</td>
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<tr>
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<td>0.80</td>
<td>24.83</td>
<td>0.45</td>
<td>1.81</td>
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<tr>
<td>Sponge cake study</td>
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<td></td>
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<tr>
<td>Low</td>
<td>4.54</td>
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<td>4.44</td>
<td>0.08</td>
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<td>6.30</td>
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<tr>
<td>High</td>
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<td>0.44</td>
<td>24.83</td>
<td>0.27</td>
<td>1.08</td>
</tr>
</tbody>
</table>

In the ILab 650 method, the glucose is oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide reacts with 4-aminoantipyrine in the presence of peroxidase to form a red quinoneimine
dye, which is proportional to the original glucose concentration in the sample. The absorbance of the dye was measured bichromatically at 510 nm. The assay principle was demonstrated in previous reactions (See reaction equations in Section 2.2.1.1.2).

Specific quality controls (QCs) were measured at the beginning and end of each run. The inter-assay CVs for the level 1 and level 2 QCs were less than 2% (1.34 and 0.49%, respectively).

2.2.3.3 Analyses of plasma TAG

The enzymatic colorimetric method on the ILab 650 (Instrumentation Laboratory, UK) was used to measure plasma TAG concentrations. TAG was hydrolysed to glycerol and free fatty acids by lipoprotein lipase. Then glycerol kinase present within the assay kit acted upon glycerol and converted it into glycerol-3-phosphate, which was subsequently oxidised by glycerophosphate oxidase. The concentration of the quinoneimine dye generated in the reaction below is proportional to the concentration of TAG in the sample. The absorbance is measured bichromatically at 505/692 nm. The assay principle is based on the following reactions:

\[
\begin{align*}
\text{Triacylglycerides} + 3\text{H}_2\text{O} & \xrightarrow{\text{Lipoprotein Lipase}} \text{glycerol} + \text{fatty acids} \\
\text{Glycerol} + \text{ATP} & \xrightarrow{\text{Glycerol Kinase}} \text{glycerol-3-phosphate} + \text{ADP} \\
\text{Glycerol-3-phosphate} + \text{O}_2 & \xrightarrow{\text{GPO}} \text{dihydroxyacetone-phosphate} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{-chlorophenol} \text{ and } 4\text{-aminoantipyrine} & \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + \text{H}_2\text{O}
\end{align*}
\]
Where GPO = glycerol-3-phosphate oxidase, ATP = Adenosine-5'-triphosphate, ADP = Adenosine-5'-diphosphate. The absorbance was measured bichromatically at 505/692 nm and was proportional to the amount of the TAG present. The QCs were included at the beginning and end of each assay. Inter-assay precision gave CVs for low and high QCs within acceptable limits (0.58 and 0.28 % respectively).

2.2.3.3.4 Analyses of plasma NEFA

The plasma NEFA concentrations were measured by the automated colorimetric method (ILab 650 analyser, Instrumentation Laboratory, UK). In this method, an enzymatic reaction of acyl CoA synthetase and acyl CoA oxidase was involved to produce peroxide; the peroxide then reacts with 4-aminoantipyrine which results in the formation of a purple dye that absorbs at a wavelength of 550 nm. The intensity of the colour is directly proportional to the NEFA concentration. The principle of the assay is given by the following reactions:

\[
\text{NEFA + ATP + CoA} \xrightarrow{\text{Acyl CoA synthetase}} \text{Acyl CoA} + \text{AMP} + \text{PPI}
\]

\[
\text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{Acyl CoA oxidase}} \text{2,3-trans-Enoyl-CoA} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{TOOS} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{purple adduct} + 4\text{H}_2\text{O}
\]
Where TOOS = \(N\)-ethyl-\(N\)-(2-hydroxy-3-sulphopropyl)-\(m\)-toluidine. Two QCs were employed and the CVs for the low and high were found to be within acceptable limits (0.25 and 1.45 % respectively).

2.2.3.3.5 Analyses of plasma insulin

An enzyme linked immunosorbant assay (ELISA) was employed for measuring insulin content of the plasma samples. Samples were left to defrost at room temperature and then centrifuged at 3000 \(x\) \(g\) for 5 min to remove insoluble debris. The quality controls, standards (Invitron Ltd, Monmouth NP25 3SR, UK) and samples were incubated with the labelled antibody solution (Invitron Ltd, Monmouth NP25 3SR, UK) at 37\(^\circ\)C for 2 h and unbound labelled antibodies were removed by the wash buffer (Invitron Ltd, Monmouth NP25 3SR, UK) according to the manufacturer's instructions. The insulin was then measured using the microtitre plate luminometer (Luminescent plate reader Centro LB 960). All readings obtained from the luminometer were multiplied by 6 to convert the units (mU/l) into pmol/l. Two quality controls were employed and their CVs were shown in Table 2.3.
Table 2.3 The two quality controls (QCs) used to determine intra- and inter-assay variations for MLT Insulin Assay.

<table>
<thead>
<tr>
<th></th>
<th>Mean (pmol/l)</th>
<th>SD</th>
<th>% CV</th>
<th>Mean (pmol/l)</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
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<td></td>
<td>intra assay</td>
<td></td>
<td></td>
<td>inter assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hassawi rice study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>QC A</td>
<td>66</td>
<td>3.0</td>
<td>4.5</td>
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<td>QC B</td>
<td>834</td>
<td>24.0</td>
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<td>1035</td>
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<tr>
<td>QC A</td>
<td>64</td>
<td>2.4</td>
<td>3.7</td>
<td>60</td>
<td>5.5</td>
<td>9.1</td>
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<tr>
<td>QC B</td>
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<td>55.7</td>
<td>6.8</td>
<td>809</td>
<td>88</td>
<td>10.8</td>
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<tr>
<td>Dates study</td>
<td></td>
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<tr>
<td>QC A</td>
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<td>2.4</td>
<td>3.1</td>
<td>81</td>
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<td>6.6</td>
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<tr>
<td>QC B</td>
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<td>14.6</td>
<td>1.5</td>
<td>905</td>
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<td>QC A</td>
<td>74</td>
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<td>QC B</td>
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<td>20.8</td>
<td>2.6</td>
<td>826.6</td>
<td>54.6</td>
<td>6.6</td>
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</tbody>
</table>

2.2.4 Calculation and statistical analysis

The incremental area under the glucose and insulin curves (iAUC) for the reference glucose drink, Hassawi rice, Uncle Ben’s rice, dates and dates with Arabic coffee was calculated as recommended by WHO (1998). The GI and II values of Hassawi rice, Uncle Ben’s rice, dates and dates with Arabic coffee for each subject was calculated as follows:
GI/II of test foods = \( \text{iAUC for the test food/ iAUC for reference} \times 100 \)

The GI/II values of Hassawi rice, Uncle Ben's rice, dates and dates with Arabic coffee were calculated as the average value obtained for 10 subjects. But the GI value for sponge cake was the average value of only 8 subjects.

Results were checked for normality using the Kolmogorov-Smirnov test (K-S test) and expressed as a means ± one standard error of the mean (SEM).

For the human studies, two factor (treatment and time) repeated measures ANOVA were used to analyse differences in the mean of glucose and insulin within the Hassawi rice, Uncle Ben's rice and standard glucose; and also within dates, dates with Arabic coffee and standard glucose. In addition, a single factor repeated measures analysis of variance ANOVA was used as well to analyse differences in the iAUC for glucose and insulin (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). If a significant interaction was obtained following ANOVA, a Bonferroni step-wise post hoc test was performed to determine the location of the variance. Also, comparison within two groups was analysed statistically using paired t-test (SPSS version 16.0 for Windows; Copyright (c) 2009 SPSS Inc., Chicago, USA). All data were examined using a two-tailed approach with a level of \( p < 0.05 \) as considered significant.
CHAPTER 3
CHAPTER 3

Establishment of the methodology for the determination of rapidly available glucose (RAG) and slowly available glucose (SAG) in selected Saudi and UK foods

3.1 Introduction

Dietary carbohydrates (CHOs) can be classified in a number of different ways and these generally ubiquitous compounds were traditionally classified on the basis of their chemical characteristics. However, the chemical nature of a CHO is not necessarily a reliable indicator of their physiological effects (Englyst et al., 1999). As a result, classifying starchy foods and other foods rich in CHO in terms of their physiological effects is a more useful approach in understanding the health effects of CHO containing diets. Indeed, Jenkins et al. introduced a new concept in 1981, the glycaemic index (GI), which allowed foods to be ranked according to their physiological effect in terms of their effect on blood glucose levels. An approach which has continued to be useful up to the present day.

Yet, it is evident that the digestion and absorption of CHO occurs at different rates and also to differing extents in the human small intestine. Nevertheless, this process is not particularly accessible to study in vivo and therefore a method for measuring the rate and extent of starch digestion in vitro was developed by Englyst et al. in 1992. In this method, two terms related to glucose release from CHO digestion in vitro were introduced, namely, slowly
available glucose (SAG) and rapidly available glucose (RAG). The latter of which has been shown to be a reliable predictor of the glycaemic response in vivo (Englyst et al., 1999) thus highlighting its potential usefulness. It has also been observed that a high positive correlation ($r = 0.8-0.9$, $p < 0.05$) exists between the rate of in vitro CHO digestibility and GI values for a selection of starchy foods (Jenkins et al., 1984; Goni et al., 1997). In fact, the indices of RAG and SAG were specifically designed to reflect the rate at which glucose from starchy foods becomes available for absorption in the human small intestine (Englyst et al., 1999). Therefore, RAG values may be used as a supplement to the GI approach to provide further data which could be useful in understanding of the impact of dietary CHO on blood glucose levels and thus insulin levels. However, the use of RAG and SAG in concert with GI may be useful still further.

Most procedures for determining starch fractions rely on measuring the liberated glucose after hydrolysis of starch with either acids or enzymes (Englyst et al., 2000). However, hydrolysis with acids may cause destruction of sugars and is very prone to error due to the destruction of not only starch, but also other polysaccharides (Bergmeyer, 1981). Enzymatic procedures are therefore preferable as they are more specific and do not cause destruction of sugars (Englyst et al., 2000). Nevertheless, the hydrolytic enzymes used must be pure and free from contaminating activities which may make the hydrolysis less specific.

The GI values of more than 750 different types of foods have already been published (Foster-Powell et al., 2002). In contrast, however, the RAG and SAG values of very few of these foods, for which the GI is available, have
been measured. RAG and SAG measurements may prove to be a useful index to help further understand the impact of CHOs in vivo. Therefore, in this study the methodology for measuring the RAG and SAG was established and used to determine these in vivo related parameters of a number of Saudi and UK foods using the Englyst method. The Englyst technique (2000) includes two versions of the main procedure for measurement of starch hydrolysis (Englyst et al., 1999; 2000). One is based on the colorimetric determination of glucose and the other is based on the measurement of glucose by high performance liquid chromatography (HPLC). The HPLC approach requires the use of an internal standard (Englyst et al., 2000) to allow accurate quantitation of the glucose present. Both methods, however, provide the analyst with values of RAG and SAG which can be used to compare different foods.

3.2 Study aims
The aim of this study was to establish the methodology of RAG and SAG measurement in a system that simulates CHO digestion; and to determine RAG and SAG for some foods.

3.3 Methods
3.3.1 Preparation of samples
Four foods were selected for study. They were Hareece grains (Al-Hassa, Saudi Arabia), palm dates (Khulas variety; Al-Mssallem Farm, Al-Hassa Dates Factories, Saudi Arabia), Long grain white rice (Express Rice, King's Lynn, Norfolk, UK), and pasta (Macaroni; made of durum wheat; Barilla,
Parma, Italy). Pasta, Hareece grains and Long grain rice were cooked in their traditional ways in distilled water for 12 min, 5 h and 12 min, respectively. Each sample was ground using a mortar and pestle and used for analysis. In addition, three reference foods were studied using the RAG and SAG method. These were potato starch (Sigma, Chemical Co. Ltd, Poole, UK), white wheat flour, and corn flakes © (Kellogg's, USA).

3.3.2 RAG and SAG measurements

For the measurement of RAG and SAG, portions of the samples (0.6-3 g) were weighed into 50 ml centrifuge tubes (polypropylene tubes from Corning Inc., NY, 14831) to the nearest ±1 mg and incubated with a mixture of hydrolytic enzymes (amyloglucosidase from Englyst Carbohydrate Services Ltd. (Southampton, UK), amylase (heat-stable) and pancreatin from Sigma Chemical Co. Ltd, Poole, UK) under controlled conditions of temperature (37 °C), pH (pH 5.2) and viscosity. Subsamples were collected from the incubation mixture at specific time points (20 and 120 min) and measured for glucose levels which were then used to calculate the RAG and SAG values, respectively (as described in Chapter 2, Section 2.2.1.1.1 and 2.2.1.5.1). Three reference samples were included in every batch of samples analysed. For the reference 1 (white wheat flour) the CVs of $G_{20}$ and $G_{120}$ were 1.7 and 4.9 %, respectively. The CVs for $G_{20}$ and $G_{120}$ in reference 2 (potato starch) were 12.3 and 6.0 %, respectively; while it was 3.9 % for $G_{20}$ and 4.5 % for $G_{120}$ in reference 3 (cornflakes) (See Chapter 2, Table 2.1).
3.3.3 Calculation and statistical analyses

Values for RAG and SAG were calculated from the measured $G_{20}$ and $G_{120}$ as the following:

$$\text{RAG (g)} = G_{20}$$
$$\text{SAG (g)} = G_{120} - G_{20}$$

Results were expressed as the means ± standard error of the mean (SEM). Comparison between three groups was performed by analysis of variance (ANOVA) for multivariate analysis with Tukey's post hoc test (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). Differences between two groups were made by paired t-test. All data were examined using a two-tailed approach with a level of $p < 0.05$ considered as significant.

3.4 Results

3.4.1 Analysis of the three reference foods (Potato starch, wheat flour, and corn flakes)

Figures 3.1 and 3.2 show the RAG and SAG values, respectively, for the three reference foods that were obtained using the three methods of determination of the released glucose (See Appendix 7). Results showed that the RAG and SAG values obtained using the three glucose analysis methods for the reference standard foods were in fact quite similar. However, the results obtained from the auto-analysers method were higher compared to those obtained from the manual method or the HPLC method.
Figure 3.1 RAG values for the three reference foods (n= 6) obtained from the two colorimetric assays and HPLC analysis (Mean ± SEM).

The highest RAG value was found in the corn flakes (88.3 g) using the auto-analyser method and also the same reference gave the lowest SAG value (1.4 g) by using the HPLC method. Wheat flour showed the highest SAG value (45.9 g) using the auto-analyser method.

The RAG value for potato starch was almost the same in all three methods of determination the glucose, however, its SAG value from the HPLC method was the lowest compared to the other two methods.
3.4.2 Test foods

The RAG and SAG values for the four test foods analysed, namely Hareece, dates, long grain rice and macaroni are presented in Table 3.1. The highest and lowest RAG values were found in dates and Hareece (31.0 and 9.8 g), respectively (see Appendix 8).

Figure 3.2 SAG values for the three reference foods obtained (n= 6) from the two colorimetric assays and HPLC analysis (Mean ± SEM).
Table 3.1 RAG and SAG values (g/100 g as eaten) for test foods (Mean ± SEM).

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<thead>
<tr>
<th></th>
<th>RAG</th>
<th>SAG</th>
</tr>
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<tbody>
<tr>
<td>Hareece</td>
<td>9.8 ± 0.2</td>
<td>1.7 ± 0.3</td>
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<tr>
<td>Dates</td>
<td>31.0 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Long grain rice</td>
<td>22.0 ± 1.0</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td>Macaroni</td>
<td>20.8 ± 1.9</td>
<td>10.8 ± 0.7</td>
</tr>
</tbody>
</table>

3.5 Discussion

3.5.1 Reference foods (Potato starch, wheat flour, and corn flakes)
The objective of this study was to establish the methodology of RAG and SAG measurement *in vitro*. In addition, the measurement of the RAG and SAG was carried out for Hareece, dates, long grain rice and macaroni. RAG and SAG measurements may provide a useful index to help understand further the impact of CHO in *vivo*. The statistically significant correlation between the GI and RAG value that has been reported previously (Englyst *et al.*, 1996a; 1999; Jenkins *et al.*, 1984; Goni *et al.*, 1997) makes it possible to rely on the *in vitro* measurement of RAG and SAG as an alternative way to predict the likely *in vivo* glycaemic impact of CHO rich foods.

Results showed that the RAG and SAG values obtained using the three methods of determination of released glucose for the reference standard foods were in fact quite similar.

The colorimetric results obtained using the manual method (spectrophotometry) showed a consistency with those in the literature for both RAG and SAG of the three reference foods (Table 3.2). For wheat flour, there was a similarity in the RAG (38.9 vs. 35.0 g) and SAG values (41.3 vs.
42.0 g) which were obtained from the colorimetric assay in comparison with those that obtained from literature. A high similarity was also observed for the RAG of corn flakes (79.1 vs. 79.0 g). However, for potato starch the RAG value obtained using the colorimetric assay (8.3 g) was significantly higher ($p=0.008$) than that presented in literature (4.0 g). Consequently, the SAG value was significantly lower ($p<0.05$) for potato starch obtained using colorimetric assay (17.2 g) compared to that obtained from the literature (22.0 g).

As the SAG value is calculated from subtracting $G_{20}$ from $G_{120}$, there was no significant difference between the $G_{120}$ from colorimetric assay and literature (25.6 and 26.0 g respectively). It is important to highlight that the potato starch with its high resistant starch (RS$_2$) content was used as a quality control for establishing the optimum stroke speed of the shaking water bath during the incubation. Values for $G_{120}$ of potato starch that are too low (compared with the target values that obtained from literature) require the stroke speed to be increased and vice versa (Englyst et al., 2000).

Overall, we can conclude that the results obtained from colorimetric assay compared well with the literature. Some of these differences can be explained by differences that occur between foods which is dependent on their origin and variety season. Therefore, RAG and SAG results for all test foods in this chapter and subsequent ones were based only on the manual colorimetric method as this proved to be a reliable and consistent method.
Table 3.2 The RAG and SAG values for the three reference foods from the colorimetric assay and literature (Mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Colorimetric assay</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAG</td>
<td>SAG</td>
</tr>
<tr>
<td>White flour</td>
<td>38.9 ± 0.2</td>
<td>41.3 ± 1.6</td>
</tr>
<tr>
<td>Potato starch</td>
<td>8.3 ± 0.4</td>
<td>17.2 ± 0.7</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>79.1 ± 1.2</td>
<td>4.0 ± 0.6</td>
</tr>
</tbody>
</table>

3.5.2 Test foods

Four test foods were selected to investigate their RAG and SAG values. The RAG value of Hareece was significantly lower ($p < 0.001$) than the RAG values obtained for dates, long grain rice and macaroni. Hareece is a whole wheat grain which is cooked and usually served with meat. The low RAG value in Hareece might be due to physical form of starch presented in Hareece. In fact, starch in Hareece is contained within whole grain structures surrounded by rigid plant cell walls. This structure can inhibit completely the swelling and dispersion of starch, and also partially block access of digestive enzymes (Englyst et al., 1992). The low RAG value of Hareece refers to the fact that Hareece is digested more slowly and its CHO is probably converted into glucose at a slower rate, producing a lower postprandial glucose concentration and subsequently lower insulin response. The SAG value in Hareece was also low indicating that the particles have not completely hydrolysed within the 2 h of incubation and the remaining starch is perhaps physically inaccessible starch (Englyst et al., 1996a).

The highest RAG value was found in the dates (31.0 g), while its SAG value (0.7 g) was the lowest (Table 3.1). This can be explained because the dates
is an excellent source of rapidly available CHO which mainly consists of simple sugars such as glucose (30.4 %) and fructose (29.4 %) and in some varieties there is a small amount (11.6 %) of sucrose (Al-Farsi & Lee, 2008). So, it was not surprising to observe the high value of RAG for dates because of the large amount of CHO hydrolysed in the first 20 min during the amylolytic incubation.

Macaroni was found to have high SAG value compared to the Hareece and dates. This high value of SAG for macaroni can be explained by the availability of the starch. Starch in macaroni is found within a dense structure which can hinder access of digestive enzymes and delay the released glucose in the first 20 min ($G_{20}$), giving most of it within a 2 h ($G_{120}$). The slow rate of starch digestion perhaps produces a lower postprandial glucose and insulin responses (Hallfrisch & Behall, 2000). Similarly, with the Long grain rice, the high SAG value could be due to starch presents as amylose. Rice with a high proportion of amylose has been shown to have a slow rate of digestion and therefore elicit lower glycaemic and insulinaemic (II) indices (Brand-Miller et al., 1992). For example, the GI value for Waxy rice (low in amylose) was 88; however, Doongara white rice (high in amylose) had a GI of 64 (Brand-Miller et al., 1992). It has been suggested that a factor such as the effect of lipid-amylose complex and NSP in high amylose rice might be associated with delaying the digestion of CHO (Goddard et al., 1984).

It is worth mentioning that there was some moderate difference among RAG (20.8 vs. 15.1 g) and SAG (10.8 vs. 13.0 g) values in macaroni from our results compared to those obtained from literature (Englyst et al., 1996a). These differences could be due to physicochemical properties of the dough.
such as the degree of kneading and the type of wheat which have both been shown to influence starch gelatinisation. However, the difference in the RAG and SAG values for Long grain rice from our results and that from literature (Englyst et al., 1996a) was much less marked (22.0 g vs. 19.3 and 9.3 g vs. 10.2, respectively).

As the Hareece is consumed in the form of a whole grains, it can provide a substantial contribution to the improvement of the diets of Saudis where there is a high rate of prevalence of diabetes (Al-Nozha et al., 2004). A consumption of three servings a day of whole grain foods was recommended due to its effectiveness in lowering glycaemic response and improving insulin resistant (Hallfrisch & Behall, 2000). Further investigation on the impact of Hareece on postprandial glucose and insulin should be carried out which could clarify the role of physical form of starch granules in lowering glucose or insulin response. Structural and inherent botanical differences of starch granules might explain the lower postprandial insulin response between starchy foods (Juntunen et al., 2003).

In conclusion, the in vitro measurement for RAG and SAG has been established showing good reproducibility and the results were generally comparable to those found in literature. As the results obtained using the colorimetric assay were more consistent with that found in the literature, the colorimetric method was adopted. Other reasons for choosing this method related to the availability and flexibility of using the instrumentation. This method of determining the released glucose was the only one that was used for the determination the RAG and SAG of the test foods.
CHAPTER 4
A study of Hassawi rice (*Oryza sativa* L.) in terms of its RAG and SAG determination *in vitro* and glycaemic and insulinaemic indices *in vivo*

4.1 Introduction

The prevalence of diabetes mellitus (DM) is increasing at an alarming rate across many countries of the developed world (Wild et al., 2004). In Saudi Arabia, epidemiological studies have shown that the prevalence of DM has increased dramatically in recent years (El-Hazmi et al., 1998). Indeed, the overall prevalence of DM in 1987 in Saudi adults was 4.3% (Fatani et al., 1987) but this has increased 6-fold in less than 20 years (Al-Nozha et al., 2004). Several factors are considered to be involved in influencing the prevalence of DM such as gender, age, obesity, socioeconomic status, genetic susceptibility and lifestyle. In Saudi Arabia, two of the main reasons for the increase in DM may be due to a major change in habitual eating patterns, including modifications in the quality and quantity of dietary carbohydrates (Musaiger, 1987) and their resultant impact on obesity. The daily intakes of finely milled cereal and grain products has increased over recent years (Alissa et al., 2005); there has been a concomitant reduction in the consumption of some healthy traditional starchy foods such as Hareece (whole grain wheat cooked with meat), Matateez and Marqooq (whole wheat dough with vegetables and meat), Jireesh (cracked wheat cooked with vegetables and meat) and Kabsa which is mainly made from rice (either a
white or the reddish brown rice variety known as Hassawi rice (Al-Mssallem, 1999).

Rice (*Oryza sativa*) is one of the most important cereal food crops in the world (Wang & Li, 2005). It is a staple carbohydrate (CHO) source in Saudi Arabia (Al-Mssallem, 1999). In Saudi Arabia, a wide range of rice varieties are available including Hassawi rice (*Oryza sativa* L.), an indigenous reddish brown rice of *Indica* variety (Figure 4.1). This type of rice is grown and consumed traditionally (by about 5% of the Saudi population) in the Al-Hassa oasis in the Eastern Province of Saudi Arabia (Al-Bahrany, 2002).

![Figure 4.1 Uncooked Hassawi rice grains.](image)

Hassawi rice (Land race) by tradition is consumed in a main dish (Kabsa) with cooked vegetables and meat (e.g. lamb, chicken or fish; Figure 4.2). Traditionally, Hassawi rice has been thought to have a better nutritional quality and to be healthier for women in the postpartum period compared to white rice (Al-Mssallem, 1999). Hassawi rice is lower in its total CHO content (CATM, 1985) and higher in total protein (Al-Mssallem & Al-Msalam 1997; Al-Mssallem, 1999) versus white long grain rice. Furthermore, levels of ash,
proximate fat and fibre are higher in Hassawi rice (CATM, 1985; Al-Bahrany, 2002).

Figure 4.2 Cooked Hassawi rice made into Kabsa.

Both the macro- and micro-nutrient content of foods can have a major impact upon the health of individuals (Leena et al., 2004). This is particularly applicable to the CHO present which can impact on both plasma glucose and the accompanying insulin levels. Indeed, a strong positive association has been shown to exist between the glycaemic index (GI, a measure of the impact a food makes on postprandial glucose levels) of foods and the risk of T2DM (Schulze et al., 2004; Salmeron et al., 1997), which supports the idea of the importance of the quality of dietary CHO in delaying the onset or preventing T2DM (Schulze et al., 2004; Sluijs et al., 2010). There is also a positive link between high GI and low cereal fibre content and increased risk of diabetes and it has been suggested that grains should be consumed in a
minimally refined form to reduce the incidence of T2DM (Salmeron et al., 1997; Willett et al., 2002). As such high GI foods may alter the risk of T2DM owing to the production of higher postprandial blood glucose concentrations and a greater insulin demand compared to low GI foods (Kalergis et al., 2005; Frost & Dornhorst, 2000).

CHO digestion and the subsequent release of glucose can be assessed *in vitro*. Indeed in 1992, Englyst developed an *in vitro* technique of dietary CHO digestion and created two terms related to glucose release from CHOs (Englyst et al., 1992). These terms were rapidly available glucose (RAG) and slowly available glucose (SAG). The way in which food CHOs are digested can have an important metabolic impact on the body and so studies *in vitro* may provide useful information about how a CHO rich food may perform *in vivo*. There is evidence that RAG can predict glycaemic response *in vivo* (Englyst et al., 1999) but at present there is little information on RAG and SAG values of rice varieties.

A greater understanding of the effects of traditional Saudi Arabian foods on blood glucose and insulin levels may lead to more effective lifestyle prevention strategies for T2DM. As such, in this study we investigated the RAG, SAG, chemical and surface composition, GI and II of Hassawi rice and compared it with another type of rice that is frequently consumed, namely Uncle Ben's rice. We hypothesised that Hassawi rice would have a beneficial effect on plasma glucose and insulin levels.
4.2 Study aims
The aim of this study was to measure the RAG and SAG value of Hassawi rice and to compare them with the same indices for Uncle Ben’s rice. This study also aimed to determine the chemical composition, GI and II for Hassawi rice and Uncle Ben’s rice in order to characterise Hassawi rice in more detail. In addition, as the two types of rice looked very different the surface composition of Hassawi rice and Uncle Ben’s rice was studied using X-ray photoelectron spectroscopy (XPS).

4.3 Methods
4.3.1 RAG and SAG measurements
4.3.1.1 Preparation of samples
Two types of rice were selected for the study, Hassawi rice (Al-Hassa, Saudi Arabia), and long grain parboiled Uncle Ben’s rice (Masterfoods, Belgium). Hassawi rice and Uncle Ben’s rice were cooked in their traditional ways in distilled water for 45 and 17 min, respectively. Each sample was then ground using a mortar and pestle to the same consistency and used for RAG and SAG measurement.
4.3.1.2 Procedure

The procedure used to determine RAG, SAG and TS (total starch) was an enzymatic hydrolysis of the food carbohydrate employing the method of Englyst et al. (2000) as described in Chapter 2 (Section 2.2.1.1 and 2.2.1.3).

4.3.2 Chemical composition

4.3.2.1 Preparation of samples

Two types of rice were selected for the study, Hassawi rice (Al-Hassa, Saudi Arabia) and long grain parboiled Uncle Ben’s rice (Masterfoods, Belgium). Hassawi rice and Uncle Ben’s rice were prepared as described above.

4.3.2.2 Procedure of chemical analysis

The chemical composition for Hassawi rice and Uncle Ben’s rice (fat, protein, ash, non-starch polysaccharides (NSP) and amylose) were determined using standard methods (AOAC, 1995, See Chapter 2, Section 2.2.2).

4.3.2.3 Mineral and vitamins

Minerals content was analysed at King Faisal Specialist Hospital and Research Centre/ Saudi Arabian/ Riyadh, using inductively coupled plasma mass spectrometry (ICP-MS). While vitamins analysis for thiamine (B1), riboflavin (B2) and niacin (B3) was carried out at Inspection diagnostics analysis consultation (IDAC) laboratory, Riyadh, Saudi Arabia using VitaFast test kits from R-Biopharm in accordance with the AOAC procedure for determining vitamins contents.
4.3.3 Surface analysis

XPS was used to study the chemical nature of atoms at and below the surface of the uncooked grains of Hassawi rice and long grain parboiled Uncle Ben's rice. The study was carried out in the Surface Analysis Laboratory at the University of Surrey by Prof. John Watts and his colleague Dr. Steven Hinder, the XPS system used was a Thermo Scientific Theta Probe. Samples were mounted on to a holder and placed in vacuum. The survey scans were taken with 1.0 eV step and 50 eV pass energy for each sample and an analysis depth of 5 nm.

4.3.4 GI and II determination

4.3.4.1 Subjects

A randomised crossover design carried out in accordance with the FAO/WHO guidelines (FAO/WHO, 1998) for GI testing was used. The study design received ethical approval from the University of Surrey Ethics Committee (EC/2004/37/SBMS) and 13 healthy volunteers were recruited from the postgraduate student and staff population at the University of Surrey by the distribution of both e-mails and posters. All volunteers gave informed written consent. The 13 individuals recruited were 6 men and 7 women, mean age 30.0 years (SEM 1.74 years; range 25 – 42 y). Weight, height, fasting blood glucose and blood pressure were measured at baseline.

4.3.4.2 Test foods

Hassawi rice and Uncle Ben’s rice were cooked in a kitchen at the Clinical Investigation Unit (University of Surrey). A portion of 120 g of Hassawi rice
and 83 g of Uncle Ben’s rice (which both contained 25 g of available CHO) were served to subjects with 250 ml of water on four separate sessions. On three other separate occasions 250 ml of water containing 25 g glucose (Fisher Scientific Ltd., Loughborough, UK) was given. Volunteers were asked to eat the rice and consume the drink within a 10 min time period.

4.3.4.3 Blood sample collection

Participants arrived at the Clinical Investigation Unit at the University of Surrey at 0830 h on each study day after an overnight fast (10-12 h). Finger pricks capillary blood samples were taken by using preset lancets (Accu-chek Softclix Pro., Brighton, UK) at fasting and at 15, 30, 45, 60, 90 and 120 minutes after consuming the Hassawi rice, Uncle Ben’s rice or standard glucose solution. Blood samples were collected into 300 µl plastic microvette tubes (Sarstedt Ltd., Leicester, UK) coated with fluoride oxalate and were immediately centrifuged at 3000 × g for 10 min at 4 °C. The resultant plasma was transferred into separate 300 µl plastic plain microvette tubes (Sarstedt Ltd.). The tubes were then frozen and kept at -20 °C until analysis for insulin concentrations (within 4 weeks) while the glucose concentrations were analysed immediately as mentioned below.

4.3.4.4 Glucose measurement

An automatic analyser (YSI 2300 STAT plus, Yellow Springs, Analytical Technologies, YSI Ltd., Fleet, UK) was used for determining plasma glucose concentrations (as described in Chapter 2, Section 2.2.3.3.2).
4.3.4.5 Insulin analysis

An enzyme linked immunosorbant assay (ELISA) was employed for measuring plasma insulin concentrations (as described in Chapter 2, Section 2.2.3.3.5).

4.3.5 Calculations and statistical analyses

The incremental area under the glucose and insulin curves (iAUC) for the reference glucose drink, Hassawi rice and Uncle Ben's rice were calculated according to the recommended method by WHO (FAO/WHO, 1998). The GI and II values of Hassawi rice and Uncle Ben’s rice for each subject were calculated as follows:

\[
\text{GI/II of Hassawi rice or Uncle Ben’s rice} = \frac{\text{iAUC for Hassawi rice or Uncle Ben’s rice}}{\text{iAUC for reference}} \times 100.
\]

The GI/II values of Hassawi rice and Uncle Ben's rice was calculated as the average value obtained for 10 subjects.

Results were checked for normality using the Kolmogorov-Smirnov test (K-S test) and expressed as a means ± one standard error of the mean (SEM).

For the human study, a two factor (treatment and time) repeated measures ANOVA was used to analyse differences in the means of the glucose and insulin levels within the two types of rice and standard glucose. In addition, a single factor (treatment) repeated measures analysis of variance ANOVA was used to analyse differences in the iAUC for glucose and insulin (SPSS 16.0 for Windows; Copyright © 2009 SPSS Inc.). If a significant interaction was obtained following ANOVA, a Bonferroni step-wise post hoc test was performed to determine the location of the variance. Differences in RAG,
SAG and TS values between Hassawi rice and Uncle Ben's rice were evaluated statistically using paired t-test (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). All data were examined using a two-tailed approach with a level of $p < 0.05$ being considered as significant.

4.4 Results

4.4.1 RAG, SAG and TS measurement

The RAG, SAG and TS values are presented in Figure 4.3 (see also Appendix 9). The RAG values for Hassawi rice and Uncle Ben's rice were $16.7 \pm 0.5$ and $21.1 \pm 0.2$ g and the values for SAG were $5.4 \pm 0.3$ and $9.65 \pm 0.9$ g, respectively. The TS value for Hassawi rice ($21.5 \pm 0.5$ g) was significantly lower ($p < 0.01$) than the corresponding value for Uncle Ben's rice ($31.0 \pm 0.5$ g).

![Figure 4.3](image)

Figure 4.3 RAG, SAG and TS values for the two types of rice given in g/100 g as eaten (Results expressed as Mean ± SEM)
4.4.2 Compositional and surface analysis

The compositional analytical data for Hassawi rice and Uncle Ben’s rice is demonstrated in Table 4.1 (see also Appendix 10). The proximate content of CHO in Hassawi rice was significantly lower than that found in Uncle Ben’s rice \((p<0.01)\), however; fat \((p<0.001)\), protein \((p<0.01)\), amylose \((p<0.01)\), ash and NSP \((p<0.001)\) were significantly higher in Hassawi rice as compared to Uncle Ben’s rice.

Table 4.1 Nutritional composition for the Hassawi rice and Uncle Ben’s rice per serving size (150 g).

<table>
<thead>
<tr>
<th></th>
<th>Hassawi rice</th>
<th>Uncle Ben’s rice</th>
<th>Significant? ((p&lt;0.05))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (KJ)</td>
<td>682.9 ± 15.6</td>
<td>873.0 ± 11.7</td>
<td>Yes ↓</td>
</tr>
<tr>
<td>Energy density (KJ/g)</td>
<td>4.5 ± 0.10</td>
<td>5.8 ± 0.07</td>
<td>Yes ↓</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.1 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>Yes ↑</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.8 ± 0.02</td>
<td>4.3 ± 0.02</td>
<td>Yes ↑</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>32.6 ± 0.9</td>
<td>46.7 ± 0.7</td>
<td>Yes ↓</td>
</tr>
<tr>
<td>Available CHO (g)</td>
<td>31.3 ± 0.9</td>
<td>45.2 ± 0.6</td>
<td>Yes ↓</td>
</tr>
<tr>
<td>Amylose (g)</td>
<td>26.3 ± 0.2</td>
<td>19.2 ± 0.3</td>
<td>Yes ↑</td>
</tr>
<tr>
<td>NSP (g)</td>
<td>0.9 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>Yes ↑</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>0.67 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>Yes ↑</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM. CHO, carbohydrate; NSP, non-starch polysaccharide.

Moisture content contributed to 62.5 % of cooked Hassawi rice and 64.6 % of cooked Uncle Ben’s rice. ↑ = Higher and ↓ = Lower compared to Uncle Ben’s rice.

* Energy was calculated from macronutrients (CHO, fat, and protein).
The total CHO in Hassawi rice was lower by about 20% than that reported in previous study (60 g vs. 75 g / 100 g dried basis), while the NSP in Hassawi rice (1.6 g / 100 g dried basis) was approximately double that found in literature (0.8 g / 100 g dried basis; Al-Bahrany, 2002). However, the protein (10.2 g vs. 10.6 g), fat (2.0 g vs. 2.0 g) and ash (1.4 g vs. 1.5 g) contents were almost the same (Al-Bahrany, 2002; Al-Mssallem & Al-Mssallem, 1997).

Table 4.2 demonstrates the mineral and vitamin content of Hassawi rice and Uncle Ben’s rice. Hassawi rice had a higher content of magnesium and zinc compared to Uncle Ben’s rice, however; the content of calcium and sodium was much lower in HR in comparison with UBR. The content of copper and iron were almost similar in the two types of rice. The lead and cadmium contents were not detectable at detection limits of 1 ppb. For the thiamine, riboflavin, and niacin; HR had higher contents of these water-soluble vitamins and they were almost double that found in UBR.
Table 4.2 Minerals and vitamins contents in Hassawi rice and Uncle Ben’s rice (cooked dried matters).

<table>
<thead>
<tr>
<th>Minerals &amp; Vitamins</th>
<th>Hassawi rice</th>
<th>Uncle Ben’s rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (μg/g)</td>
<td>146 ± 16</td>
<td>729 ± 11</td>
</tr>
<tr>
<td>Cu (μg/g)</td>
<td>4 ± 0.01</td>
<td>3 ± 0.01</td>
</tr>
<tr>
<td>Fe (μg/g)</td>
<td>13 ± 0.1</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Mg (μg/g)</td>
<td>1287 ± 12</td>
<td>301 ± 15</td>
</tr>
<tr>
<td>Na (μg/g)</td>
<td>45 ± 4</td>
<td>217 ± 41</td>
</tr>
<tr>
<td>Zn (μg/g)</td>
<td>30 ± 0.1</td>
<td>14 ± 0.9</td>
</tr>
<tr>
<td>Pb (μg/g)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cd (μg/g)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B1 (μg/g)</td>
<td>0.5 ± 0.01</td>
<td>0.34 ± 0.001</td>
</tr>
<tr>
<td>B2 (μg/g)</td>
<td>0.87 ± 0.06</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>B3 (μg/g)</td>
<td>0.63 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM. Ca, calcium; Cu, copper; Fe, iron; Mg, magnesium; Na, sodium; Zn, zinc; Pb, lead; Cd, cadmium; B1, thiamine; B2, riboflavin; B3, niacin. ND, not detected.

Figure 4.4 and 4.3 show XPS survey of Hassawi rice and Uncle Ben’s rice. The peaks of P_{2p}, C_{1s}, O_{1s}, N_{1s}, and Si_{2p} occurred in the regions around 136, 285, 536, 402 and 103 eV, respectively. The surface compositions of the two rice samples are presented in Table 4.3. The carbon-to-oxygen (C/O) ratio is also shown in Table 4.3.
Table 4.3 Surface composition and carbon-oxygen ratio of the two types of rice as obtained from the XPS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface composition (atomic %)</th>
<th>Element ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>O</td>
</tr>
<tr>
<td>Hassawi rice</td>
<td>80.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Uncle Ben's rice</td>
<td>84.6</td>
<td>14.0</td>
</tr>
</tbody>
</table>

For both types of rice, the dominant element was carbon, followed by oxygen and nitrogen in decreasing order. It must be noted that XPS is not able to analyse for hydrogen content. As shown in Table 4.3 the C/O ratio in Hassawi rice was lower compared to Uncle Ben’s rice.

Figure 4.4 XPS survey spectrum of Hassawi rice.
Figure 4.5 XPS survey spectrum of Uncle Ben's rice.

4.4.3 GI and II determination

The subjects' characteristics are displayed in Table 4.4. They had an average age of 30 years and were modestly overweight according to the average BMI score but had normal blood pressure and fasting blood glucose levels.
Table 4.4 Descriptive characteristics of study participants at baseline.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.0 ± 1.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.6 ± 4.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ± 1.0</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74.1 ± 2.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>116.3 ± 2.9</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.0 ± 0.1</td>
</tr>
</tbody>
</table>

Results presented as Mean ± SEM (n=13).

The incremental area under the curve (iAUC) for glucose was calculated for the reference (glucose drink), Hassawi rice and Uncle Ben's rice (Figure 4.6; see also Appendix 11). The iAUC for the reference glucose was significantly higher (p < 0.01) than that demonstrated for either Hassawi rice or Uncle Ben's rice. The GI of Hassawi rice and Uncle Ben's rice was calculated and found to be 59 ± 5 and 54 ± 7, respectively. No significant difference was observed between these two rice varieties.

On closer scrutiny of the glucose profiles it is evident that the peak glucose level which occurred at 30 min for standard glucose solution was significantly higher than that achieved for Hassawi rice (p = 0.01) and Uncle Ben's rice (p = 0.004). For each of the rice meals the glucose level declined over the next 30 min and then remained steady for the subsequent 60 minutes. In contrast,
however, for the standard glucose drink plasma glucose levels continued to drop to below the baseline at 90 min by approximately 0.33 mmol/l, and then levels increased towards the baseline during the last 30 min.

![Graph showing plasma glucose responses during 2 h following reference glucose, Hassawi rice and Uncle Ben's rice. Results presented as Mean ± SEM.]

**Figure 4.6** Plasma glucose responses during 2 h following reference glucose, Hassawi rice and Uncle Ben’s rice. Results presented as Mean ± SEM.

The iAUC for insulin was also calculated from the data presented in Figure 4.7 (see Appendix 12) and it was found that the II for Hassawi rice was 56 ± 10 and for Uncle Ben’s rice was 78 ± 17. The insulin response observed after Hassawi rice consumption was significantly lower than that observed after the consumption of the reference glucose and Uncle Ben’s rice ($p = 0.005$ and $p = 0.007$ respectively).
Figure 4.7 Plasma insulin responses during 2 h following reference glucose, Hassawi rice and Uncle Ben's rice. Results expressed as Mean ± SEM.

4.5 Discussion

Hassawi rice is a variety of rice commonly consumed in Saudi Arabia which is considered to be good nutritionally, in part, owing to the fact that it is frequently consumed by women post partum as well as in other traditional settings. In this study we evaluated the nutritional composition of Hassawi rice and studied some metabolically relevant parameters related to its CHO content in vitro and in vivo which may be important in terms of diabetes. This study has also provided the first documentation of the GI and II for Hassawi rice.

The total CHO in Hassawi rice in this study was lower by about 20 % than that reported in a previous study (Al-Bahrany, 2002). This is similar to that found in brown rice where the total CHO was 23 % lower compared to white
rice from the same batch and variety (Panlasigui & Thompson, 2006). Amylose content of Hassawi rice and Uncle Ben's rice was determined by using a modified procedure based on the Concanavalin A (Con A) method (Gibson et al., 1997). This method has been shown to correlate well ($r > 0.99$) with the iodine-based procedure. The advantage of Con A method is that it is applicable to flour samples without the need for prior purification and does not require a calibration curve compared to the iodine-based method (Gibson et al., 1997; Zhong et al., 2006; Benmoussa et al., 2007; Chen & Bergman, 2007; Li et al., 2008). However, the iodine-binding procedure for determination of amylose is still preferred by some researchers (Herrero-Martinez et al., 2004; Xu et al., 2008).

In this study, the mineral and vitamin content of Hassawi rice and Uncle Ben's rice were evaluated. It is interesting to see that the magnesium and zinc content were much higher in HR compared to UBR. However, the calcium content was higher in UBR and this could be due to the fact that UBR is fortified with calcium and other minerals and vitamins as stated on the package. The iron content is similar in both types of rice. Despite the fact that HR is non-fortified with any minerals, its level of iron was considerably higher than that of white rice (Ma et al., 2005). A serving size (150 g) of HR would provide 4% of the requirement for iron and 11% of the zinc requirement for adults according to the Reference Nutrition Intake (RNI, the daily recommended values used in UK). In terms of vitamin content, HR had higher levels of vitamins B1, B2, and B3 compared to that found in UBR. It has been reported that preparing and cooking may result in substantial losses of some water-soluble vitamins especially vitamin B1. Polishing and
boiling of rice also causes losses of vitamin B1, therefore polished rice is a poor source of this vitamin. It has been found that the thiamine level of polished rice is only 0.15 mg/100 g, however; it is 0.46 mg/100 g in brown rice (Lynch & Young, 2000). A serving size (150 g) of HR would thus provide 5% of the requirement for thiamine and 8% for riboflavin for adults according to the Reference Nutrition Intake data (RNI, the daily recommended values used in UK).

Hassawi rice was shown to have the lowest RAG, SAG and TS values of the two rice varieties measured. The difference compared to the other rice variety was significant (p< 0.01) for all these three measurements of CHO hydrolysis. However, the RAG and SAG values of the two types of rice found in this study were comparable to those found in literature which ranged from 16 to 26 g for RAG and from 4 to 10 g for SAG (Englyst et al., 1996a; Patindol et al., 2010).

Interestingly, when the RAG and SAG values are expressed as a percentage of TS for the two types of rice, it is observed that there is no significant difference in the RAG value between the two rice varieties (p>0.05). However, if this normalisation with respect to TS is carried out for SAG the SAG value is still significantly lower for Hassawi rice (p< 0.05) compared to the Uncle Ben's rice (Table 4.5). This low SAG value observed for Hassawi rice may be due to the fact that most of its starch content is converted to glucose within the first 20 min of incubation. Complete hydrolysis of the Hassawi rice starch was then achieved during the next 60 min and this was essentially lower than that observed for Uncle Ben's rice. Nevertheless, these differences may be due to the high level of NSP in Hassawi rice which is
more than double that observed in Uncle Ben's rice (Table 4.1). This will be discussed in more detail below.

**Table 4.5** Rapidly available glucose (RAG) and slowly available glucose (SAG) relative values in Hassawi rice and Uncle Ben's rice.

<table>
<thead>
<tr>
<th></th>
<th>Hassawi rice</th>
<th>Uncle Ben's rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG (%)</td>
<td>66.2 ± 5.8</td>
<td>62.0 ± 1.3</td>
</tr>
<tr>
<td>SAG (%)</td>
<td>23.3 ± 0.5</td>
<td>32.8 ± 2.0</td>
</tr>
</tbody>
</table>

Values (Mean ± SEM) are given as a percentage of the total starch value.

The reddish brown colour observed for Hassawi rice was considered important in terms of the surface composition of this type of rice which may impact on the digestion of the rice in vivo. As such it was thought that the surface of Hassawi rice would have a different elemental composition. Carbon was found to have the greatest surface concentration for the two types of rice (~82 %) followed by the oxygen (~15 %). Both the carbon and oxygen could arise from CHO components in the rice such as polysaccharides and cellulose (Rouxhet et al., 2008). As shown in Table 4.3, Hassawi rice had a higher oxygen percentage, but its carbon content was lower when compared to Uncle Ben's rice. This resulted in a lower C/O ratio in Hassawi rice compared to other rice variety. This could be due to the difference in the carbon structure. Uncle Ben’s rice was shown to have lower nitrogen percentage with a value of 0.6 % compared to 1.7 % for Hassawi rice. The nitrogen might be attributed to amide and amine (Rouxhet et al.,
The source of the silicon and phosphorus is unknown but silicon is an inorganic element and may arise as a result of transfer from equipment or packaging to the product. The phosphorus may originate from an additive in the polymer packaging (Park et al., 2004).

Hassawi rice was shown in this study to have a medium GI and II with values of 59 ± 5 and 56 ± 10, respectively (glucose = 100). However, Uncle Ben's rice had a low GI (54 ± 7) and a high II (78 ± 17). The classification of GI was proposed by Wolever et al. (1991) and within this definition high GI foods are those with an index of greater than or equal 70 in the case of using glucose as a standard reference, while low GI foods have a value less than or equal 55.

It has been observed that the glucose response is correlated positively with the RAG value for a number of starchy foods (Garsetti et al., 2005; Englyst et al., 2003). Foods with a high RAG value show a rapid digestion and absorption due to the fact that their CHO's are fully gelatinised and available for digestion, such as cornflakes, which have a RAG value of 46 g (per portion size similar to that used for GI testing) and a GI of 93 (Englyst et al., 2003). In contrast, the RAG values for 24 varieties of plain sweet biscuits range from 23 to 37 g (per portion size similar to that used for GI testing) with a GI which ranges from 38 to 60 (Garsetti et al., 2005). As such RAG values positively correlate with GI, while there is a negative correlation between the SAG and GI (Garsetti et al., 2005; Englyst et al., 2003).

In this study we observed that although there was a difference in the amylose content between Hassawi rice (17.5 %) and Uncle Ben's rice (12.7 %) this did not impact upon the GI to any observable extent. There may be a good
explanation for this as it is well known that high amylase rice (28 %) produces lower GI and II (Brand-Miller et al., 1992), but the content of amylase in Hassawi rice and Uncle Ben’s rice are much lower than the high amylase rice and fall within the low amylase content region. For example, the high amylase Bangladeshi rice (28 %) had a GI value of 38 while the GI for the waxy rice with 0-2 % amylase was 88 (Foster-Powell et al., 2002).

The GI value correlated strongly ($r = 0.76, p < 0.0001$) with II value (Garsetti et al., 2005) and the latter was usually lower on the relative scale than was the GI of the foods (Brand-Miller et al., 1992).

It is important to consider both the GI and II of foods in the dietary management of an individual with diabetes because there are some foods with a low GI but their II is high. Several studies have included measures of II values because the role of insulin in glucose homeostasis is well known and because of the association between the large insulin demand with a high GI food which has been proposed to be involved in the aetiology of diabetes (Salmeron et al., 1997; Wolever, 2000a; 2000b; Englyst et al., 2003). Incorporating the use of GI and II values should be considered in planning the optimal dietary CHO's for people with diabetes.

Despite the fact that there was no significant difference between the GI of Hassawi rice (59) and Uncle Ben’s rice (54); the GL of Hassawi rice was significantly lower ($p<0.01$) than Uncle Ben’s rice in terms of a similar serving size basis (Table 4.6). This reduced level could be explained by the lower total CHO content of Hassawi rice in conjunction with the amount of available CHO which was lower than that present in Uncle Ben’s rice. Energy density was also lower for Hassawi rice compared to that found in Uncle Ben’s rice.
(4.5 and 5.8 KJ/g, respectively, $p<0.001$, Table 4.1). All these characteristics of Hassawi rice may be important, suggesting that Hassawi rice will have a lower glycaemic and insulinaemic impact than an equivalent cooked weight of Uncle Ben’s rice. It has been observed that a lower risk of developing diabetes is associated with a higher consumption of low GL diets (Salmeron et al., 1997). Similarly, the II of Hassawi rice was significantly lower than Uncle Ben’s rice ($p = 0.007$) and this finding could be due to the higher content of NSP in Hassawi rice ($p<0.001$, Table 4.1) compared with Uncle Ben’s rice. It is evident that NSP-rich foods may have played a role in the reduced insulinaemic response (Stevenson et al., 2008; Jenkins et al., 2000) and their beneficial effect in reducing the risk of developing diabetes has been studied in large cohort epidemiological studies. Indeed, many studies have shown a significant association between dietary NSP and reduced risk of diabetes (Schulze et al., 2004; Salmeron et al., 1997; Meyer et al., 2000; Stevens et al., 2002). The role of NSP in influencing glycaemic response may depend on the type of NSP whether soluble or insoluble. Soluble NSPs, such as guar gum, pectin, and β-glucans, slow digestion and absorption of the starch due to the fact that they increase viscosity within the stomach and work as a barrier around the CHO to be digested (Frost & Dornhorst, 2000). However, the role of a certain amount of NSP in reducing GI value is still unclear.
Table 4.6 Comparison between Hassawi rice and Uncle Ben’s rice in terms of their GI, II and GL (serving size = 150 g).

<table>
<thead>
<tr>
<th></th>
<th>Hassawi rice</th>
<th>Uncle Ben’s rice</th>
<th>Significant? (p&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>59 ±5</td>
<td>54 ±7</td>
<td>No</td>
</tr>
<tr>
<td>II</td>
<td>56 ±10</td>
<td>78 ±17</td>
<td>Yes (\downarrow)</td>
</tr>
<tr>
<td>GL</td>
<td>18.5± 0.5</td>
<td>24.4 ±0.3</td>
<td>Yes (\downarrow)</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SEM. GI, glycaemic index; II, insulinaemic index; GL, glycaemic load.

In conclusion, the lower GL and insulinaemic response possessed by Hassawi rice suggest that this type of rice may have benefits on postprandial glycaemic and insulinaemic levels and may have a role to play in the management and prevention of T2DM. In addition, Hassawi rice contains a high level of NSP which is significantly higher than that found in Uncle Ben’s rice. It is well documented that dietary NSP have beneficial effects in improving insulin sensitivity and therefore reducing the risk of developing T2DM. As diabetes is becoming more common in developed countries, including Saudi Arabia, steps need to be taken to address this problem. Nutritional advice, including the use of staple foods, such as Hassawi rice on a regular basis, could be given to the general public and in particular individuals with diabetes to overcome this disease. However, further research, particularly long term studies, may be required to ascertain the clear benefits of Hassawi rice.
CHAPTER 5
CHAPTER 5

Does a meal with the same GI but different SAG value produce the same glycaemic response?

5.1 Introduction

Carbohydrates (CHOs) are the major source of dietary energy for all people and exist in a wide range of forms which are for the most part heterogeneous in terms of their physical and chemical characteristics (Cummings et al., 1997). Nevertheless, in order to understand further the dietary impact of this important macronutrient it is necessary to classify CHOs in a manner appropriate to their effects. However, classifying CHOs based simply on their chemical structure is not a reliable indicator of their physiological effects (Englyst et al., 1999; Cummings et al., 1997). As such, the approach of classifying foods according to their physiological effects can be considered a more useful method in terms of understanding the health effects of diets containing CHOs. Indeed, Jenkins et al. (1981) introduced a concept, known as the glycaemic index (GI), which allows foods to be ranked according to their effects on blood glucose levels. However, it is appreciated that the GI approach can have its limitations. GI, as a physiological measurement of CHO containing foods, helps to rank foods according to their digestibility and absorption in the human small intestine (Jenkins et al., 1981). However, this index is not related directly to the actual amount of CHOs usually present in foods 'as consumed'. As such, the terms
RAG and SAG have been devised relatively recently and are used as a direct measurement of absolute CHO which is available for absorption in the human small intestine (Englyst et al., 1992; 1999; 2003; Englyst & Englyst, 2005). RAG and SAG measurements are based solely on the hydrolysis of dietary CHO in vitro using a mixture of enzymes resembling those present in the GI tract. The measurements of RAG give values for glucose that are likely to be absorbed in the human small intestine and, thus likely to influence blood glucose and insulin responses (Englyst et al., 1996a). The relationship between the food RAG values and GI has been studied and it has been found that there is a significant positive correlation between these values for 39 starchy foods ($r = 0.76$, $p < 0.001$). It is therefore apparent that the RAG but also the SAG measurements of CHO containing foods may be used as a supplement to the GI approach and may provide further information which could be useful in understanding the impact of different foods containing CHO on blood glucose and insulin levels (Englyst et al., 2003; Englyst & Englyst, 2005).

As highlighted, RAG and SAG measurements may provide a useful index to help understand the metabolic impact of CHO in vivo. However, before this can be put into place greater attention needs to be paid in terms of understanding the metabolic impact of CHO on the basis of their RAG and SAG values in combination with the GI approach. As such, we hypothesised that studying the effect of two CHO rich meals one with a high value of SAG and the other with a low SAG could help us to understand the differences in the metabolic effects of different dietary CHO in blood glucose and insulin levels versus GI values.
5.2 Study aims

This study aimed to examine the metabolic impact of two meals with similar Gl and macronutrient content but different (high and low) SAG values.

5.3 Methods

5.3.1 In vitro measurement of RAG and SAG

The in vitro procedure was based on the enzymatic hydrolysis of the food CHO using the method of Englyst et al. (2000) as described in Chapter 2, Section 2.2.1.1.

5.3.2 In vivo study

5.3.2.1 Subjects

Twelve healthy volunteers were recruited from the postgraduate student population of the University of Surrey by the distribution of both e-mails and posters. Inclusion criteria included the male gender, non-smoking status and age between 20 and 65 y. Subjects also needed to have a normal weight in relation to their height, normal resting blood pressure and normal fasting plasma glucose levels. Subjects who were either overweight (BMI > 25), or had abnormal blood pressure (> 140/120 mmHg) or abnormal fasting plasma glucose (≥ 7.0 mmol/l) were excluded from the study. All subjects gave informed written consent. Weight, height, blood pressure and other measurements were measured at baseline.
5.3.2.2 Study design

A randomised controlled crossover trial of a single meal (either a low SAG meal followed by a high SAG meal or the reverse) was employed. Each subject was randomly assigned to receive one of these two meals first. The study design received ethical approval from the University of Surrey Ethics Committee (EC/2007/78/FHMS). Subjects were asked to consume the meal at their breakfast time (at ~0830 h).

5.3.2.3 Meal design

In order to test the hypothesis, the two identical meals were designed to achieve as large a difference as possible in the content of SAG, but with no significant differences between the two meals regarding the overall GI, energy content, absolute available CHO, fat or protein levels. The low SAG meal consisted of 204 g of Hassawi rice (previously characterised by Al-Mssallem et al., submitted), a plain yoghurt drink (225 g) and Arabic dates (45 g). This meal had a total SAG of 11.8 g and an overall GI of 47 (see Table 5.1). The high SAG meal contained Uncle Ben's rice (204 g), a plain yoghurt drink (250 g) and Arabic dates (20 g); with a total SAG of 20.2 g and an overall GI of 46 (see Appendix 13). The GI values were based on glucose as the standard reference taken from our own data (Al-Mssallem, unpublished observations). All foods were prepared in the kitchen unit of the Clinical Investigation Unit at the University of Surrey, UK.
5.3.2.4 Blood samples collection

Participants arrived at the Clinical Investigation Unit at the University of Surrey at 0830 h on the day of the study after an overnight fast (10-12 h). Subjects were cannulated via the antecubital forearm vein prior to sampling. Blood samples of 5 ml were drawn fasting and at 15, 30, 45, 60, 90, 120, 180 minutes after consuming either the low or high SAG meal and analysed for glucose, insulin, free fatty acids and triacylglyceride levels. Blood samples were collected into 5 ml dipotassium EDTA polystyrene tubes for insulin, non-esterified fatty acids (NEFA) and triacylglyceride (TAG) (Teklab Ltd, Durham, UK) levels and 2 ml fluoride oxalate polystyrene tubes (Teklab Ltd.) for plasma glucose levels, and were immediately centrifuged at 3000 $\times$ g for 10 min. The centrifuged plasma was transferred into separate 500 $\mu$l plastic plain microvette tubes (Alpha Laboratories Ltd, Eastleigh, Hampshire, UK). The tubes were then frozen and kept in the freezer at -20°C until analysis (within 4 weeks).

5.3.2.5 Glucose, NEFA and TAG measurement

The enzymatic automated colorimetric method on the ILab 650 (Instrumentation Laboratory, UK) was applied to measure plasma glucose, TAG and NEFA concentrations using glucose GOD/PAP test kit, NEFA kit and TAG kit along with two quality control (QC) samples (Randox Laboratories Ltd., County Antrim, UK). The intra-assay coefficient of variation of the level 1 and 2 QCs for glucose was less than 2 % (1.34 and 0.49 %, respectively). For the TAG and NEFA, the
precision of the analysis gave CVs for QC1 and QC2 within acceptable limits (0.58 & 0.28 %, and 0.25 & 1.45 %, respectively).

5.3.2.6 Insulin measurement
An enzyme linked immunosorbant assay (ELISA) was used for measuring plasma insulin concentrations (MLT, Cardiff, UK). The quality controls, standards (Invitron Ltd, Monmouth, UK) and samples were incubated with the labelled antibody solution (Invitron Ltd) at 37 °C for 2 h and unbound labelled antibodies were removed using the wash buffer (Invitron Ltd) according to the manufacturer’s instructions. The insulin was then measured using the microtitre plate luminometer (Luminescent plate reader Centro LB 960). All readings obtained from the luminometer were multiplied by 6 to convert the units (mU/l) into pmol/l. Two quality controls (one high and one low insulin levels) were employed and their CVs were 9 % and 6 %, respectively.

5.3.3 Statistical analyses
Results were expressed as a means ± one standard error of the mean (SEM) and checked for normality using the Kolmogorov-Smirnov test (K-S test). Comparison within the two groups was analysed statistically using paired t-test (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). All data were examined using a two-tailed approach with a level of \( p < 0.05 \) being considered as significant.
5.4 Results

5.4.1 RAG and SAG levels of the two meals

RAG and SAG values for the low and high SAG meals are shown in Table 5.1. For the high SAG meal, the RAG and SAG values were $49.6 \pm 0.58$ and $20.2 \pm 1.86$ g, respectively; and $50.0 \pm 1.2$ and $11.8 \pm 0.63$ g, respectively in the low SAG meal. The SAG value in the latter meal was found to be significantly lower than that present in the high SAG meal ($p=0.002$). However, there was no significant difference between the two meals in terms of their content of RAG ($p=0.85$).

Table 5.1 High and low SAG meal composition.

<table>
<thead>
<tr>
<th></th>
<th>High SAG meal</th>
<th>Low SAG meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>492</td>
<td>492</td>
</tr>
<tr>
<td>RAG (g)</td>
<td>49.6</td>
<td>50.0</td>
</tr>
<tr>
<td>SAG (g)</td>
<td>20.2</td>
<td>11.8</td>
</tr>
<tr>
<td>Energy* (KJ)</td>
<td>2145 (517 Kcal)</td>
<td>2118 (510 Kcal)</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>86.6</td>
<td>84.7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>12.2</td>
<td>12.4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>14.5</td>
<td>14.9</td>
</tr>
<tr>
<td>GI</td>
<td>46</td>
<td>47</td>
</tr>
</tbody>
</table>

RAG = rapidly available glucose, SAG = slowly available glucose, CHO = carbohydrate, GI = glycaemic index.

*Energy was calculated from macronutrients (CHO, fat, and protein).
5.4.2 Subjects' characters

Subjects' characters are displayed in Table 5.2. They each had a normal BMI, resting blood pressure values and fasting blood glucose levels.

Table 5.2 Characteristics of volunteers.

<table>
<thead>
<tr>
<th>Subjects' characteristics</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>26.5 ± 1.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.1 ± 3.8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 0.6</td>
</tr>
<tr>
<td>Blood pressure (diastolic) mm Hg</td>
<td>70.7 ± 1.7</td>
</tr>
<tr>
<td>Blood pressure (systolic) mm Hg</td>
<td>119.9 ± 2.1</td>
</tr>
<tr>
<td>Fat (%) (normal range 8-20 %)</td>
<td>17.04 ± 1.10</td>
</tr>
<tr>
<td>Fat mass (normal range 5-14 kg)</td>
<td>12.56 ± 1.11</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.3 ± 0.06</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SEM (n=12)

5.4.3 Glucose, insulin, TAG and NEFA

The incremental area under the glucose curve (iAUC) was calculated for the low and high SAG meals and was 106.3 ± 14.46 and 88.1 ± 10.67 mmol/l/min, respectively (Figure 5.1; see also Appendix 14). These figures showed that the iAUC for the glucose response to the low SAG meal was higher than that
observed for the high SAG meal although the difference between the responses to the two meals did not achieve statistical significance ($p = 0.21$). Closer scrutiny of the plasma glucose profile revealed that the maximum glucose concentration was observed at the 30 min time point after meal consumption. The glucose concentration then dropped to the base line level at 60 min. In the high SAG meal, however, the plasma glucose concentration rose again and then levelled off for the next 1 h after the consumption of the high SAG meal. In the low SAG meal the plasma glucose concentration also rose and gave a second peak at 120 min and then declined again reaching the baseline at 180 min after the consumption the low SAG meal (Figure 5.2).

![Image](image.png)

**Figure 5.1** The iAUC for the glucose, TAG and NEFA in the low and high SAG meals (mean ± SEM).

iAUC = incremental area under the curve, SAG = slowly available glucose, TAG = triacylglycerides, NEFA = non-esterified fatty acids.
Figure 5.2 Plasma glucose responses during 3 h following low and high SAG meal (mean ± SEM).

Responses of the other metabolic parameters measure also showed some small differences. For the insulin, TAG and NEFA measurements the iAUC were 26550 ± 3266 pmol/l/min, 46.9 ± 6.62 and 33.5 ± 7.19 mmol/l/min respectively in terms of the responses to the low SAG meal and 23701 ± 3065 pmol/l/min, 50.6 ± 6.50 and 34.8 ± 3.78 mmol/l/min, respectively, in terms of the responses to the high SAG meal (Figures 5.1 and 5.3; see also Appendix 14). The insulin response to the low SAG meal was higher than that observed for the high SAG meal, however; this difference was not significant (p = 0.33). On closer scrutiny of the time course of insulin concentrations it was observed that at 45 min the low SAG meal produced an insulin response that was considerably higher than
that observed with the high SAG meal ($p = 0.053$). The consumption of the high SAG meal resulted in a lower insulin peak at 30 min, while the insulin response to the low SAG meal peaked at 45 min. Subsequently, the insulin concentration started to drop gradually towards the baseline level at 180 min after the consumption of the two meals.

**Figure 5.3** Plasma insulin responses during 3 h following low and high SAG meal (Mean ± SEM, n=12).

The fasting TAG level was 1.1 ± 0.09 mmol/l for subjects prior to the low SAG meal and it was 1.2 ± 0.17 mmol/l prior to the high SAG meal ($p=0.52$). The mean fasting NEFA concentration was 0.44 ± 0.06 and 0.44 ± 0.05 mmol/l ($p=0.96$) for subjects prior to the low and high SAG meals, respectively. There was a similar increase in the postprandial TAG concentration response to both of the
meals. As expected, the plasma NEFA concentrations were suppressed after the consumption of both meals and remained suppressed throughout the postprandial period (data not shown).

5.5 Discussion

The main target of this study was to investigate the metabolic effect of two meals with similar GI but with different SAG values. It is clear that some foods with similar GI values can have different metabolic impacts on the blood glucose and insulin responses due to differences in their in vitro CHO digestibility (Anderson et al., 2010). This dissimilarity could be explained by small differences in the RAG and SAG values of the foods.

In this study the low SAG meal was shown to produce higher glucose and insulin responses despite the fact that the two meals had similar GI values. Although these differences were not significant \((p = 0.21\) and 0.33, respectively), it is evident that the differences are in the direction that would be expected. Foods with a high content of SAG contain an amount of available glucose that is likely to be absorbed more slowly, and thus delay the elevation of blood glucose and insulin levels. The two meals (high and low SAG) were well designed meals with similar GI (46 and 47, respectively) and macronutrient levels (Table 5.1), but it worthy of note that it was possible to create meals that were significantly different in their SAG value \((p = 0.002)\).

It has been previously observed that GI is positively correlated \((r = 0.50; p = 0.01)\) with RAG values and negatively \((r = - 0.42; p = 0.04)\) with SAG values for
24 varieties of plain sweet biscuits (Garsetti et al., 2005). Similar results have been reported for 23 cereal products (Englyst et al., 2003) and 39 starchy foods (Englyst et al., 1996a). Similarly, there was positive correlation between II and RAG values and an inverse correlation between II and SAG values (Garsetti et al., 2005; Englyst et al., 2003). The inverse correlation between the SAG and glycaemic and insulinaemic indices suggests that aiming for a high SAG content can be an alternative way to lower the glucose and insulin responses (Garsetti et al., 2005). Our findings are consistent with that stated above where we observed that a high SAG meal elicited lower glucose and insulin responses; however, this is particularly interesting given that the GI values of the two meals were in essence the same.

The RAG and SAG measurement provided values that could be used as a supplement to the GI approach which may be useful in the understanding of the impact of CHO on blood glucose and insulin levels (Englyst et al., 2003; Englyst & Englyst, 2005). There was, as expected, a higher increase in the blood glucose and insulin levels in the low SAG meal compared to high SAG meal. On the other hand, there was no significant difference in the iAUC for TAG ($p = 0.61$) and NEFA ($p = 0.77$) between low and high SAG meals. The plasma NEFA concentration fell rapidly after consuming both meals and reached lower concentrations with value of 0.12 mmol/l at 120 min after consuming low SAG meal compared to 0.20 mmol/l in subjects who consumed high SAG meal ($p < 0.001$).
A finding such as this supports the idea that a reductive effect on blood glucose and insulin responses can be achieved by increasing the consumption of foods with a high content of SAG (Englyst et al., 2003). A dietary preference for foods with a slow-release CHO may provide health benefits, especially for those with diabetes (Englyst et al., 2005). It is well established that RAG and SAG values are good indicators in predicting the metabolic impact of CHOs on blood glucose and insulin profiles. It is important to know that the RAG and SAG values relate to the food as eaten and are expressed as g/100 g which allowed food to be compared on an equal weight basis (Englyst et al., 1999; 2000). This is consistent with the American Diabetes Association (1994) recommendations which focus on the total of the CHOs consumed rather than only their source. For people with diabetes, it is critical for them to know the quantity and quality of their intakes of dietary CHOs, therefore it has been suggested that the RAG value should be used with the GI value where RAG value indicates the amount of glucose likely to be rapidly absorbed in the human small intestine (Englyst et al., 1999). In addition, it has been found that RAG and SAG explain more of the variation in GI and II than macronutrient composition of the starch foods (Garsetti et al., 2005). And we found that RAG and SAG values have shown to be a good indicator for the postprandial glucose and insulin responses. As shown in Figure 5.3, the insulin does not get as high with the high SAG meal versus the low SAG meal is interesting and as people with diabetes wish to keep their glucose and insulin levels as stable as possible. This work has some important implications for people with diabetes but also for people without
diabetes as maintaining glucose levels on a more level basis is also important for them.

To our knowledge, this study is the first of its kind for investigation the metabolic impact of CHO's on the basis of their RAG and SAG contents. It was not possible to ascertain with certainty the responses that would be achieved on the two meals. Further studies are needed to understand more about the effects of high SAG foods on postprandial blood glucose and insulin levels in comparison with low SAG foods. Also, an intervention clinical trial should be carried out to investigate the long term impact of consumption of high SAG meals on blood glucose and insulin levels in people with and without diabetes.
CHAPTER 6
An investigation into the effect of Arabic coffee on the glycaemic and insulinaemic responses to date consumption

6.1 Introduction

Coffee is believed to be the most widely consumed beverage in the world (Higdon & Frei, 2006). Two varieties of coffee beans exist namely Coffea Arabica and Coffea Canephora Robusta (Briandet et al., 1996; Chandrasekar & Viswanathan, 1999; Aloys & Marc, 2005). The relationship between coffee consumption and the risk of developing type 2 diabetes mellitus (T2DM) has been studied in short-term randomized controlled trials and also in epidemiologic studies (Van Dam & Hu, 2005; Van Dam et al., 2006; Bidel et al., 2008). These studies have shown that a high consumption of coffee beverage is associated with a better glucose tolerance and a substantially lower risk of T2 DM (Van Dam & Hu, 2005; Bidel et al., 2008). This may, in part, be related to the coffee phenolic constituents where coffee is considered the major source of chlorogenic acids (CGA) in the human diet (Clifford, 1999; 2000). CGA and other phenolic compounds in coffee may inhibit glucose absorption via their interference with glucose transporters (Bidel et al., 2008; Higdon & Frei, 2006; Johnston et al., 2003). Also, CGA have been shown to affect glucose metabolism by increasing insulin sensitivity (Clifford, 1999; 2000). Nevertheless, ingestion of coffee has also been shown to increase the area under the glucose
and insulin curves and to reduce insulin sensitivity in response to high and low glycaemic index (GI) meals (Graham et al., 2001). However, this effect is considered to be associated with the presence of caffeine in the coffee beverage which has also been shown to have important physiological effects (Graham et al., 2001; Moisey et al., 2008).

There is evidence that dietary habits and lifestyle play important roles in developing or preventing chronic diseases such as diabetes. Saudi populations habitually enjoy consuming Arabic coffee which is mainly prepared from Arabica coffee beans which are lightly roasted and then mixed with a small amount of cardamom (El Shabrawy & Felimban, 1993). Arabic coffee (see Appendix 15) is customarily served along with dates (a traditional Saudi snack) on a daily basis (Figure 6.1).

Figure 6.1 The traditional Saudi snack (Dates with Arabic coffee).
In Saudi Arabia, there are about 450 varieties of palm dates that are locally grown and their production reaches about 15% of the total international production of dates (Yang et al., 2010). The most common varieties are shown in Figure 6.2. Ajwa, is grown in Al-Maddina Almonnawarah, and is the most expensive variety of dates, while Khulas, from Al-Hassa, is the most famous date variety (Al-Jaber & Al-Jaber, 2006).

![Figure 6.2 Most common varieties of dates that consumed in Saudi Arabia.](image)

Saudi patients with diabetes are commonly advised without a sound scientific basis, to avoid the consumption of dates (Ahmed et al., 1991). This is mainly due to the fact that the main sugar in dates is glucose (Al-Farsi & Lee, 2008) which can be readily absorbed in the human small intestine and thus influence diabetic control. However, the GI for different varieties of dates is generally in the low range (47 to 57; Ali et al., 2008). Interestingly, the first documentation of the GI for dates came from a Saudi Arabian variety known as Khulas, which had
a GI of 57 (Ahmed et al., 1991). Furthermore, a later study showed that the GI of dates can be reduced to 36 when consumed with sour milk (Miller et al., 2003). This reduction could be due to the effect of protein and fat presented in sour milk (Gulliford et al., 1989; Miller et al., 2003; Henry et al., 2005; 2007) or due to increase acidity which can have an effect in lowering the GI (Liljeberg et al., 1995; Ostman et al., 2002).

A typical adult Saudi may consume between 20 and 120 g of dates with 60-300 ml of Arabic coffee at one sitting (El Shabrawy & Felimban, 1993). However, although the effect of consuming dates with a drink such as sour milk is known the metabolic impact of consuming dates with another popular drink, Arabic coffee has not been investigated previously. It is apparent that using our knowledge of the constituents of coffee that both a beneficial and detrimental effect could occur in terms of the impact on glucose and insulin levels. The study presented in this chapter investigated the role of traditionally consumed Arabic coffee on the glycaemic and insulinaemic profiles after date consumption.

6.2 Study aims

This study aimed to investigate the glucose and insulin responses to the ingestion of an equivalent amount of 50 g of available carbohydrate from dates alone and dates with Arabic coffee.
6.3 Methods

6.3.1 Compositional analysis of Saudi Arabian dates

The chemical composition of dates (i.e. moisture, fat, protein and ash) was determined using standard analytical methods (See Chapter 2, Section 2.2.2).

6.3.2 Total phenol content determination

The level of total phenols in Arabic coffee was determined colorimetrically using the Folin-Ciocalteu’s reagent (Sigma Chemical Company Ltd., Poole, UK) and the method of Vinson et al. (2001). The coffee beverage was prepared by dissolving coffee particles (4 g) in 100 ml boiled Milli-Q water. Ten ml of coffee beverage was then added to a screw-capped tube containing 8 ml of 1.2 M hydrochloric acid in 50 % methanol/water. The samples were then placed in a water bath at 80 °C for 3 h with mixing by vortex every 30 min. For the coffee samples to be analysed 100 µl was used and reacted with the diluted Folin-Ciocalteu’s reagent. A reagent blank was made using Folin-Ciocalteu’s reagent diluted 1 in 9. The calibration standards were made using epicatechin at a concentration range of 0.025 - 0.3 mg/ml in which 100 µl of these solutions were also reacted with Folin-Ciocalteu’s reagent. The colorimetric measurement was carried out at 720 nm.

6.3.3 Liquid chromatography-mass spectrometry (LC-MS) analysis

Arabic coffee (Coffea Arabica) was analysed for chlorogenic acid (CGA), other components with a similar structure to CGA and also other phenolic compounds.
Coffee particles (375 g) were dissolved into 20 ml of boiling Milli-Q water and left to stand for 2 min. Then 10 ml of this coffee solution was transferred to a test tube and 0.5 ml of each of Carrez A and B reagents were added separately and vortex mixed. The mixture was then centrifuged at 4000 x g for 20 min at 4 °C and 7 ml supernatant withdrawn and evaporated to dryness with nitrogen at 30 °C. The sample was resuspended in 1 ml of solvent A (2 % acetonitrile, 0.5 % acetic acid, 97.5 % Milli-Q water) then filtered and injected on to the LC-MS and analysed according to the method of Clifford et al. (2003). Peaks were identified corresponding to the retention times and fragmentation patterns of the compounds found within the Arabic coffee.

6.3.4 GI and II determination

6.3.4.1 Subjects

A randomised crossover design carried out in accordance with the FAO/WHO guidelines (FAO/WHO, 1998) for GI testing was used. The study design received ethical approval from the University of Surrey Ethics Committee (EC/2004/37/SBMS) and ten healthy volunteers were recruited from the postgraduate student and staff population at the University of Surrey by the distribution of both e-mails and posters. Inclusion criteria included being male or a non-pregnant healthy female, aged 20-65 years, having normal resting blood pressure, normal fasting plasma glucose levels and being willing to comply with the study protocol. Subjects who were had abnormal blood pressure (> 140/120 mm-Hg) or abnormal fasting plasma glucose (≥ 7.0 mmol/l), had gastrointestinal
condition affecting digestion or absorption of nutrients, the use of drugs affecting gastrointestinal motility or nutrient digestion or absorption, hepatitis, surgery or infection within the last three months were excluded from the study. All volunteers gave informed written consent. The 10 individuals recruited were 5 men and 5 women, aged 30.8 ± 2.8 y. Weight, height, fasting blood glucose and blood pressure were measured at baseline.

6.3.4.2 Test foods
Dates (*Phoenix dactylifera* L.) Khulas variety and Arabic coffee (*Coffea Arabica*) were prepared in a kitchen at the Clinical Investigation Unit (University of Surrey). A portion of 82 g of dates (which contained 50 g of available carbohydrate) were served to subjects with 180 ml of hot water (60 °C) or Arabic coffee (60 °C) on four separate sessions. On a further three separate occasions 250 ml of water containing 50 g pure glucose (Fisher Scientific, UK) was given. Volunteers were asked to eat the dates and consume the drink within 10 min.

6.3.4.3 Blood sample collection
Participants arrived at the Clinical Investigation Unit at the University of Surrey at 0830 h each study day after an overnight fast (10 - 12 h). Finger pricks capillary blood samples were taken by using preset lancets (Accu-chek Softclix Pro., Brighton, East Sussex, UK) at fasting and at 15, 30, 45, 60, 90 and 120 minutes after consuming the dates with water, dates with Arabic coffee or standard glucose solution. Blood samples were collected into 300 µl plastic
microvette tubes (SARSTED Ltd., Leicester, UK) coated with fluoride oxalate, and were immediately centrifuged at $3000 \times g$ for 10 min at 4 °C. The resultant plasma was transferred into separate 300 μl plastic plain microvette tubes (SARSTED Ltd.). The tubes were then frozen and kept at -20 °C until analysis for insulin concentrations (within 4 weeks) while the glucose concentrations were analysed immediately as mentioned below.

6.3.4.4 Glucose measurement
An automatic analyser (YSI 2300 STAT plus, Yellow Springs, Analytical Technologies, YSI Ltd., UK) was used for determining plasma glucose concentrations. Twenty four samples were analysed within each run along with three quality controls (QC) samples. Within each run the coefficient of variation of the QC1, 2 and 3 was 2.5 %, 3.6 % and 1.8 %, respectively (See Chapter 2, Section 2.2.3.3.2).

6.3.4.5 Insulin determination
An enzyme linked immunosorbant assay (ELISA) was employed for measuring plasma insulin concentrations (See Chapter 2, Section 2.2.3.3.5).

6.3.6 Calculation and statistical analyses
The recommended method for calculating the iAUC (FAO/WHO, 1998) was used to calculate the iAUC for the reference glucose drink, dates and dates with coffee.
The Gl/II values of dates and dates with coffee were calculated as the average value obtained for 10 subjects. Results were expressed as a means ± one standard error of the mean (SEM) and checked for normality using the Kolmogorov-Smirnov test (K-S test). For the human study, a two factor repeated measures ANOVA was used to analyse differences in the mean responses of glucose and insulin to the dates with water, to dates with Arabic coffee and to the standard glucose solution. In addition, a single factor repeated measures analysis of variance ANOVA was used as well to analyse differences in the iAUC for glucose and insulin (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). If a significant interaction was obtained following ANOVA, a Bonferroni step-wise post hoc test was performed to determine the location of the variance. All data were examined using a two-tailed approach with a level of \( p < 0.05 \) as considered significant.

6.4 Results

6.4.1 Chemical composition

The compositional analytical data for dates is presented in Table 6.1. Dates as expected mainly consisted of sugars with value of 62.2 g/100 g. Protein and fat were present in small amounts with values of 1.4 and 0.3 g/100g, respectively.
Table 6.1 Nutritional composition (g/100 g) of dates.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g)</td>
<td>14.7 ± 0.04</td>
</tr>
<tr>
<td>Energy* (KJ)</td>
<td>1073.6 ± 18.36</td>
</tr>
<tr>
<td>Energy density (KJ/g)</td>
<td>10.7 ± 0.18</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.4 ± 0.03</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>62.2 ± 1.03</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>1.5 ± 0.07</td>
</tr>
<tr>
<td>Fibre* (g)</td>
<td>17.9 ± 0.9</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SEM (n=4).

*Energy was calculated from macronutrients (CHO, fat, and protein).

*Fibre was estimated by subtracting the sum of moisture, protein, fat, ash and sugar from 100.

6.4.2 Phenols analysis

The standard curve for the quantitation of total phenols is presented in Figure 6.3. Using this standard curve the amount of total phenols in Arabic coffee was calculated to be 1.2 mmol Epicatechin equivalents/l.

A number of phenols were positively identified within the Arabic coffee sample. The LC-MS analysis showed that the Arabic coffee contained CGA and caffeine. The most abundant derivative was caffeoyl quinic acid. The Figure 6.4 below shows the compounds that were identified within the Arabic coffee sample. The highest peak was shown for 5-Caffeoyl quinic acid followed by 3-Caffeoyl quinic...
acid, Caffeoyl quinic acid and Caffeine. However, $p$-Coumaroyl quinic acid, Feruloyl quinic acid and Dicaffeoyl quinic acid were also observed in relatively small amounts. Identification was based on their retention time, absorbance spectrum and MS fragmentation pattern.

![Figure 6.3 Standard calibration curve for epicatechin.](image)

Figure 6.3 Standard calibration curve for epicatechin.
6.4.3 Determination of GI and II

The subjects' characteristics are displayed in Table 6.2. They had an average age of 30 years and were of average weight, blood pressure and fasting blood glucose levels.

As shown in Figure 6.5, peak glucose levels occurred at 30 min for standard glucose, dates and dates with Arabic coffee. At this time the response to the standard glucose solution was significantly higher ($p<0.05$) than the response to dates, however, this response was not significant with dates with Arabic coffee ($p = 0.11$). Similarly, the glucose response at 45 and 60 min for dates was
significantly lower than that for dates with Arabic coffee ($p= 0.017$ and $p= 0.041$, respectively). It appeared that coffee ingestion increased the glucose peak rise by approximately 0.4 mmol/l.

### Table 6.2 Characteristics of subjects

<table>
<thead>
<tr>
<th>Subjects' characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>30.8 ± 2.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.0 ± 5.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.0 ± 1.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72.5 ± 3.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122.1 ± 2.8</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.0 ± 0.1</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SEM (n=10 subjects).

The iAUC for plasma glucose concentrations over 2 h postprandial period following consumption of standard glucose solution, dates and dates with Arabic coffee was 180 ± 13, 96 ± 11 and 112 ± 11, respectively (see Appendix 16). From this, the GI for dates with water and dates with Arabic coffee was calculated and was found to be 55 ± 6 and 63 ± 5, respectively. The mean GI value for dates with water was lower than that for dates with Arabic coffee but the difference did not quite reach statistical significance ($p= 0.08$).
Figure 6.5 Plasma glucose responses during 2 h following reference glucose, dates with water and dates with Arabic coffee (Mean ± SEM).

Plasma insulin for the standard glucose, dates with water and dates with Arabic coffee peaked at 30 min after the ingestion of the foods and then declined towards the baseline level by 120 min (Figure 6.6). Similar patterns were evident for all. The iAUC for plasma insulin concentrations was also calculated and the II for dates with water was 64 ± 7 and for dates with Arabic coffee was 62 ± 8 (see Appendix 16). The iAUC of insulin for dates with water and dates with Arabic coffee was much lower than that observed for standard glucose solution. This lower iAUC, however, was not sufficiently low enough to be significantly lower than the iAUC for glucose. However, the p-value (p= 0.07) was close enough to 0.05 to be worthy of further consideration. There was no significant difference in
the plasma insulin response between dates and dates with Arabic coffee ($p = 1.00$).

![Graph showing plasma insulin responses](image)

**Figure 6.6** Plasma insulin responses during 2 h following reference glucose, dates with water and dates with Arabic coffee (Mean ± SEM).

### 6.5 Discussion

This study investigated both the food composition of Saudi Arabian Khulas dates and examined the impact these dates on glucose and insulin levels in the presence and absence of Arabic coffee. For clarity the discussion will focus on the compositional features of the dates in the first instance.

In this study the Khulas dates was chosen as it is the most famous variety consumed in Saudi Arabia. The moisture content of this variety of dates was
14.7%. Similar results have been reported for 16 varieties of dates, including Khulas, for which the average moisture content was 15.2% (Al-Shahib & Marshall, 2002; Al-Farsi & Lee, 2008). Furthermore, the ash (1.5 g/100g), fat (0.3 g/100 g) and protein (1.4 g/100 g) content of the dates from this study fell in between those values from the previous studies (1.1 - 1.8 g, 0.36 - 1.3 and 1.2 - 1.7 g, respectively; Ali et al., 2008; Al-Farsi et al., 2005; 2008). The total sugar content of the dates in this study was 62.2 g, similar to that reported previously with a value of 59.9 g - 62.2 g (Ali et al., 2008; Al-Farsi et al., 2005). The sugar content in dates is mostly in the form of glucose and fructose (Al-Farsi et al., 2005; 2007). Also, dates is a good source of NSP which has been found to range between 6 to 11% (Al-Shahib & Marshall, 2002; Al-Farsi et al., 2005; 2007). In this study, our estimation of NSP in dates was 17%. However, this is prone to be overestimation by about 6% due to accumulative errors arising from the other individual analyses. As reported previously, the daily consumption of dates was estimated to be about 100 g and this amount would provide about 50% of the recommended daily amount of dietary NSP (Al-Shahib & Marshall, 2002; Al-Farsi et al., 2008).

In this study, the GI investigation resulted in a relative low GI with value of 55 for dates. Previous studies have found that the GI for different varieties of dates ranges from 47 to 57 (Ali et al., 2008). In more recent study for GI of dates, the mean GI value for 9 varieties of Saudi dates was 55 (ranged from 47 to 64; Bajaber, 2006); so the result obtained here compares well with this. The result also compares well with the first documentation of the GI for dates which came
It is well known that coffee contains many phenolic compounds such as CGA which may have a potential effect on glucose and insulin levels (Bidel et al., 2008; Higdon & Frei, 2006; Johnston et al., 2003). The possible role of CGA (350 mg) in glucose metabolism could be via its effect on inhibiting glucose transporters (Na+-dependent glucose transporter) which would influence the amount of glucose absorbed. Furthermore these compounds may influence α-glucosidase activity which would lower the amount of glucose made available within the intestine and thus lower plasma glucose concentration (Bidel et al., 2008). Also, consumption of coffee (400 ml containing 350 mg CGA) may affect the secretion of gastrointestinal peptides (glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)) by decreasing GIP and increasing GLP-1, leading to slow intestinal glucose absorption (McCarty, 2005; Johnston et al., 2003). In addition, the CGA may reduce plasma glucose output from the liver by inhibiting glucose-6-phosphatase (Bidel et al., 2008; Higdon & Frei, 2006; McCarty, 2005). The beneficial effects of CGA would be appear at a higher volume of coffee beverages or for long periods of frequent coffee consumption. This might be the reason behind not seeing that effect in our study because it was randomised clinical trial for investigation the effect of Arabic coffee in postprandial glucose and insulin levels. Also, the concentration of Arabic coffee beverage in our study was too low as discussed in further details below. This finding is similar to the findings of Louie et al. (2008), who
observed that there was no difference between the postprandial glucose and insulin levels of decaffeinated coffee vs. water owing to a lower CGA content that presented in the small volume of decaffeinated coffee beverage consumed. It is evident that the phenols are poorly absorbed from the human small intestine and are likely metabolised to their metabolites. For example, about 33 % of CGA is only absorbed and about two-thirds of it reach the colon and may be metabolised to caffeic acid and quinic acid (Higdon & Frei, 2006). It has reported that a 200 ml cup of coffee contains 70-350 mg of CGA (Clifford, 1999; 2000), and the beneficial health effect of lowering the risk of developing T2DM was found in people who consumed ≥ 7 cups of coffee (Van Dam & Hu, 2005; Bidel et al., 2008). Thus, the level of CGA that seem to have health benefits would range from 0.5 – 2.5 g/ day. Our LC-MS results for Arabic coffee have shown that Arabic coffee used in this study contained CGA (Figure 6.4). The level of CGA which has found to have an effect in delaying intestinal glucose absorption was 2.5 mmol/l (Johnston et al., 2003), but the total phenols concentration in our Arabic coffee was just 1.2 mmol/l. This concentration may therefore be too low to elicit significant difference in glucose and insulin levels. It is well known that the effects of phenols will depend on the amount consumed and also on their bioavailability (Manach et al., 2004).

Our study has indicated that the consumption of coffee with dates exacerbates the plasma glucose response in healthy volunteers compared to the consumption of dates with water. Interestingly, however, there was no significant difference in the insulin response between the two treatments. A possible
explanation for these non-significantly different responses of glucose and insulin in the dates with Arabic coffee compared to dates might be due to the fact that the concentration of the Arabic coffee constituents was not high enough to influence glucose absorption and thus elicit a significant effect on the blood glucose levels and subsequently the insulin levels. Similarly, it has been observed that an increase in the area under glucose and insulin curves and reduction in the insulin sensitivity for ingestion of coffee with meals (Moisey et al., 2008; Johnston et al., 2003; Graham et al., 2001). All these effects were proposed to be associated with the presence of caffeine in the coffee which increases AUC of glucose and insulin by about 25 to 50 % (Moisey et al., 2008; Graham et al., 2001). Caffeine is phosphodiesterase inhibitors which can increase the concentration of cyclic adenosine monophosphate (cAMP). Increased concentration of cAMP was associated with impaired glucose tolerance after consumption caffeinated coffee beverage (Johnston et al., 2003). Caffeine can also inhibit muscle glucose uptake as it acts as adenosine receptor antagonist (Keijzers et al., 2002). Our results have shown that coffee increased the AUC of glucose by 15 % but not the insulin, therefore; the GI rose to 63. Most of the physiologic effects of coffee can attribute to the presence of caffeine. It is clear that the consumption of Arabic coffee with dates had a modest effect bordering on a significant effect. In contrast to these short term controlled trials which has shown the effects of caffeine, this effect might be modified during long periods of coffee consumption among heavy and chronic coffee consumers (Bidel et al., 2008). The better glucose tolerance and a substantially lower risk of
T2DM was associated with high consumption of coffee (Bidel et al., 2008; Van Dam & Hu, 2005).

In conclusion, consumption dates with Arabic coffee is a traditional practice in Saudi Arabia. We found that this habit of ingestion dates, at the same time with drinking Arabic coffee, increased slightly glucose response in healthy ($p = 0.08$) people and this is in accordance with the previous clinical trials which have found that caffeine administration impairs glucose tolerance and decreases insulin sensitivity. We would assume that consumption of dates would have an effect on plasma glucose levels in diabetic individuals similar to that effect seen in healthy people. The significantly lower postprandial glucose response for dates either with water or Arabic coffee as compared to that for standard glucose solution, suggests that dates could be consumed by people with diabetes as a good source of simple sugar as well as dietary NSP in a moderate amount (5-7 pieces) in a daily basis.
7.1 Introduction

Carbohydrates (CHOs) of dietary origin are key macronutrients in terms their capacity to influence blood glucose levels. They can be digested at differing rates and to different extents in the human small intestine and this can impact on their resultant differential effects on plasma glucose levels (Englyst et al., 1999). These features of dietary CHO have been identified using the concept known as the glycaemic index (GI) which allows foods to be ranked on the basis of the rate of digestion and absorption of their CHOs (Jenkins et al., 1981).

In terms of food choice guidance, it is recommended to use the GI of foods in conjunction with information about food composition (FAO/WHO, 1998). Some scientists advocate the use of diets rich in low GI foods for controlling blood glucose profiles in people with diabetes (Thomas & Elliott, 2010; Heilbronn et al., 2002). A list of foods with low GIs is available in the International Table of GI and GL (Foster-Powell et al., 2002) or from GI database (www.glycemicindex.com). One of these foods, of particular note, is sponge cake which is often highlighted in the media and healthy eating websites as a food that people can consume while on a low GI diet owing to its low GI. Sponge cake is a light, porous cake based on the ingredients wheat flour, sugar, and eggs. It gets its leavening power entirely from incorporating air in the beating, whisking and sieving stages. The GI value for
sponge cake is widely reported to be 46 as a result of the early GI studies conducted by Jenkins et al. (1981). Indeed, this value for sponge cake appears to be the only measurement published. However, a low GI value for sponge cake appears to be counterintuitive if we consider the ingredients. Indeed, it is difficult to rationalise the fact that sponge cake has a low GI when we consider the key ingredients which generally have a high GI or their presence in other foods results in a high GI. Nevertheless, the GI value cannot be predicted from the composition of a food, it has to be measured (Jenkins et al. 1981).

There may well be reasons for the low GI for sponge cake. It is evident that food components such as protein and fat have both been found to lower the GI of foods. However; the quantity of protein from the eggs used in sponge cake preparation (10.0 g/100 g) does not reach the amount required (50 g) that could influence the GI (Paul & Southgate, 1979; Gannon et al., 2001; Wolever et al., 1991). Furthermore, the standard recipe for sponge cake does not contain shortening of any kind and so is reasonably low in fat. Of course there are recipes for sponge cake in which fat is used and for which the fat content can increase to 25 g/100g (Paul & Southgate, 1979).

It has been observed that there is a highly significant positive correlation \((r = 0.76, p < 0.001)\) between GI value of starchy foods and their in vitro CHO hydrolysis during first 20 min of incubation with digestive enzymes (Englyst et al., 1996a). The glucose released from the in vitro CHO hydrolysis in the first 20 min is known as rapidly available glucose (RAG), while slowly available glucose (SAG) is that glucose released during the next 100 min. This high correlation supports the idea that RAG and possibly SAG measurements
CHOs in vivo. It can be hypothesised therefore that a low GI food would have a low RAG and consequently a high SAG value. Conversely, a food with a high GI would have a high RAG and low SAG. In order to test this, sponge cake which is considered to have a low GI value, based on published literature values, was selected and it’s RAG and SAG measurements were determined. In addition, the GI of the sponge cake was tested to confirm the literature results.

7.2 Study aims
The aim of this study was to measure the RAG and SAG for sponge cake which has been previously shown to have a low GI value (as indicated in the International table of GI and GL (Foster-Powell et al., 2002). The GI was determined in our Clinical Investigation Unit at the University of Surrey. A further aim was to investigate the relationship between RAG and GI of a variety of foods including sponge cake.

7.3 Methods
7.3.1 The RAG and SAG measurement
For the measurement of RAG and SAG, portions of the samples (0.6-3 g) were weighed into 50 ml centrifuge tubes (polypropylene tubes from Corning Inc., NY, 14831) to the nearest ± 1 mg and incubated with an amylglucosidase (Englyst Carbohydrate Services Ltd., Southampton, UK), amylase (heat-stable) and pancreatin (Sigma Chemical Co. Ltd., Poole, UK) under controlled conditions of temperature (37 °C), pH (pH 5.2) and viscosity
(standardised by guar gum). Subsamples were collected from the incubation mixture at specific time points (20 and 120 min) and measured for glucose levels which were then used for the RAG and SAG values, respectively (See chapter 2, Section 2.2.1.1.1 and 2.2.1.5.1). Further treatment and incubations were performed to disperse any remaining starch present in the samples in order to determine the total glucose (TG) as described in Chapter 2 (Section 2.2.1.3).

Two reference samples (potato starch and cornflakes) were included in every batch of samples analysed. The CVs of $G_{20}$ and $G_{120}$ were 10.4 and 6.7 %, respectively for potato starch; while it was less than 5 % for both $G_{20}$ and $G_{120}$ in reference cornflakes.

7.3.2 Determination of GI
7.3.2.1 Subjects
A randomised crossover design carried out in accordance with the FAO/WHO guidelines (FAO/WHO, 1998) for GI testing was used. The study design received ethical approval from the University of Surrey Ethics Committee (EC/2004/37/SBMS) and 8 healthy volunteers were recruited from the postgraduate student and staff population at the University of Surrey by the distribution of both posters and e-mails. All volunteers gave informed written consent. The 8 participants recruited were 4 men and 4 women, mean age 31.0 years (SEM 2.1 years; range 25 – 42 y). Weight, height, fasting blood glucose and blood pressure were measured at baseline.
CHAPTER 7

7.3.2.2 Test food selection

A number of low GI foods from the International Table of GI and GL (Foster-Powell et al., 2002) were selected and their RAG and SAG were measured (Long grain rice, Uncle Ben's rice, macaroni, sponge cake and dates). We found that the sponge cake had a high RAG value despite the fact that its GI was low (just 46) as stated in the International Table of GI. Therefore, sponge cake was chosen to test its GI value in our Clinical Investigation Unit to ensure that it had a low GI despite its RAG value being high.

The sponge cake was prepared using 3 medium eggs, 100 g caster sugar (super fine sugar), 100 g plain flour and pinch of salt. It was made using a standard method by beating the eggs with sugar until they were light and creamy, then carefully sieving and folding in the flour. The mixture was then poured into the chosen cake tin and baked. Then each portion was packed into a plastic plate and stored in a fridge in the kitchen at the Clinical Investigation Unit (University of Surrey). A portion of 53 g of sponge cake, which contained 25 g of available CHO, was served to subjects with 250 ml of water on two separate sessions. On three other separate occasions 250 ml of water containing 25 g glucose (Fisher Scientific, Loughborough, UK) was given. Participants were asked to eat the sponge cake and consume the drink within a 10 min time period.

7.3.2.3 Blood sample collection

Participants arrived at the Clinical Investigation Unit at the University of Surrey at 0830 h on each study day after an overnight fast (10 -12 h). Capillary finger pricks blood samples were taken by using preset lancets
(Accu-check Softclix Pro., Brighton, UK) at fasting and at 15, 30, 45, 60, 90 and 120 minutes after consuming the sponge cake or standard glucose solution. Blood samples were collected into 300 µl plastic microvette tubes (Sarstedt Ltd., Leicester, UK) coated with fluoride oxalate and were immediately centrifuged at 3000 × g for 10 min at 4 °C. The resultant plasma was transferred into separate 300 µl plastic plain microvette tubes (Sarstedt Ltd.). The plasma samples were then analysed immediately for glucose as described below.

### 7.3.2.4 Glucose measurement

Plasma glucose concentrations were determined by using an automatic analyser (YSI 2300 STAT plus, Yellow Springs, Analytical Technologies, YSI Ltd., Fleet, UK) as described in Chapter 2, Section 2.2.3.3.2.

The incremental area under the glucose curve (iAUC) for the reference glucose drink and sponge cake was calculated according to the recommended method by WHO (FAO/WHO, 1998). The GI values of sponge cake for each subject were calculated as follows:

\[
\text{GI of sponge cake} = \frac{\text{iAUC for sponge cake}}{\text{iAUC for reference}} \times 100.
\]

The GI value of sponge cake was calculated as the average value obtained for 8 subjects.

### 7.3.3 Statistical analyses

Results were checked for normality using the Kolmogorov-Smirnov test (K-S test) and expressed as a means ± one standard error of the mean (SEM). Paired t-test was used to analyse differences in the iAUC for reference
glucose and sponge cake (SPSS 16.0 for Windows; Copyright © 2009 SPSS Inc.). All data were examined using a two-tailed approach with a level of $p < 0.05$ being considered as significant.

7.5 Results

7.5.1 RAG, SAG and TG results

The $G_{20}$, $G_{120}$ and TG values for sponge cake were 33.9, 36.4 and 36.9 g respectively. These figures indicate that about 91% of CHO in sponge cake was released during the first 20 min. The RAG and SAG values for sponge cake were calculated and these values were $33.9 \pm 0.4$ and $2.5 \pm 0.7$ g, respectively (see Appendix 17).

7.5.2 GI determination

The subjects' characteristics are displayed in Table 7.1. They had an average age of 31 years and were modestly overweight according to the average BMI score but had normal blood pressure and fasting blood glucose levels.

The peak blood glucose level occurred at 30 min for both the standard glucose solution and sponge cake (Figure 7.1). The glucose level then declined over the next 60 min and continued to drop to below the baseline at 90 min for the standard glucose drink while it remained steady at the baseline level for the sponge cake. The incremental area under the curve (iAUC) for glucose was calculated for the reference (glucose drink) and sponge cake (see Appendix 18). The iAUC for the reference glucose was higher than that
for sponge cake, but this was not significant \( (p = 0.53) \). The GI of sponge cake was calculated to be high and found to be 78 ± 9.

**Table 7.1** Descriptive characteristics of study participants at baseline.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>31 ± 2.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ± 6.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 1.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78.3 ± 2.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>119.2 ± 3.2</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.9 ± 0.06</td>
</tr>
</tbody>
</table>

Results presented as Mean ± SEM (n=8).
**Figure 7.1** Plasma glucose responses during 2 h following reference glucose and sponge cake. Results presented as Mean ± SEM.

### 7.6 Discussion

The objective of this study was to investigate the RAG level of sponge cake and its accompanying GI value. A further objective was to compare and contrast the RAG and GI values for other carbohydrate rich foods. The sponge cake was first selected as a food with a low GI value (Foster-Powell *et al.*, 2002) despite the fact that the presence of its ingredients in other foods results in a high GI. For example, the GI values for wholemeal bread is 69 (Appendix 19); and its RAG value is 36 g (Englyst *et al.*, 1996a) similar to the RAG value found for sponge cake in this study (33.9 g). These confounding results led us to take a decision to undertake the determination of the GI of sponge cake. In this study we found that sponge cake had a GI of 78. This was in agreement with the expected association as foods with a high
GI have been shown to have a high RAG value as there is a high positive correlation \((r = 0.8-0.9, p < 0.05)\) between GI and RAG values (Englyst et al., 1999). Using the correlation between the GI and RAG values for some starchy foods (Figure 7.2) an approximation of GI may be derived from a predetermined RAG value using the equation of the regression line. By doing so a RAG value of 33.9 g, would give a GI of around 58. However, our finding fits well with the fact that high GI foods generally have high RAG and low SAG values.

![Figure 7.2 The correlation between GI and RAG values for some starchy foods (Englyst et al., 1996a; 2003; Gasetti et al., 2005).](image)
To investigate the relationship further a correlation was created for foods with some similar ingredients to sponge cake. Indeed, when the GI values were correlated with RAG values for just wheat flour based foods, the predicted GI value for sponge cake using the formula (shown in the top of Figure 7.3) could be calculated to be around 60. These foods are also plotted in Figure 7.3. Clearly therefore the sponge cake GI value in the literature appears to be much lower than one would anticipate for a number of reasons.

Figure 7.3 The correlation between GI and RAG values for 7 wheat based foods (Englyst et al., 1996a; 2003; Gasetti et al., 2005).

The GI study of Jenkins et al. (1981) found that sponge cake has a low GI (46). Our finding is not consistent with this value. This discrepancy could be attributed to the differences in the contents and preparation methods. Despite contacting the author of the Wolever and Jenkins paper (1981) it was
apparent that the method and recipe which had been used to prepare the sponge cake in 1981 was not recorded and could not be recalled. As stated in the introduction, the presence of other components, such as protein and fat, could lower the GI value of foods. As we followed the standard recipe for sponge cake we would not expect to get a large difference in protein content between our sponge cake and that used in Jenkins’ study as a typical sponge cake contains the same number of eggs. However; there could potentially be a difference in the fat content between recipes. The percentage of fat in sponge cake prepared without fat is 6.7 g/100, while this figure increases to 26.5 g/100 g in sponge cake prepared with fat (Paul & Southgate, 1979). However, the figure of 26.5 g/100 g is still under that required to have an effect on the GI value of the food (Wolever et al., 1991). Nevertheless, it is important to note that whatever method was used to prepare the sponge cake it would have been difficult to achieve a GI value similar to that which was reported in 1981. As such we believe that the GI value of 78 for sponge cake in this study is more reliable by considering the high RAG value of sponge cake and also the number of participants (8) used in this study. Indeed, it is important to note that the GI for sponge cake reported in the 1981 study was calculated from data obtained for just 5 participants. It is appreciated that that GI values can vary and that between 8 and 12 participants are required to obtain an accurate GI value. In effect the GI value recorded previously for sponge cake has been obtained without following FAO/WHO procedures and thus this value may be potentially mis-leading.

In conclusion, the RAG value obtained for sponge cake was shown to relate well with its new GI value. When using other data as well it appears that the
correlation between the GI and RAG value suggests the latter can be used as a direct comparison for starchy foods on an equal weight basis. However, it is appreciated that this approach has its limitations. Other factors which can influence the GI value, such as the degree of chewing and gastrointestinal emptying, can not be reflected and measured from the RAG measurement and as such variations can occur.
8.0 General discussion

One of the greatest health problems associated with excess morbidity and mortality around the world wide is DM. The disease afflicts many living in affluent industrialised nations and it is apparent that the incidence of the disease is increasing. Saudi Arabia is a country with a population of approximately 27 million. Its people have experienced great changes in the last 70 years as a result of the discovery of oil. This has resulted in large social changes and now the population is experiencing a high prevalence of DM which is increasing at an alarming rate. One of the main causes for this phenomenon is due to rapid changes in habitual eating patterns, in particular the quantity and quality of dietary CHOs. Several strategies have been investigated to prevent or alleviate the acuteness of this problem. A modification in the type and amount of the dietary CHOs intakes is known to alter the impact of dietary CHOs on the plasma glucose and insulin profiles and this presents a useful strategy to alleviate some of the problems associated with DM.

The only way of ranking foods based on their type of CHOs in a physiological way is by using the GI concept. Other indices, although determined in vitro, are available and it is appreciated that the RAG value of foods could be used as a complementary tool for determining the influence of starchy foods on glycaemic response. We considered that as there has been a dramatic change in the habitual diets in Saudi Arabia and that the population has generally moved away from long established foods that these traditional foods may have a beneficial effect on plasma glucose and insulin levels owing to the fact that their CHO may be slowly hydrolysed and thus absorbed
CHAPTER 8

at a slower rate in the small intestine. As such the RAG measurement was
developed and tested for a range of UK and Saudi foods and compared with
a more commonly used index, the GI.

8.1 Glycaemic and insulinaemic indices of Hassawi rice and Uncle
Ben's rice

The first objective of this study was to determine the glycaemic and
insulinaemic indices of Hassawi rice in comparison to Uncle Ben's rice. We
hypothesised that Hassawi rice would have a beneficial affect on plasma
glucose and insulin responses as it was an unprocessed traditional rice.
Interestingly, although no difference was observed in glucose response
between Hassawi rice and Uncle Ben's rice, the plasma insulin response to
Hassawi rice was significantly lower than that for Uncle Ben's rice. This could
be perceived as beneficial for people with diabetes especially those who
have a hyperinsulinaemia. The precise reason for the difference in II between
the two rice varieties will be discussed later.

The results showed that Hassawi rice can be classified as a food with a
medium GI (59) while the GI value of Uncle Ben's rice fell within the low GI
bracket (54). However, the difference between the two types of rice regarding
their glycaemic response was not significant. It is important to note that the
GI of the Uncle Ben's rice tested in this study compared well with the
published one (54 vs. 52; Foster-Powell et al., 2002). This is important as it
validates the methods that were used in this study to generate and calculate
the GI. It is noteworthy, however, that results from our study can be deemed
to be the first documented GI for Hassawi rice.
Classifying rice as a high or low GI food depends to a large extent on the amylose to amylopectin ratio. High amylose rice varieties (25-33 % amylose) have been shown to elicit a lower GI and II value (Brand-Miller et al., 1992) compared to low amylose rice varieties. For the rice varieties tested in this thesis it was observed that although there was a small difference in the amylose content between Hassawi rice (17.5 %) and Uncle Ben’s rice (12.7 %) this did not impact upon the GI to any observable extent. This confirms that the amylose content alone may not be a good predictor of glucose response to rice (Panlasigui et al., 1991; Benmoussa et al., 2007).

The relatively low glucose response to Uncle Ben’s rice compared to Hassawi rice could also be explained by the fact that the Uncle Ben’s rice is parboiled rice whereas Hassawi rice is not. The process of parboiling can influence the digestibility of rice and therefore the release of glucose. Changes in starch structure can occur during parboiling and this can affect the gelatinisation and degradation properties which have been shown to slow the digestion and absorption of the starch (Benmoussa et al., 2007; Panlasigui et al., 1991). However, this effect depends on the severity of parboiling process. Studies have shown that traditional parboiling of rice had no effect on the glycaemic response compared to pressure parboiling which reduces the GI of rice (Larsen et al., 2000). This may explain why the GI values were generally similar for the two rice varieties. Nevertheless, it must be noted that the Uncle Ben’s rice was chosen in this study as it is commonly consumed in Saudi Arabia and because of its standardised nature to which other rice varieties can be compared.
It is noteworthy that the II for Hassawi rice was lower than its GI value. This finding was in accordance with that found for varieties of Australian rice (Brand-Miller et al., 1992).

The lower insulin response to Hassawi rice could be due to the higher content of NSP in Hassawi rice compared to that present in Uncle Ben's rice. Indeed, it is well known that a high NSP containing diet is associated with a favourable effect on insulin sensitivity and may protect against the development of DM (McMillan-Price & Brand-Miller, 2006; Liese et al, 2005).

Hassawi rice had a low GL (serving size is 150 g) and this was significantly lower than that observed for Uncle Ben's rice. This important finding suggests that this type of rice may have important benefits on postprandial glycaemic and insulinaemic levels. Therefore, Hassawi rice may have a role to play in the management and prevention of T2DM in Saudi Arabia where rice is considered as a major staple food. The rice is not usually consumed individually but it is served with cooked vegetables and meat. These complementary components add valuable nutrients to the consumption of the rice.

As this study on Hassawi rice is the first document the physiological effects of this rice, the information may be applied for further research to ascertain the clear benefits of Hassawi rice.
8.2 Metabolic impact of high and low SAG meals on blood glucose, insulin and lipids levels

The second objective of this series of studies was to understand whether the RAG and SAG values can be used to explain more about the metabolic difference of starchy foods/meals. In this respect, two meals with similar GIs but different SAG contents were employed to examine their metabolic effects on blood glucose, insulin, TAG and NEFA levels in healthy people. It is well established that the RAG value is a good indicator in predicting the metabolic impact of CHO's on blood glucose and insulin profiles (Englyst et al., 1996a; 1999; 2003). But this study examined the SAG effect specifically.

As expected, the high SAG meal elicited slightly lower glucose and insulin responses compared to the low SAG meal. A possible explanation might be due to the fact that foods with a high content of SAG contain an amount of available glucose that is likely to be hydrolysed and absorbed more slowly, and thus delay the elevation of blood glucose and insulin levels. However, it is important to note that the reduction in blood glucose and insulin levels did not achieve statistical significance overall although there were small differences in insulin at some time points (45 min). This might be due to the fact that there was no difference in the RAG values between the two meals and this will perhaps have a greater role to play on the GI and II. Nevertheless, this was a study looking at the effects of two meals in an acute setting. It would be interesting to study this further by examining the effect of a longer term intervention on the metabolic parameters associated with insulin resistance and glucose control.
It was not easy to create two meals with a similar GI and RAG but with a large difference in SAG content. However, this finding supports the idea that high SAG foods could be a target for planning diets which can improve the postprandial glucose and insulin responses in people with diabetes (Englyst et al., 2003).

8.3 The RAG and SAG measurement

The in vitro method for starch hydrolysis has been proposed as a faster and more cost effective method for predicting the GI of starchy foods, however; there is no official standard method for the assessment of the glucose released at specific time points and these can vary between methods. Clearly Englyst has defined RAG and SAG measurements and these relate to glucose released at 20 and 120 min after the start of hydrolysis (Englyst et al., 20000; Germaine et al., 2008; Zhang & Hamaker, 2009). Differences between the methods for the in vitro measurement of starch digestibility rates include variations in the initial food breakdown procedures and incubation system as well as the timing of samples (Zhang & Hamaker, 2009; Germaine et al., 2008; Goni et al., 1997; Granfeldt et al., 1995; Englyst et al., 1992). Nevertheless, the Englyst system adopted provided a method for which a relatively large number of foods had been previously analysed and as such was the best starting point in this thesis.

It is well known that GI values of more than a thousand different foods have so far been published (Brand-Miller et al., 2009). However, the number of foods for which both GI and RAG and SAG are available is rather small (about 80 foods). RAG and SAG measurements may prove to be a useful
index to help further understand the impact of CHO~s in vivo. Therefore, it was necessary to establish the methodology of RAG and SAG determination during the preliminary stages of the project so that the method could be used for RAG and SAG determination for some common Saudi and UK foods as well as three reference foods.

Three methods were used to determine the concentration of released glucose for the reference foods during the RAG and SAG measurements. Overall, the results for RAG and SAG generated from these foods in this study generally confirm the observations in the literature. For example, the RAG and SAG for wheat flour obtained from our results were 39.7 g and 43.0 g, respectively, compared to 35.0 g for the RAG and 42.0 g for the SAG in literature. Nevertheless, the colorimetric results obtained from the manual spectrophotometric assay were more consistent with those in the literature for both RAG and SAG of the three reference foods. As such this method was taken forward to analyse RAG and SAG in other foods.

For RAG and SAG values for some foods, our results were comparable with those obtained from previous study (Englyst et al., 1996a). For example, we found that the RAG and SAG for Long Grain rice were 22.0 g vs. 19.3 g and 9.3 g vs. 10.2, respectively. We also found that Hareece as a traditional Saudi food, consumed as whole grains wheat, showed a lower RAG value (9.8 g/100 g as eaten) compared to wheat-based products such as macaroni (20.8 g/100 g as eaten). Interestingly, the GI of Hareece was found to be low with a value of 52 (Nasib, 2003). This is an important feature of Hareece which could provide substantial contribution to the improvement of the diets in Saudis populations.
In these series of studies, the Englyst method for RAG and SAG measurement was applied. This method has shown to be applicable to a variety of foods and is widely accepted in the food industry (Zhang & Hamaker, 2009). Moreover, Englyst’s method analyses foods as eaten without requiring further pretreatment of samples compared to, for example, Goni’s method in which lipids and proteins need to be removed from the samples prior to starch analysis (Goni et al., 1997).

The correlation between the GI and RAG and SAG value has been studied and found that GI is correlated positively with RAG \((r = 0.5; p = 0.01)\) while there is a negative correlation \((r = -0.41; p = 0.04)\) between GI and SAG value (Garsetti et al., 2005). This was consistent with our results for sponge cake. The RAG and SAG for sponge cake showed that it had high RAG and low SAG values, however, its GI value was low (46) as stated in the International Table of GI and GL. When the GI of sponge cake was determined in our Clinical Investigation Unit, it showed that sponge cake had a high GI value (78) indicating that the RAG value correlates well with the GI value of sponge cake. The relationship between the GI and RAG value was also examined for other Saudi and UK foods showing that the GI values of these foods correlated positively with their RAG values \((r = 0.7; p = 0.06)\). Although this did not achieve significance \((p > 0.05)\) a clear trend was observed. This lack of a significant correlation might be due to the small number of foods tested in this study. The exception to the rule was that GI of dates which was found to be 55, despite the fact that dates had a high RAG value (31) and low SAG value (0.7). This could be understood by the fact that dates have a considerable amount of fructose present. It has been found that
the glucose to fructose ratio in dates was 1.1 (Ali et al., 2008) and fructose has an effect in lowering the plasma glucose response by inhibiting gluconeogenesis (Louie et al., 2008; Heacock et al., 2002; Wolever et al., 1994). In addition, dates is considered as a good source of phenolics, antioxidant, and NSP (Al-Shahib & Marshall, 2002; Al-Farsi et al., 2007; Al-Humaid et al., 2010) and all these components may play a role in lowering plasma glucose response to date by reducing CHO digestion (Frost & Dornhorst, 2000) or glucose absorption from the small intestine (Bidel et al., 2008; Higdon & Frei, 2006).

8.4 The traditional Saudi practice

It is evident that Saudi habitual dietary patterns have recently changed in accordance with the rapid improvement in socioeconomic status of the country. However, there is still some common place traditional practice that remains, such as the consumption of dates with Arabic coffee. Although there are some studies evaluating the effects of coffee on glucose absorption and metabolism there appeared to be no studies with dates. The results of our study showed that the consumption of Arabic coffee with dates slightly worsened the plasma glucose profile in healthy volunteers. As the levels of phenols in this beverage are low, this effect is unlikely to be due to the phenolic acids (e.g. CGA) present. Indeed, the effect of coffee phenols has been shown using a large volume (~500 ml) of decaffeinated coffee which produced a lower postprandial glucose response and higher insulin sensitivity (Battram et al., 2006). The effect, however, could be explained by the presence of caffeine in the coffee (Moisey et al., 2008) as this methylxanthine
has been shown to exacerbate the metabolism of glucose. As the consumption of dates with Arabic coffee is a traditional Saudi practice that could be difficult to change, we suggest that the volume of the traditional Arabic coffee beverage consumed during dates consumption could be reduced. Another suggestion is the consumption of an alternative coffee such as a decaffeinated Arabic coffee, barley coffee (Sha’aer coffee) or nucleons dates coffee (Nawat altamer coffee). The latter two types of coffee have recently been introduced and are now commonly consumed in Saudi Arabia as they have met people’s desires. It is evident that these two types of beverage are free of caffeine which has been shown to be linked to impaired glucose tolerance and reduced insulin sensitivity (Louie et al., 2008; Battram et al., 2006; Keijzers et al., 2002). These suggested alternative coffees could be useful in allowing a normal digestion and absorption of dates and in the meanwhile preventing the adverse impacts of caffeine presented in coffee. It is noteworthy that it recommended that caffeine intake should be limited for children and pregnant women as it can result in some behavioural effects, such as increased nervousness or sleep disturbances as well as its effect on glucose and insulin responses as discussed previously (Higdon & Frei, 2006).

Nevertheless, experiments would need to be conducted to ascertain whether this consumption of an alternative coffee alongside dates would have any beneficial or detrimental effects. It is noteworthy that long term coffee consumption is associated with a decreased incidence of T2DM. Whether this alternative Arabic coffee would have a beneficial health effect is unknown and rather speculative and would require long term studies to establish this.
8.5 Concluding remark

- This study suggests that RAG value could be used as a complementary tool along with the GI value with some starchy foods, and the SAG value may be a good determinant of physiological impact of foods.

- A major practical implication from the findings of this thesis is to encourage the consumption of some selected Saudi traditional foods.

- This study is the first to measure the GI and II of some traditional Saudi foods, revealing that these foods have intermediate glycaemic and insulinaemic indices.

- Despite a similar GI value, a meal with a high SAG value produced a small reduction in peak glucose concentration and a general lowering of insulin response. Foods with high SAG values may therefore be useful in controlling postprandial glucose levels in both healthy and diabetic people.

- For the studies involving the consumption of Arabic coffee with dates, we would recommend that diabetic people may be able to continue consuming the dates which have a low GI and are a good source of NSP. The influence of the coffee beverage indicates that some detrimental effects can occur and the consumption of a decaffeinated coffee may be wise.
8.6 Further works

- Further studies are needed to confirm the impact of long term consumption of traditional foods (e.g. Hassawi rice and Hareece) on blood glucose and insulin levels in healthy people and those with diabetes.

- Measurement of GI and II for Saudi traditional meals rather than individual foods such as Hassawi rice Kabsa, Hareece with meat and dates with nuts.

- A long term study investigating the impact of consuming high SAG meals on blood glucose and insulin profiles is also recommended.
PUBLICATIONS
Full Papers


Abstracts


REFERENCES
REFERENCES


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APPENDICES
APPENDICES

Appendix 1

Examples of suitable sample weights (taken from Englyst et al., 1992).

<table>
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<tr>
<th>Dry matter (%)</th>
<th>Examples</th>
<th>Weight (g)</th>
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<tbody>
<tr>
<td>75-100</td>
<td>Starch</td>
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<tr>
<td>75-100</td>
<td>Flours, breakfast cereals</td>
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<tr>
<td>55-75</td>
<td>Bread, cakes</td>
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<tr>
<td>35-55</td>
<td>Beans, pasta, rice</td>
<td>1.5-3</td>
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<td>15-35</td>
<td>Canned foods, sauces</td>
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</table>
Appendix 2

Health and Lifestyle Questionnaire

The effect of Low and High SAG Meals on Postprandial Blood Glucose and Lipid Levels of Healthy Volunteers

Name: ..................................................  DOB: ......................
Address: ............................................................
............................................................
............................................................
Daytime Tel: ........................................... Evening Tel: ..............................
GP Name: ............................................................
GP Address: ............................................................

Anthropometric measurements
Weight (kg): .............. Height (m): ..............

Blood pressure

<table>
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<th>3&lt;sup&gt;rd&lt;/sup&gt;</th>
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<tbody>
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<td>Diastolic</td>
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Blood samples

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General Health

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<td>Psychiatric Disorders</td>
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<td></td>
</tr>
<tr>
<td>Drug/Alcohol dependence</td>
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<td></td>
</tr>
<tr>
<td>Epilepsy</td>
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<td></td>
</tr>
<tr>
<td>Sleep Disorders e.g. Insomnia</td>
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<td></td>
</tr>
<tr>
<td>Endocrine Disorders (inc. Diabetes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure-Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal Disease</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you take any dietary supplements such as vitamins, minerals or fish oils?
YES/NO
If yes, please state which type and how often.

Are you currently on a weight-reducing diet or other dietary restrictions?
YES/NO
If yes, please give details.

Do you exercise regularly?
YES/NO
If yes, what type of exercise and how often?

Have you been involved in a clinical trial in the last 3 months?
YES/NO
In the study day, do you have a problem eating the following foods at breakfast time (please tick appropriate box):

<table>
<thead>
<tr>
<th>Meal (1)</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hassawi rice (brown rice) with fried onion &amp; tomato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabic dates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meal (2)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncle Ben's rice with fried onion &amp; tomato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabic dates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you smoke?

YES/NO

If yes, how many per day?

Do you drink alcohol?

YES/NO

If yes, how many units per week? (See next page describing units of alcohol)

Thank you for your time in completing this questionnaire. All information will be kept strictly confidential at all times.
What is a unit of Alcohol?

The list below shows the approximate number of units of alcohol in common drinks:

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Measure</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary strength lager (4 %) e.g. Carling, Fosters</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Strong lager (5.2%) e.g. Stella Artois, Kronenburg</td>
<td>Pint</td>
<td>3</td>
</tr>
<tr>
<td>Strong lager e.g. Stella Artois, Carlsberg Export, Grolsch</td>
<td>440ml can</td>
<td>2.2</td>
</tr>
<tr>
<td>Beer/ordinary strength Ale e.g. John Smith's, Guinness</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Std 175ml</td>
<td>2</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Lg. 250ml</td>
<td>3</td>
</tr>
<tr>
<td>Spirits</td>
<td>Std 25ml</td>
<td>1</td>
</tr>
<tr>
<td>Spirits</td>
<td>Lg. 35ml</td>
<td>1.4</td>
</tr>
<tr>
<td>Alcopop e.g. Smirnoff Ice, Bacardi Breezer, Reef</td>
<td>275ml</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Appendix 3

UNIVERSITY OF SURREY CONSENT FORM FOR THE DONATION OF BLOOD FOR TEACHING OR RESEARCH

To be completed by all volunteers donating blood for use in the University.

Please read this carefully before signing. Your signature on the consent form is taken as evidence that you have read the statements.

The purpose of this consent form is to act as an obvious means of screening for situations which could put the donor, phlebotomist (trained person taking the blood) or user of the specimen at risk. It allows the donor the opportunity at any time to withdraw from blood donation without having to justify a reason for doing so.

For volunteers intending to donate blood on more than one occasion for a particular study it is sufficient to sign this form only once on the understanding that if reasons arise which make donation inadvisable, no further samples are donated.

SECTION A (to be completed by ALL donors)
*delete as appropriate

I ..............................................................Name in BLOCK CAPITALS)

An *undergraduate/*postgraduate/*member of staff/*person from the general public

of the .............................................................. Department

or *visitor/*volunteer with no connection to the University

Agree to give blood on the following terms:

1. The procedure has been explained to me
2. I understand that participation in the procedure shall be entirely at my own risk and that the University of Surrey will not accept liability for any effects suffered by me as a result of such participation except to the extent that it can be shown to have been negligent. I understand that this does not affect my statutory rights.

3. I am over 18 years of age and am knowingly suffering from any medical condition which would make it inadvisable for me to donate blood.

4. I agree to cease donating blood should reasons arise for doing so.

Signed ................................................. Date..............................................
Appendix 4

Sample Consent Form

Please select the point(s) which are relevant to your protocol:

- I the undersigned voluntarily agree to take part in the study on .................

- I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill-effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

- I agree to comply with any instruction given to me during the study and to cooperate fully with the investigators. I shall inform them immediately if I suffer any deterioration of any kind in my health or well-being, or experience any unexpected or unusual symptoms.

- I agree to the investigators contacting my general practitioner about my participation in the study, and I authorise my GP to disclose details of my relevant medical or drug history, in confidence.

- I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998). I agree that I will not seek to restrict the use of the results of the study on the understanding that my anonymity is preserved.

- I understand that I am free to withdraw from the study at any time without needing to justify my decision and without prejudice.

- I acknowledge that in consideration for completing the study I shall receive the sum of £.... I recognise that the sum would be less, and at the discretion of the Principal Investigator, if I withdraw before completion of the study.

- I understand that in the event of my suffering a significant and enduring injury (including illness or disease) as a direct result of my participation in the study, compensation will be paid to me by the University (or sponsor where a clinical trial is sponsored by a pharmaceutical company), subject to certain provisos and limitations. The amount of compensation will be appropriate to the nature, severity and persistence of the injury and will, in general terms, be consistent with the amount of damages commonly awarded for similar injury by an English court in cases where the liability has been admitted.

- I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

Name of volunteer (BLOCK CAPITALS)........................................................................................................
Appendix 5

Blood sampling sheet (GI study)

<table>
<thead>
<tr>
<th>Time</th>
<th>Start study</th>
<th>YSI results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Time 0 is before taking test drink/food. Take your first blood sample and then drink/eat your test meal and note time down) in the Start study column)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (0 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 min after eating</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 6

**Blood sampling sheet (Low & High SAG study)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Actual time</th>
<th>Blood volume</th>
<th>Blood tube</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL (G) Pink: 4mL (I, NEFA, TG)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td>5 mL</td>
<td>Yellow:1mL Pink: 3mL</td>
<td></td>
</tr>
</tbody>
</table>
## Appendix 7

RAG and SAG values (g/100 g as eaten) for the three reference foods that were obtained using the three methods of determination of the released glucose.

<table>
<thead>
<tr>
<th>Methods</th>
<th>R1 (White flour)</th>
<th>R2 (potato starch)</th>
<th>R3 (Cornflakes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAG</td>
<td>SAG</td>
<td>RAG</td>
</tr>
<tr>
<td></td>
<td>39.019</td>
<td>41.325</td>
<td>7.657</td>
</tr>
<tr>
<td></td>
<td>39.976</td>
<td>39.447</td>
<td>7.657</td>
</tr>
<tr>
<td></td>
<td>39.051</td>
<td>47.896</td>
<td>9.869</td>
</tr>
<tr>
<td></td>
<td>39.477</td>
<td>37.893</td>
<td>8.801</td>
</tr>
<tr>
<td></td>
<td>38.303</td>
<td>37.566</td>
<td>8.801</td>
</tr>
<tr>
<td>Mean</td>
<td>38.997</td>
<td>41.375</td>
<td>8.336</td>
</tr>
<tr>
<td>SEM</td>
<td>0.282</td>
<td>1.637</td>
<td>0.405</td>
</tr>
<tr>
<td>Auto-analysers</td>
<td>43.818</td>
<td>51.891</td>
<td>6.15</td>
</tr>
<tr>
<td></td>
<td>43.818</td>
<td>47.278</td>
<td>8.456</td>
</tr>
<tr>
<td></td>
<td>43.818</td>
<td>48.432</td>
<td>8.918</td>
</tr>
<tr>
<td></td>
<td>43.818</td>
<td>46.122</td>
<td>8.456</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>43.8</td>
<td>42.2</td>
<td>6.15</td>
</tr>
<tr>
<td>Mean</td>
<td>42.845</td>
<td>45.987</td>
<td>7.188</td>
</tr>
<tr>
<td>SEM</td>
<td>0.969</td>
<td>1.758</td>
<td>0.427</td>
</tr>
<tr>
<td>HPLC</td>
<td>35.352</td>
<td>42.831</td>
<td>8.405</td>
</tr>
<tr>
<td></td>
<td>35.322</td>
<td>44.261</td>
<td>9.859</td>
</tr>
<tr>
<td></td>
<td>39.588</td>
<td>39.904</td>
<td>10.586</td>
</tr>
<tr>
<td></td>
<td>35.322</td>
<td>44.261</td>
<td>9.859</td>
</tr>
<tr>
<td></td>
<td>39.588</td>
<td>39.904</td>
<td>10.586</td>
</tr>
<tr>
<td>Mean</td>
<td>37.497</td>
<td>41.844</td>
<td>9.738</td>
</tr>
<tr>
<td>SEM</td>
<td>0.96565</td>
<td>0.8934</td>
<td>0.3466</td>
</tr>
</tbody>
</table>
### Appendix 8

RAG and SAG values (g/100 g as eaten) for test foods

<table>
<thead>
<tr>
<th>N</th>
<th>Hareece</th>
<th>Dates</th>
<th>Long grain rice</th>
<th>Macaroni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAG</td>
<td>SAG</td>
<td>RAG</td>
<td>SAG</td>
</tr>
<tr>
<td>1</td>
<td>9.69</td>
<td>1.05</td>
<td>29.77</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>11.00</td>
<td>2.93</td>
<td>30.88</td>
<td>0.55</td>
</tr>
<tr>
<td>3</td>
<td>9.63</td>
<td>1.83</td>
<td>30.94</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>10.22</td>
<td>0.52</td>
<td>31.07</td>
<td>1.17</td>
</tr>
<tr>
<td>5</td>
<td>9.40</td>
<td>2.05</td>
<td>31.63</td>
<td>0.64</td>
</tr>
<tr>
<td>6</td>
<td>9.10</td>
<td>2.35</td>
<td>32.11</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean</td>
<td>9.84</td>
<td>1.79</td>
<td>31.07</td>
<td>0.74</td>
</tr>
<tr>
<td>SEM</td>
<td>0.27</td>
<td>0.35</td>
<td>0.32</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### Appendix 9

The RAG, SAG and TS values (g/100 g as eaten) for Hassawi rice and Uncle Ben's rice.

<table>
<thead>
<tr>
<th>N</th>
<th>Hassawi rice</th>
<th>Uncle Ben's rice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAG</td>
<td>SAG</td>
</tr>
<tr>
<td>1</td>
<td>15.67</td>
<td>6.456</td>
</tr>
<tr>
<td>2</td>
<td>15.16</td>
<td>6.38</td>
</tr>
<tr>
<td>3</td>
<td>18.69</td>
<td>5.23</td>
</tr>
<tr>
<td>4</td>
<td>15.53</td>
<td>5.165</td>
</tr>
<tr>
<td>5</td>
<td>17.79</td>
<td>4.79</td>
</tr>
<tr>
<td>6</td>
<td>17.4</td>
<td>4.79</td>
</tr>
<tr>
<td>SEM</td>
<td>0.5899</td>
<td>0.3096</td>
</tr>
</tbody>
</table>
Appendix 10

The macronutrients values (g/100 g as eaten) for Hassawi rice and Uncle Ben’s rice.

<table>
<thead>
<tr>
<th>No</th>
<th>Hassawi rice</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moisture%</td>
<td>CHO%</td>
<td>Fat%</td>
<td>Protein%</td>
<td>Ash%</td>
<td>NSP%</td>
<td>Amylose%</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>63.113</td>
<td>22.127</td>
<td>0.789</td>
<td>3.7757</td>
<td>0.543</td>
<td>0.6</td>
<td>17.27</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>62.488</td>
<td>21.54</td>
<td>0.7982</td>
<td>3.7288</td>
<td>0.5337</td>
<td>0.61</td>
<td>17.56</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>62.014</td>
<td>23.924</td>
<td>0.7689</td>
<td>3.7255</td>
<td>0.544</td>
<td>0.62</td>
<td>17.79</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20.695</td>
<td>0.7431</td>
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</tr>
<tr>
<td>5</td>
<td>20.593</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>62.53</td>
<td>21.52</td>
<td>0.77</td>
<td>3.74</td>
<td>0.54</td>
<td>0.61</td>
<td>17.54</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.31</td>
<td>0.55</td>
<td>0.012</td>
<td>0.016</td>
<td>0.003</td>
<td>0.005</td>
<td>0.150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>Uncle Ben’s rice</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moisture%</td>
<td>CHO%</td>
<td>Fat%</td>
<td>Protein%</td>
<td>Ash%</td>
<td>NSP%</td>
<td>Amylose%</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>63.655</td>
<td>32.305</td>
<td>0.2625</td>
<td>2.8883</td>
<td>0.2449</td>
<td>0.26</td>
<td>13.04</td>
<td></td>
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<tr>
<td>2</td>
<td>65.332</td>
<td>30.471</td>
<td>0.2569</td>
<td>2.9044</td>
<td>0.2516</td>
<td>0.25</td>
<td>12.43</td>
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</tr>
<tr>
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<td>63.528</td>
<td>31.816</td>
<td>0.2244</td>
<td>2.818</td>
<td>0.2457</td>
<td>0.26</td>
<td>13.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>64.971</td>
<td>29.99</td>
<td>0.2172</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>32.322</td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>Mean</td>
<td>64.37</td>
<td>31.07</td>
<td>0.24</td>
<td>2.87</td>
<td>0.24</td>
<td>0.25</td>
<td>12.85</td>
</tr>
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<td></td>
<td>SEM</td>
<td>0.45</td>
<td>0.50</td>
<td>0.01</td>
<td>0.02</td>
<td>0.002</td>
<td>0.003</td>
<td>0.210</td>
</tr>
</tbody>
</table>
Appendix 11

The individual iAUC of glucose for reference glucose, Hassawi rice and Uncle Ben’s rice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>iAUC for reference glucose</th>
<th>iAUC for Hassawi rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>116</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>60.8</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>125.1</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>120.6</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>97.5</td>
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<td>94</td>
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### Appendix 12

The individual iAUC of insulin for reference glucose, Hassawi rice and Uncle Ben's rice.

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<td>5253</td>
</tr>
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<td>2667</td>
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<td>6</td>
<td>2501</td>
<td>1597</td>
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<td>7</td>
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<td>6391</td>
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<tr>
<td>8</td>
<td>2985</td>
<td>711</td>
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<tr>
<td>9</td>
<td>1349</td>
<td>472</td>
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<tr>
<td>10</td>
<td>6081</td>
<td>3754</td>
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<td>Mean</td>
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<td>2224.2</td>
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<td>2</td>
<td>4617</td>
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<tr>
<td>3</td>
<td>688</td>
</tr>
<tr>
<td>4</td>
<td>4968</td>
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<td>3882</td>
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<td>1407</td>
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<td>1506</td>
</tr>
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<td>9</td>
<td>2425</td>
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<tr>
<td>10</td>
<td>758</td>
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240
Appendix 13

Low and high SAG meals composition.

<table>
<thead>
<tr>
<th></th>
<th>Weight (KJ)</th>
<th>Energy (Kcal)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Food GI</th>
<th>Meal GI</th>
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<tbody>
<tr>
<td><strong>Low SAG meal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hassawi rice, cooked</td>
<td>200</td>
<td>990.44</td>
<td>238.66</td>
<td>48</td>
<td>1.54</td>
<td>8.2</td>
<td>59</td>
</tr>
<tr>
<td>tomato paste</td>
<td>5</td>
<td>17.8</td>
<td>4.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td></td>
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<tr>
<td>Onion</td>
<td>10</td>
<td>14.94</td>
<td>3.6</td>
<td>0.8</td>
<td>0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td>8</td>
<td>298.8</td>
<td>72</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sour milk</td>
<td>225</td>
<td>394.66</td>
<td>95.1</td>
<td>9.2</td>
<td>3.5</td>
<td>6.7</td>
<td>36</td>
</tr>
<tr>
<td>Arabic dates</td>
<td>45</td>
<td>479.49</td>
<td>115.54</td>
<td>27.9</td>
<td>0.14</td>
<td>0.67</td>
<td>41</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>493</td>
<td>2196.13</td>
<td>529.2</td>
<td>86.2</td>
<td>13.4</td>
<td>15.7</td>
<td>49.6</td>
</tr>
</tbody>
</table>

| **High SAG meal**      |             |               |         |         |             |         |         |
| Uncle Ben's rice,      | 200         | 1150.63       | 277.26  | 62      | 0.46        | 6.28    | 54      | 37.5    |
| cooked                 |             |               |         |         |             |         |         |
| tomato paste           | 5           | 17.8          | 4.3     | 0.3     | 0.3         | 0.1     |         |         |
| Onion                  | 10          | 14.94         | 3.6     | 0.8     | 0           | 0.1     |         |         |
| Oil                    | 8           | 298.8         | 72      | 0       | 8           | 0       |         |         |
| Sour milk              | 250         | 489.7         | 118     | 11.3    | 4.4         | 8.3     | 36      | 5.2     |
| Arabic dates           | 20          | 213.21        | 51.376  | 12.4    | 0.064       | 0.3     | 41      | 6.5     |
| **Total**              | 493         | 2185.08       | 526.54  | 86.8    | 13.2        | 15.08   | 49.2    |         |
Appendix 14

The individual iAUC of glucose, insulin, TAG, and NEFA for low and high SAG meals.

<table>
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<tr>
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<th>insulin</th>
<th>TAG</th>
<th>NEFA</th>
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<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
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<td>96.64</td>
<td>94.5</td>
<td>13523</td>
<td>13360</td>
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<td>2</td>
<td>106</td>
<td>88</td>
<td>47952</td>
<td>36018</td>
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<td>3</td>
<td>213.8</td>
<td>36.96</td>
<td>36785</td>
<td>13188</td>
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<td>4</td>
<td>100.9</td>
<td>64.93</td>
<td>17396</td>
<td>19808</td>
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<td>5</td>
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<td>85.01</td>
<td>36947</td>
<td>35977</td>
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<td>127.2</td>
<td>72.4</td>
<td>33719</td>
<td>36310</td>
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<td>49.1</td>
<td>59.75</td>
<td>24549</td>
<td>38995</td>
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<td>8</td>
<td>71.66</td>
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<td>20064</td>
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<td>68.9</td>
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<td>35879</td>
<td>22641</td>
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<td>10</td>
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<td>142</td>
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<td>13971</td>
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<td>11</td>
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<td>77.02</td>
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<td>12</td>
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<td>88</td>
<td>26550</td>
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<td>SEM</td>
<td>15</td>
<td>11</td>
<td>3266</td>
<td>3065</td>
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Appendix 15

Arabic coffee is prepared in a different way from western and other oriental types of coffee. The ingredients and standard preparation of Arabic coffee is mentioned below.

**Ingredient:**

1/2 cup green Arabic coffee beans
2 tea spoon cardamom
1/2 tea spoon saffron
1 litre boiled water

Coffee beans are roasted instantly over an open fire for a period which does not lead to darkening. The roasted coffee beans and cardamom are coarsely ground in a metal mortar. Then boiled water is added to the grounded coffee beans and left over a moderate fire for almost 5 minutes. The boiled coffee is then poured into a special pot contained the cardamom and saffron.
**Appendix 16**

The individual iAUC of glucose and insulin for reference glucose, dates and dates with Arabic coffee.

<table>
<thead>
<tr>
<th>Subject</th>
<th>iAUC of glucose</th>
<th>Reference glucose</th>
<th>Dates with water</th>
<th>Dates with Arabic coffee</th>
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<td>63.504</td>
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<td>130.393</td>
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<td>173.185</td>
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<td>173.97</td>
<td>140.775</td>
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<td>112.1876</td>
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<th>Reference glucose</th>
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<th>Dates with Arabic coffee</th>
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<td>7092.37</td>
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**Appendix 17**

RAG, SAG and TG for sponge cake.

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**Appendix 18**

The individual iAUC of glucose for reference glucose and sponge cake.

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<td>89.7</td>
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<tr>
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<td>5</td>
<td>97.6</td>
<td>103.6</td>
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<td>143.8</td>
<td>58</td>
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<tr>
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<td>103.6</td>
<td>126.6</td>
</tr>
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<td>94</td>
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Appendix 19

The RAG and GI values for 28 starchy foods (Englyst et al., 1996a).

<table>
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<th>Food</th>
<th>RAG (g/100)</th>
<th>GI (Glucose=100)</th>
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<td>Haricot bean</td>
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<td>29</td>
</tr>
<tr>
<td>Chickpea (garbanzo)</td>
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</tr>
<tr>
<td>Red lentil</td>
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<td>29</td>
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<td>Pearled barley</td>
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<tr>
<td>Kidney bean (canned)</td>
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</tr>
<tr>
<td>Chickpea (canned)</td>
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</tr>
<tr>
<td>Sweet potato</td>
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<td>49</td>
</tr>
<tr>
<td>Butter bean</td>
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<td>36</td>
</tr>
<tr>
<td>Pinto bean</td>
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<td>49</td>
</tr>
<tr>
<td>Instant potato</td>
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</tr>
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<td>Buckwheat</td>
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<td>Macaroni</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Brown rice</td>
<td>16.3</td>
<td>67</td>
</tr>
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<td>All Bran</td>
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</tr>
<tr>
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<td>70</td>
</tr>
<tr>
<td>Potato crisps</td>
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<td>51</td>
</tr>
<tr>
<td>Oatmeal biscuits</td>
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<td>57</td>
</tr>
<tr>
<td>French baguette with chocolate</td>
<td>52</td>
<td>72</td>
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<tr>
<td>Shredded Wheat</td>
<td>55.0</td>
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</tr>
<tr>
<td>Cracker</td>
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<td>64</td>
</tr>
<tr>
<td>Weetabix</td>
<td>65.3</td>
<td>76</td>
</tr>
<tr>
<td>Puffed Wheat</td>
<td>70.4</td>
<td>77</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>78</td>
<td>93</td>
</tr>
<tr>
<td>Rice Krispies</td>
<td>80.3</td>
<td>82</td>
</tr>
<tr>
<td>Terminology</td>
<td>Definition</td>
<td></td>
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<tr>
<td><strong>Glycaemic index (GI)</strong></td>
<td>is defined as the incremental area under the glucose response curve (AUC) of a 50g available carbohydrate-containing food expressed as a percentage of the response to the same amount of carbohydrate from standard food taken by the same subject.</td>
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<tr>
<td><strong>Glycaemic load (GL)</strong></td>
<td>measuring the effect of the GI of a food multiply by its available carbohydrate content in grams.</td>
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<tr>
<td><strong>Insulinaemic index (II)</strong></td>
<td>is defined as the area under the curve of the insulin responses to a CHO-containing food compared to either a specific glucose dose or a specific amount of white bread, the so-called insulinaemic response.</td>
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<tr>
<td><strong>Rapidly available glucose (RAG)</strong></td>
<td>reflect the rate of glucose release from food, which is an important determinant of glycaemic available glucose (SAG) index.</td>
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<tr>
<td><strong>Insulin resistance</strong></td>
<td>an inability of cell to respond to insulin.</td>
<td></td>
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<tr>
<td><strong>Insulin sensitivity</strong></td>
<td>the ability of insulin to help move glucose from the bloodstream into the cell.</td>
<td></td>
</tr>
</tbody>
</table>