Glycaemic response and Glycaemic index to five varieties of rice in people of European and Chinese ethnicity

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Abstract

Glycaemic Index (GI) may be used to guide choice of carbohydrate containing foods. GI has typically been determined in small groups of European volunteers and the value thus obtained is assumed to apply to all populations. The aim of this study was to determine whether there are ethnic differences in glycaemic responses and GI to various varieties of rice in people of European and Chinese ethnicity.

Sixty-two healthy volunteers, 31 Chinese and 31 Europeans (18-50yr) consumed 50g of available carbohydrate portions on separate mornings after a 10hr overnight fast. Capillary blood glucose was measured at baseline and over a 2hr period following ingestion of foods (glucose beverage, tested two occasions, and five rice varieties: Jasmine, Basmati, Brown, Doongara® and Parboiled, each tested on a single occasion).

Age, height, and sex distribution were not different between the two groups, but body weight and body mass index (BMI) were significantly lower in the Chinese than the European group (p<0.05). Incremental blood glucose areas under the curve (iAUC) of all tested foods were greater in Chinese than in Europeans (p<0.05). The largest difference was for Parboiled rice for which the Chinese iAUC was 77% (95%CI: 38, 226, p<0.001) higher than the European iAUC. In the Chinese and European groups, respectively, the GI of Doongara® (67, 55), Jasmine (81, 68), and Parboiled rice (72, 57) were significantly higher in the Chinese.

The greater glycaemic response to carbohydrate in Chinese compared with Europeans and the higher glycaemic index for several rice varieties has potential clinical relevance. Regression analysis including variables which might have explained the ethnic differences suggested that age, sex, salivary alpha-amylase and extent of chewing contributed little to the ethnic difference.
Preface

This research project was initiated by Professor Jim Mann, my principal supervisor, after meeting with potential research collaborators from Singapore at a conference in Hong Kong 2009. Professor Mann had a primary responsibility for my supervising research and write-up. Dr. Bernard Venn, who was a co-supervisor, was responsible for funding application, supporting both clinical and laboratory works and writing. Associate Professor Sheila Williams was involved in statistical analysis and advice for the study results. Dr. Lisa Te Morenga was provided much assistance with regard to data analysis and writing up.

I was responsible for:

- Study design
- Submission of ethics application
- Conducting pilot study to determine rice to water ratio
- Conducting pilot study to determine carbohydrate content of test foods
- Ordering and managing laboratory supplies, and calibrating machines
- Recruitment for study participants
- Running the study between Nov 2009 and May 2010
- Preparing reference and test foods
- Developing study protocols
- Managing the research assistants
- Conducting laboratory analysis for salivary alpha-amylase
- Conducting anthropometric measurements
- Data entry and analysis
- Carrying out basic statistical test
- Conducting laboratory tests

I have presented the results of my study at the New Zealand Society for the Study of Diabetes, Dietitians New Zealand, and Nutrition Society conferences during 2011. The funding to support the research was contributed by the Riddet Institute (Palmerston North) and the University of Otago Performance-Based Research Fund (PBRF).
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<td>iAUC</td>
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1 Introduction

The concept of the glycaemic index (GI) is intended to guide choice of carbohydrate-rich foods. Implicit in the concept is that GI is solely a property of the food and that the GI determined in a relatively small group of volunteers applies to all people regardless of their glucose tolerance status or other characteristics such as age, sex or ethnicity (Wolever TM et al., 1991, Wolever TM et al., 1991, Wolever TMS et al., 2003, Wolever TM et al., 2008). Some studies have been carried out to test the assumption that GI is the same in everyone and although it has been concluded that GI appears to be independent of subject status (Brouns F et al., 2005), the evidence is contradictory and potentially confounded by small samples. For example, 15 foods were tested in groups of people with normal glucose tolerance and people with diabetes (Jenkins DJA et al., 1983). A significant correlation between the GIs obtained in the two groups was reported, but on average the GIs in the diabetic group were 22 GI units higher than the GIs obtained in the normal group. In another study involving the testing of five foods in healthy individuals and those with type 2 diabetes, there was variable agreement between groups with differences ranging from 1 to 24 GI units higher in the group with diabetes (Perry T et al., 2000). Despite some large numerical between group differences in GI, none of the GIs were statistically significantly different probably as a consequence of large variability and small samples. If GI is to be used as a guide to food choice, it is important to verify that the same GI applies to everyone, particularly to people with diabetes irrespective of ethnicity. This generalizability is uncertain because differences in iAUC (Dickinson S et al., 2002) and GI (Venn BJ et al., 2010) have been shown between ethnic groups.

A cornerstone of diabetes management is good glycaemic control as assessed by glycated haemoglobin (HbA1c). Poor control has been linked with an increased risk of diabetic complications (Diabetes Control and Complications Trial (DCCT) Research Group, 1995, Stratton IM, 2000). Although variable in results, data from short-term studies indicate that diets selected on the basis of GI have a modest impact on HbA1c (Brand-Miller J et al., 2003). The proposed mechanism is that low GI foods are more slowly absorbed and have a more favourable glycaemic impact compared with high GI foods. In support of this mechanism, drugs that interfere with starch absorption such as Acarbose have been found to be effective at lowering HbA1c (Hanefeld M et al., 2004, Nathan D et al., 2009, Van de Larr FA et al., 2009).
In New Zealand as in many other countries, the recommendation to choose food with a low GI is an important component of the advice given by dietitian to diabetic patients. To further inform the discussion regarding the generalisability of published GI figures the research presented in this thesis compares glycaemic responses to ingested carbohydrate in those of Chinese and European descent. Responses to five varieties of rice were examined, as rice is a staple food for many groups of Asian people and an important carbohydrate source for many Europeans.

A vast body of literature reports on many different aspects of the science relating to GI. This literature review is therefore principally confined to issues relevant to ethnicity. In particular the extent to which Asians might differ from Europeans in their blood glucose response to ingested carbohydrate and why the glycaemic response to rice appears to differ markedly according to ethnicity are explored.
2 Literature Review

2.1 Definition of ethnicity

There has been discussion as to which term should be used when classifying people based on their origin: ‘ethnicity’ or ‘race’. The problem is that these terminologies are often used interchangeably. ‘Race’ is a biological term that differentiates humans by their physical characteristics (Senior PA and Bhopal R, 1994, McKenzie KJ and Crowcroft NS, 1994), whereas, ‘ethnicity’ is a term derived from social theory (McKenzie KJ and Crowcroft, 1994). People of the same ethnicity share origins, social background, distinctive culture and traditions, and have a sense of identity and common language (Senior PA and Bhopal R, 1994). In epidemiological studies, the word ethnicity is preferred to race (Gill PS and Johnson M, 1995), and thus has been used as a variable to describe the population of interest (Senior PA and Bhopal R, 1994).

Statistics New Zealand defines ethnicity as ‘the ethnic group or groups with which people identify or to which they feel they belong. Ethnicity is a measure of cultural affiliation, as opposed to race, ancestry, nationality or citizenship’ (Statistics New Zealand 2006). In this study, I have decided to use ‘ethnicity’, because the definition of the two groups was based on self-identification and dietary preference: ‘rice-based diets’ characteristic of most Asian diets, or typical European diets. Since ethnic identification was self-reported in this study, the way in which individuals chose to identify themselves from a fixed set of categories is reported in the Methods section. The definition of the two ethnic groups for this study (i.e. European and Chinese) is also given in that section.

In this literature review, however, the terms for ethnic groups have been used as they are reported in the literature. For example, the term ‘white’, ‘Caucasian’, ‘black’ and ‘Asian’ are commonly used. Since the study population of particularly interest in this project was Chinese and comparison population was European, I have also focused on comparisons between Chinese and European people.
2.2 Prevalence of type 2 diabetes (T2D) in Asian countries

Diabetes mellitus is a metabolic disorder, which is characterized by chronic hyperglycaemia and disordered macronutrient (i.e. fat, carbohydrate, and protein) metabolism (WHO, 1999).

According to the International Diabetes Federation (IDF), the number of adults with diabetes as well as the prevalence have been increasing. In 2010, it was estimated that there were 285 million adults (age 20-79yr) worldwide with diabetes, representing 6.6% of the world’s adult population (i.e. 4.3 billion) (International Diabetes Federation, 2010a). This has increased from 151 million (of 3.3 billion adults) with 4.6% prevalence in 2000, and it is projected to increase even more, to 438 million by 2030 resulting in 54% increase (International Diabetes Federation, 2010b).

Asia is the most populous region (1.5 billion) in the world and consequently has the greatest number of adults with diabetes, 77 million in the Western Pacific region of the WHO. It was previously estimated that there were 43 million people with diabetes in China alone (International Diabetes Federation, 2010c). However, a recent large Chinese cross-sectional study suggests this IDF prediction is an underestimate (Thomson, 2011). A recent study that conducted oral glucose tolerance tests (OGTTs) in over 46,000 Chinese people revealed that there were 92.4 million adults with diabetes in China giving a national prevalence of 9.7% (Yang W et al., 2010).
2.3 Characteristics of Asian populations

In comparison to western countries, the increase in prevalence of diabetes in Asian countries has been more rapid, and occurred in people with lower body mass index (BMI). For example, in China, the national prevalence rates tripled between 1980 and 1996 from 1.0% to 3.2% (Yoon KH et al., 2006), and further tripled to 9.7% (adults ≥ 20y) in 2010 (Yang W et al., 2010). In Taiwan, the rates doubled from 4.9% to 9.2% in just over a decade (Chang CJ et al., 2000 ). Meanwhile, the prevalence has increased more gradually by one-percentage point per decade in the US, from 1.8 to 5.8% during the last four decades (Gregg EW et al., 2004). However, amongst Asians living in the US, the situation was rather more comparable with that in Asian countries. In a retrospective study in the US, the greatest percentage increase (68.0%) in diabetes prevalence was seen among Asians, in comparison with an increase of 36.3% in whites (1993-2001) (McBean AM et al., 2004).

2.3.1 Nutrition transition in Asian countries

The rapid increase in prevalence has been attributed to nutrition transition. Asian countries have undergone marked economic and epidemiologic transitions during the last few decades (Chan JCN et al., 2009). Nutrition transition is a term, which means a series of changes in dietary and nutritional patterns that is associated with economic, sociodemographic, and epidemiologic changes (Popkin BM, 1993, Kim S and Popkin BM, 2000, FAO, 2010). Compared to the US and European countries, where there was a more gradual shift in the structure of the diet (Popkin BM, 1994), dietary change in Asian countries occurred extremely rapidly (Popkin BM, 1994, Drewnowski A and Popkin BM, 1997, Wang Y et al., 2007). For example, China has the world’s fastest-growing economy (by 10% GDP each year) (The World Bank, 2008), and is still in the middle stage of nutrition transition (Drewnowski A and Popkin BM, 1997). Economic growth increases family income enabling people to buy more high fat and energy-dense foods such as meats and processed foods. Moreover, the growth of global trade and exchange of food products between countries means more foods are available at cheaper prices than ever before (Wang Y et al., 2007). As economies have increased, the proportion of dietary energy from fats and sugar has also increased (Drewnowski A and Popkin BM, 1997). Energy from dietary fat in China has increased sharply from 19.3% (1989) to 27.3% (1997) within a decade (Du S et al., 2002), due to an increase in intake of foods from animal sources (Popkin BM, 1994, Drewnowski A and Popkin BM, 1997, Du S, et al. 2002). Although overall protein intake has changed little, the
proportion of protein from animal source has increased. Thus, the consumption of animal foods tripled from 46.7 to 178.2g per capita/d in urban areas of China during this period (Du S et al., 2002). Moreover, traditionally consumed coarse grains and starchy tubers were replaced with more rice and wheat (Drewnowski A and Popkin BM, 1997). This trend has been commonly seen in other Asian countries (Kim S and Popkin BM, 2000). Nutritional transition is considered to have predisposed people to both obesity and T2D (Yoon KH et al., 2006). Increase in the prevalence of overweight and obesity in Asia has occurred concurrently with the dietary changes. Consequently, the proportion of overweight and obesity (BMI $\geq 25\text{kg/m}^2$) in Chinese adults has increased by 49% from 14.6% (1992) to 21.8% (2002) (Wang Y et al., 2006).

2.3.2 BMI and diabetes

Despite the observations made above, obesity prevalence does not fully explain the increasing diabetes prevalence in Asia (Hu FB, 2011). In comparison with European countries, the prevalence of obesity (BMI $\geq 30\text{kg/m}^2$) is still small in China (2.9%) (Wang Y et al., 2007). Body Mass Index (BMI, kg/m$^2$) is a simple anthropometric measurement of body weight in relation to height, and is the most commonly used indicator to assess health risk associated with adiposity (WHO, 2000, WHO, 2004). However, the metric needs to be interpreted in the context of body composition and ethnicity. Epidemiological studies provide evidence that Asians have lower mean BMI than Europeans. In a 1999/2000 population survey in China, the mean BMI of Chinese men and women was approximately 23.5kg/m$^2$ (Wildman RP et al., 2004), while that of US adults was 28.0kg/m$^2$ (Gregg EW et al., 2004). In New Zealand, the age-adjusted mean BMI (adults aged $\geq 15\text{yrs}$) was 24.9kg/m$^2$ for Asian, in comparison with 26.8kg/m$^2$ for the European/Other group, 29.8kg/m$^2$ for Maori, and 33.2kg/m$^2$ for Pacific (New Zealand Ministry of Health, 2008). Therefore, the proportion of obese adults tends to be smaller in Asian (11.0%) compared with European population (24.3%) in New Zealand (New Zealand Ministry of Health, 2008). Despite the lower mean BMI and lower obesity rates in Chinese, the prevalence of type 2 diabetes is higher in the Asian population (6.5%) than in Europeans (4.3%) (New Zealand Ministry of Health, 2008). Therefore, the application of standard BMI cut-offs for overweight (25-29.9) and obese ($\geq 30$) derived from people of European ethnicity to Asian people, for the assessment of health risk, has been questioned (Deurenberg P et al., 2002, WHO expert consultation, 2004).
The WHO Western Pacific region along with IASO (International Association for the Study of Obesity), and IOTF (International Obesity Task Fore) have thus suggested ethnic-specific criteria for Asian-Pacific people (WHO, 2000), and a cut-off of 23kg/m² has been widely used to identify increased the risk of co-morbidities in Asians (WPRO, Regional Office for the Western Pacific, 2000). However, the people comprising the general Asian population have diverse cultural and anthropometric backgrounds (Deurenberg-Yap M et al., 2002). Even among Asian ethnicities, BMI varies from country to country (WHO, 1995). For example, lowering the cut-off value for overweight by three units of BMI might be appropriate for Hong Kong Chinese and Singaporeans, but may be too much for Northern Chinese and Japanese (WHO, 1995). Thus, some studies suggest that BMI 23 and 27 kg/m² (for overweight and obesity) may be appropriate for Singaporeans (Deurenberg-Yap M et al., 2002) but it has been suggested to be 24 and 28kg/m² for Chinese living in China (Zhou BF et al., 2002).

2.3.3 Adiposity and diabetes

Asian people are more likely to fit the “metabolically obese” phenotype meaning they might be at high risk of diabetes even though they are of an apparently healthy BMI (Chan JCN et al., 2009, Wen JYJ et al., 2010). Although BMI correlates highly with adiposity (WHO, 2000, WPRO, Regional Office for the Western Pacific, 2000), the relationship between BMI and adiposity is different among ethnic groups (Deurenberg P et al., 2002, Wang J et al., 1994). At a given BMI Asian populations have a higher body fat percentage (3-5 percentage points) compared to European people of the same age, sex and BMI (WHO expert consultation, 2004), in both men and women (Wang J et al., 1994) (Deurenberg P et al., 2002). On the other hand, at any given body fat percentage, the BMI of Singaporeans has been estimated to be about three units lower than that of Caucasians (Deurenberg-Yap M et al., 2002). A similar trend has been shown in Chinese living in New Zealand. The same amount of body fat (40.1%) found in the European subjects with BMI 30 kg/m² were shown in the Chinese men and women at BMI 27-28 kg/m² (Wen JYJ et al., 2010).

Chinese living in New Zealand have been shown to have more abdominal fat than Europeans after adjustment for weight and height (Wen JYJ et al., 2010). Abdominal fat strongly correlates with the amount of visceral adipose tissue (Despres JP et al., 2001), and excessive visceral adipose tissue has been associated with glucose intolerance, hyperinsulinaemia and metabolic syndrome (WPRO, Regional Office for the Western Pacific,
Less muscle mass has been also reported in Chinese living in New Zealand compared with Europeans (Wen JYJ et al., 2010). Greater abdominal adiposity and less muscle potentially make Asian people more likely to fit the “metabolically obese” phenotype, which is strongly associated with insulin resistance (Ouyang F et al., 2010, Wen JYJ et al., 2010, Hu FB, 2011). This in turn means that Asian people might be at higher risk of diabetes than Europeans, even though they are of an apparently healthy BMI (Chan JCN et al., 2009, Wen JYJ et al., 2010, Hu FB, 2011).

2.4 Glycaemic Index (GI)

Good glycaemic control is a primary goal in the management of diabetes (Diabetes Control and Complications Trial (DCCT) Research Group, 1995, Stratton IM et al., 2000). To optimise HbA1c, it is necessary to reduce both fasting and postprandial glucose levels (Ceriello A et al., 2008). The concept of Glycaemic index (GI) was initially introduced by Jenkins et al. in the early 1980’s as a means of reducing postprandial glycaemia following ingestion of carbohydrate containing food (Jenkins DJ et al., 1981). GI is to be used for food providing greater than 80% of energy content from carbohydrate such as bread, rice, pasta and potatoes (Brouns F et al., 2005) or foods with 15-20g of available carbohydrate per normal serving portion (Arvidsson-Lenner R et al., 2004). In general, GI is measured using the methodology recommended by WHO (FAO/WHO, 1998). Capillary blood is taken immediately prior to eating and then, 15, 30, 45, 60, 90 and 120min following the start of the ingestion of a food (Brouns F et al., 2005). The reference food is either glucose (Brouns F et al., 2005) or white bread (Wolever TM et al., 1991), and both reference and test foods should contain equivalent amounts of available carbohydrate (i.e. 50g) (Brouns F et al., 2005). Postprandial glycaemic response is taken to be the incremental area under the blood glucose curve (iAUC) (Wolever TMS et al., 2008), which is calculated from the sum of the areas below the blood glucose curve ignoring the area below the fasting blood glucose level (Wolever TM et al., 1991, Brouns F et al., 2005). GI is expressed as a proportion of the iAUC of the test food to the iAUC of the reference food (Wolever TMS et al., 2003, Brouns F et al., 2005, Wolever TMS et al., 2009). Thus, GI may be used to rank carbohydrate containing foods according to their effect on postprandial glycaemia (Sheard NF et al., 2004). The food is sometimes classified as low, medium, or high GI using an arbitrary classification system (Brand-Miller JC, 2003).
There have been a number of studies in many countries to test the GI of food, and the values have been compiled into *The International Tables of GI and GL Values* (Foster-Powell K et al., 1995, Foster-Powell K et al., 2002, Atkinson FS et al., 2008). These tables have also been used in epidemiological studies to calculate daily GI values of the diet (Liu S et al., 2000, Amano Y et al., 2004, Hodge AM et al., 2004, Murakami K et al., 2006, Liese AD et al., 2007, Beulens JWJ et al., 2007, Levitan EB et al., 2007).

However, the values of particular food items in these tables are highly variable. Table 3 shows the studies tested GI of various rice varieties. Within the same rice varieties, the variability of GI is large. For example, there are six GI values listed for Basmati rice (item #557-#562). The five values were tested by three studies (Holt SHA and Miller JB, 1995, Henry CJK et al., 2005). One value (item #561) is an unpublished observation. The GI of Basmati rice ranges from 43 to 69 (GI glucose= 100). Possible reason for this large discrepancy would be: small sample size (n 8-10), different blood collection method, different rice cooking method (e.g. boiling for various periods of time and at different intensity). It is therefore not surprisingly that the clinical relevance of GI has been questioned (Pi-Sunyer FX, 2002, Franz MJ, 2003, Sheard NF et al., 2004). On top of the methodological variability mentioned above, there are factors both external and internal to the food which affects the glycaemic response. These factors are discussed in section 2.7.

Since GI is purported to be a property of food, GI should be independent of subject status. Wolever et al. suggest that GI values may be applied to all people regardless of characteristics such as age, sex, BMI and ethnicity (Wolever TM et al., 1991, Wolever TMS et al., 2003, Wolever TM et al., 2008). Theoretically, these variables should not affect GI, because GI is a ratio and calculated on an individual basis. However, the evidence is contradictory and this critically important issue is discussed further in the following section as well as in Discussion later.
2.5 Ethnic difference in glycaemic response

There are very few studies that have examined ethnic difference in postprandial glycaemic response (Table 1). The studies were performed among mixed Asian (Chan HMS et al., 2001, Dickinson S et al., 2002, Venn BJ et al., 2010), Chinese (Dickinson S et al., 2002), South East Asian (Henry CJK et al., 2008), Asian Indian (Dickinson S et al., 2002, Henry CJK et al., 2008), non-Caucasian (Wolever TMS, 2009), and South African (Walker ARP, and Walker BF, 1984) populations in comparison with European or Caucasian groups (Table 1). The results have been inconsistent. Some observational studies have shown that Non-European people have greater postprandial glycaemic response than their European counterparts (Dickinson S et al., 2002, Wolever TMS et al., 2009, Venn BJ et al., 2010), but this has not been a consistent finding (Walker ARP and Walker BF, 1984, Chan HMS et al., 2001, Henry CJK et al., 2008).

Two studies claimed that GI is not affected by ethnicity (Chan HMS et al., 2001, Henry CJK et al., 2008). No significant differences in iAUC and GI values to nine Vietnamese foods were observed between mixed-Asian and Caucasian groups (Chan HMS et al., 2001). A collaborative study in UK and India concluded that there were no ethnic differences in GI values to biscuits and cereals, although Indian subjects had significantly higher iAUC in two test foods (Henry CJK et al., 2008). However, small sample size in these studies (n=11 and 10, respectively) and the deletion of outliers (Chan HMS et al., 2001), if the individual GI’s were greater than 2SD from the mean, are major limitations in these studies. Having different ethnic groups comprise the “Asian” group (Chan HMS et al., 2001) may also be inappropriate. Not surprisingly, these studies have shown (Henry CJK et al., 2008) large 95% confidence intervals for each GI value. An African study that reported on the applicability of GI to different ethnic groups cannot be generalised due to its study design (Walker ARP and Walker BF, 1984). There was no direct comparison between African and European in this study. The data obtained from young African subjects were compared with the results from a previous study on Europeans (Jenkins DJ et al., 1981).

Two recent studies with larger sample sizes have suggested ethnic difference (Wolever TMS et al., 2009, Venn BJ et al., 2010). Venn et al. report greater iAUC following reference and test foods in mixed-Asian (Chinese, Indian, Japanese, Vietnamese, Cambodian and Korean) compared with Caucasian ethnic difference (29%, p=0.002 and 63%, p<0.001, respectively) (Venn BJ et al., 2010). This resulted in significantly higher GI value of the breakfast cereal in Asians than Caucasians (77 vs. 61 respectively, p=0.012). Wolever et al.
also found that the Non-Caucasian group (ethnic groups not specified) had a higher GI to white bread than the Caucasian group (78 and 66 respectively, p<0.05) as well as in iAUC (Wolever TMS et al., 2009). However, no such difference was found in the reference food (i.e. 50g glucose) and other test foods, chocolate chip cookie and a strip of fruit leather (Wolever TMS et al., 2009). Larger sample sizes were the strength of these studies, but the Asian or Non-Caucasian groups were composed of different ethnic groups. Since many different ethnicities may be represented in a group of Asian participants, this must be regarded as a weakness. A further limitation is that only a small number of different foods were tested. A study not specifically examining GI is also relevant to a discussion relating to ethnicity. This study compared postprandial blood glucose levels and insulin levels after a 75g carbohydrate load (white bread) in five different ethnic groups: European Caucasian, Chinese, South East Asian, Asian Indians, and Arabic Caucasian groups (Dickinson S et al., 2002). Despite being young and healthy, markedly greater postprandial glycaemia and insulinaemia were observed in the Chinese compared with the European Caucasian individuals. Although the fasting glucose concentrations did not differ among the groups, the incremental area under the glucose curve (iAUC) for the reference food (white bread) was 50% greater in the Chinese and 100% greater in South East Asians than that of European Caucasians (p<0.001) (Dickinson S et al., 2002).

None of the studies examined possible explanations for the greater iAUC shown in Asian groups, and surprisingly, did not examine the effects of ethnicity on rice, which is the staple food of most Asian population groups.
2.6 Observational and intervention studies linking GI and diabetes

Observational and experimental studies in which the relationship between GI and diabetes have been investigated are discussed in Appendix 1. Consideration of these studies indicates that there is no consistent result both in observational and experimental studies on GI with regard to T2D risk. The studies that showed a significant improvement in HbA1c in Low GI (LGI) diet group had short study duration. Its effect seems to attenuate after six months of intervention. It still remains unknown whether LGI diet can improve HbA1c in long term. Most experimental studies were conducted in North America or Europe, not in Asia. The association of rice intake and T2D is not clear due to a lack of evidence, however the magnitude of the association may be different in Asian population from European population. Since GI has more contexts in populations that consume higher energy from carbohydrate and rice, conducting further long-term experimental studies in Asian countries would be more relevant to these populations. Further discussion is in Appendix 1.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Country</th>
<th>Subjects</th>
<th>Age &amp; BMI index (European &amp; Non-European)</th>
<th>Reference &amp; Test foods</th>
<th>Main results (in comparison with Europeans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al. 2001</td>
<td>Australia</td>
<td>6 Caucasian, 6 Asian (Chinese, Indonesian, Vietnamese)</td>
<td>23yr &amp; 21yr BMI 20.6 (Eur) 22.1 (Asian) - NS</td>
<td>Glucose powder (50g CHO) 9 test foods (rice, rice noodles, canned lychee, milk etc.)</td>
<td>• iAUC: NS (p=0.40)</td>
</tr>
<tr>
<td>Henry et al. 2008</td>
<td>UK (multi-centre)</td>
<td>34 Caucasian, 13 Asian Indian (n=10 tested each food)</td>
<td>37yr &amp; 24.8yr BMI 24.4 &amp; 22.1 (p&lt;0.001)</td>
<td>Glucose (50g CHO) Two sweet biscuits Three breakfast cereals</td>
<td>• Fasting glucose levels: NS • iAUC for all test foods: higher in the Indian, but significant only in sweet biscuits &amp; malted wheat cereal (p&lt;0.05) • GI: NS • GI is not affected by ethnicity</td>
</tr>
<tr>
<td>Wolever et al. 2009</td>
<td>Canada</td>
<td>40 Caucasian, 37 Non-Caucasian (ethnicity unknown)</td>
<td>39.9yr &amp; 37.4yr BMI 26.1 &amp; 26.2 NS</td>
<td>Glucose (50g CHO) White bread Chocolate chip cookie Fruit leather</td>
<td></td>
</tr>
<tr>
<td>Venn et al. 2010</td>
<td>New Zealand</td>
<td>73 Caucasian, 27 Asian (Chinese, Japanese, Indian, Vietnamese, Cambodian, and Korean)</td>
<td>22yr &amp; 27yr BMI 23.2 &amp; 22.2 (p=0.12)</td>
<td>Glucose beverage (50g CHO) Breakfast cereals (Kellogg’s Sustain) with skimmed milk</td>
<td>• Fasting glucose levels: NS • iAUC of white bread was significantly greater in Non-Caucasians (p&lt;0.05) • iAUC of other foods: NS • GIwhitebread: significantly greater in Non-Caucasians (p&lt;0.05)</td>
</tr>
<tr>
<td>Dickinson et al. 2002</td>
<td>Australia</td>
<td>60 (five ethnic groups: 20 Eur, 10 Chi, 10 SE Asian, 10 Indian, 10 Arabic Caucasian)</td>
<td>Mean age 20-22yr, Mean BMI 20.7-22.9 (Age, BMI, WC were comparable among the groups)</td>
<td>White bread (75g CHO) Not GI study</td>
<td>• Fasting glucose levels: NS • iAUC in Chi: greater than Eur by 50% (p&lt;0.001) • Fasting insulin levels - NS • iAUCinsulin: 90% greater in Chi (p&lt;0.001)</td>
</tr>
</tbody>
</table>

BMI: body mass index, WC: waist circumference, CHO: carbohydrate, iAUC: incremental Area Under the Curve (for blood glucose, otherwise stated), Eur: European, Chi: Chinese, SE: South East, NS: non-significant difference, pp: postprandial.
2.7 Rice

Rice (*Oryza Sativa L.*) is the most important cereal crop in the world (Juliano BO, 1993). It provides 20% of the dietary energy for the world (FAO, 2004), but the dependence on rice for the dietary energy supply is higher in Asian countries than others (Juliano BO, 1993). For example, rice contributes more than 30% of daily dietary energy in China, Malaysia, and India (Kennedy G et al., 2003). Since many Asian countries have 60-70% of total daily energy intake from carbohydrate (Yang YX et al., 2006, Murakami K et al., 2006, Kim K et al., 2008, Mohan V et al., 2009, Wen W et al., 2009) rice plays an important role as a staple food providing more than 50% of their carbohydrate intake (Kim K et al., 2008, Mohan V et al., 2009), and therefore it is a major contributor of GI and GL in the Asian diet (Amano Y et al., 2004, Murakami K et al., 2006, Villegas R et al., 2007, Wen W, 2009). The annual consumption of rice is therefore much greater in Asia (generally, >80kg/person/yr) compared with some western countries (typically, <10kg/person/yr) (United Nations Conference on Trade and Development (UNCTAD)). For example, 90kg/person/yr of rice is consumed in China, whereas only 9kg/person/yr in the US, or 4kg/person/yr in France (United Nations Conference on Trade and Development (UNCTAD)). Asian countries are often said to have ‘rice-based’ diet.

Some epidemiological studies have shown a positive relationship between rice intake and the prevalence of type 2 diabetes (T2D) (Table 2). A prospective study of Chinese women (n= 64,277) showed 78% increase in risk of T2D in the highest quintile of rice intake (raw ≥300g/d, cooked ≥750g/d) compared with the lowest quintile (raw <200g/d, cooked <500g/d) (Villegas R et al., 2007). The increased risk of T2D was still associated with rice intake after BMI adjusted. A Japanese cross-sectional study also found 65% increase in risk of T2D in the highest quintile of rice intake (608g/d) compared with the lowest (150g/d) (Nanri A et al., 2008), although the association was only observed in women. These studies suggest that the effect of rice is independent from BMI (Villegas R et al., 2010, Nanri A et al., 2010). A similar relationship has been shown in men and women in the US. Those in the highest quintile of white rice intake had a 17% greater risk of T2D compared with those in the lowest quintile (Sun Q et al., 2010). However, compared with the two Asian studies, the rice intake in the US study was small, and mean rice consumption in the highest quintile in this study was only 107g/d (Sun Q et al., 2010). It is noteworthy that the risk of diabetes was not evident in Japanese men (Nanri A et al., 2010), and also that a beneficial effect of rice on coronary heart disease risk has been reported in Japanese men (Eshak ES et al., 2011).
Although the evidence is very limited, the findings from these studies raise an interesting possibility of relationship between rice consumption and T2D risk. It is still unknown what effect rice consumption has on postprandial glycaemic response short-term as well as long-term. And what consequences are likely to occur if rice is consumed on daily basis. Exploring this would be worthwhile to suppress the increase in T2D prevalence in Asian populations.
Table 2: Observational studies on rice consumption and prevalence of type 2 diabetes

<table>
<thead>
<tr>
<th>Author &amp; Country</th>
<th>Study</th>
<th>No.</th>
<th>Age (y)†</th>
<th>Sex</th>
<th>Follow up (y)</th>
<th>Rice intake (g/d)</th>
<th>Outcome</th>
<th>Association</th>
<th>Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cross-sectional study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sun et al. 2010 US</td>
<td>NHS I, NHS II, HPFS</td>
<td>197228</td>
<td>26-87</td>
<td>M+F</td>
<td>-</td>
<td>WR: &lt;1serv/mo, 1-3serv/mo, 1serv/wk, 2-4 serv/wk, ≥5 serv/wk (i.e. ≥107g/d)</td>
<td>T2D</td>
<td>***</td>
<td>RR(95%CI) =1.17(1.02, 1.36)</td>
</tr>
<tr>
<td>Villegas et al. 2007 China</td>
<td>Shanghai Women’s Study</td>
<td>64227</td>
<td>40-70</td>
<td>F</td>
<td>4.6</td>
<td>&lt;500, 500-624, 625-749, ≥750g/d</td>
<td>T2D</td>
<td>(p-value not mentioned)</td>
<td>RR(95%CI) = 1.78 (1.48, 2.15) in Q4 vs. Q1</td>
</tr>
<tr>
<td>Nanri et al. 2010 Japan</td>
<td></td>
<td>125666</td>
<td>45-75</td>
<td>M</td>
<td>5</td>
<td>280, 420, 560, 700g/d</td>
<td>T2D</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Eshak et al. 2011 Japan</td>
<td></td>
<td>33622</td>
<td>F</td>
<td></td>
<td></td>
<td>165, 315, 420, 560g/d</td>
<td>T2D</td>
<td>**</td>
<td>RR(95%CI) = 1.65 (1.06, 2.57) in Q4 vs. Q1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83752</td>
<td>M</td>
<td>40-79</td>
<td>14.1</td>
<td>280, 420, 449, 583, 711g/d</td>
<td>Mortality from CVD</td>
<td>*</td>
<td>CHD: HR(95%CI) = 0.70 (0.49, 0.99) in Q5 vs. Q1 Total CVD: HR(95%CI) = 0.82(0.70, 0.97) in Q5 vs. Q1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>279, 259, 420, 453, 560g/d</td>
<td>Mortality from CVD</td>
<td>NS</td>
<td>-</td>
</tr>
</tbody>
</table>

†Age at baseline (range), NHS: Nurses’ Health Study, HPFS: Health Professionals Follow-up Study, M: male, F: female, WR: white rice, BR: brown rice, serv: serving(s), T2D: Type 2 diabetes, NS: non significant, RR: relative risk, HR: hazard ratio, CHD: coronary heart disease, CVD: cardiovascular disease, *p<0.05, **p<0.01m, ***p<0.001.
2.8 GI values for rice varieties

Rice is a food which has been reported to have a wide range of GI values in different studies (Miller JB et al., 1992, Larsen HN et al., 1996, Ranawana DV et al., 2009) (Table 3). Even for the same variety of rice (e.g. Basmati rice), there is a variation in GI values as previously mentioned (Atkinson FS et al., 2008). Thus, Basmati rice may be considered to have low, medium, or high GI. The differences may be both due to the character difference in rice varieties, and also due to the other variability which affects postprandial glycaemic response and GI.

2.8.1 Variability in rice variety

2.8.1.1 High Amylose content

The best known factor that influences postprandial glycaemic response is the type of starch (Goddard MS et al., 1984). Rice starch is composed of two polysaccharides: amylose (15-20% by weight) and amylopectin (80-85% by weight) (Miller JB et al., 1992, Benmoussa M et al., 2007). There is convincing evidence that high-amylose content rice (25-32%) has lower GI values than rice with low-amylose content (<20%) (Juliano BO and Hicks PA, 1996) in healthy subjects (Miller JB et al., 1992, Yang YX et al., 2006) (Table 3) and in diabetic subjects (Larsen HN et al., 1996) and in vitro (Frei M et al., 2003, Hu P et al., 2004). The insulin response after consumption of high-amylose content rice was lower in healthy subjects (Goddard MS et al. 1984, Miller JB et al., 1992) and in diabetic subjects (Larsen HN et al., 1996) than consumption of low-amylose rice. The possible mechanism of slower digestion of high-amylose rice is likely to be its molecule shape and size. Amylose has a linear formation with α-D-(1,4) linkage, whereas the amylopectin is a branched molecule with α-D-(1,4) and α-D-(1,6) linkages (Sajilata MG et al., 2006). Amylose has more extensive hydrogen bonding than amylopectin, and it promotes crystallinity in starch structure (Panlasigui LN et al., 1991, Kavita MS and Prema L, 1997). Additionally, in comparison with amylose, amylopectin is more susceptible to digestive enzymes, due to greater surface area of the molecule (Behall KM et al., 1989, Yang YX et al., 2006, Singh J et al., 2010). Moreover, the lipid-starch complex in high amylose rice (Juliano BO and Goddard MS, 1986) also contributes to a slower digestion rate (Guraya HS et al., 1997) as the complex is insoluble and stable at a relatively high temperature. It also restricts swelling and solubilisation of starch.
Amylopectin does not form this complex (Guraya HS et al., 1997), as its side-branches may be too short for complex formation (Frei M, et al. 2003). However, to emphasize the complexity of factors influencing starch digestibility, the evidence suggests that for intermediate (20-25%) and high (25-32%) amylose rice (Juliano BO and Hicks PA, 1996), the amylose content was not the only factor determining starch digestibility (Hu P et al., 2004). Even among the same varieties of high-amylose rice, the GI value may vary (Panlasigui LN et al., 1991). These are likely to be due to differences in: granule size, relative porosity of endosperm, nature and amount of non-starch components and molecular size and structure of the carbohydrate (Panlasigui LN et al., 1991, Frei M et al., 2003).

2.8.1.2 Fat and protein content

While milled rice (i.e. white rice) has lost the outer layer and germ during the milling process, these are still intact in brown rice. Thus, brown rice has a higher content of B vitamins (Juliano BO, 2003), fat and protein than milled rice. Fat and protein content of foods have been shown to have a significant negative relationship with GI (Jenkins D et al., 1981). Therefore, theoretically, brown rice is likely to have lower GI compared to white rice, and this has been demonstrated in some GI studies (Table 3) (Jenkins DJ et al., 1981, Miller JB et al., 1992, Ito Y et al., 2005, Panlasigui LN et al., 2006). Possible effects of fat and protein include delayed gastric emptying and enhanced gastric inhibitory polypeptide (Collier G and O’Dea K, 1983, Collier G et al., 1984).

However, there are other studies that show the GI value of brown rice to be as high as that of white rice (Miller JB et al., 1992, Noriega E et al., 1993, Yang YX et al., 2006).

Furthermore, brown rice generally requires double amount of water than white rice resulting in longer cooking time, and possibly greater extent of cooking. This is likely to contribute to greater digestion rate.
2.8.1.3 Parboiling

In the parboiling procedure, the grain is soaked, pressure steamed, and dried (Heinemann RJB et al., 2006). Since this hydrothermal process is undertaken prior to the milling (i.e. removal of hull), cooked parboiled rice has a pale subtle yellow colour and distinctive flavour (Heinemann RJB et al., 2006) making cooked rice kernels firmer than the non-parboiled rice (Priestley RJ, 1976). *In vitro* studies suggest that the strong heat treatment (Walter M et al., 2005) may modify the starch structure and starch molecules during the process (Walter M et al., 2005). The starch pattern changes after parboiling creating a helical amylose complex (Walter M et al., 2005) with other molecules such as lipids (Mikus FF et al., 1946). This complex is insoluble and resistant to digestive enzymes (Walter M et al., 2005, Priestley RJ, 1976) resulting in lower glycaemic response in healthy (Perry T et al., 2000) (Table 3) as well as in people with type 2 diabetes (Larsen HN et al., 2000). Furthermore, resistant starch is formed during the parboiling process (Walter M et al., 2005, Eggum BO et al., 1993). This may further explain the lower GI value of parboiled rice, although this does not appear to be the case if the parboiling method is mild (i.e. with low heat treatment) (Kavita MS and Prema L, 1997, Larsen HN et al., 2000).
<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>No. &amp; sex</th>
<th>Age (y)</th>
<th>Tested rice (g)</th>
<th>Referenc food</th>
<th>Cooking method</th>
<th>Blood collection method</th>
<th>GI† (when glucose=100) (listed number)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>WR – non specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilawari et al.</td>
<td>India</td>
<td>6M</td>
<td>36</td>
<td>Rice (raw 64g)</td>
<td>Dextrose (50gCHO)</td>
<td>Boiled (unknown how long)</td>
<td>Venipuncture</td>
<td>Not mentioned 43 (#510) (AUC was calculated ‘over the 60min’) 72 (#511)</td>
</tr>
<tr>
<td>Jenkins et al.</td>
<td>Canada</td>
<td>7 M+F</td>
<td>29</td>
<td>WR (quantity unknown)</td>
<td>Glucose (50gCHO)</td>
<td>Boiled (in a minimum of water with 2g salt)</td>
<td>Finger prick</td>
<td>WR 72±9</td>
</tr>
<tr>
<td>Kanan et al.</td>
<td>India</td>
<td>8</td>
<td>30</td>
<td>WR (64g): freshly cooked (RFC) vs. Refrigerated &amp; re-warmed (RRR)</td>
<td>White bread (50gCHO)</td>
<td>Cooked in pressure cooker</td>
<td>Not mentioned</td>
<td>Not mentioned 72 (#512)</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>China</td>
<td>9-12</td>
<td>20-45</td>
<td>Rice Sticky rice (SR) Sticky rice2 Sticky rice (high AM) Rice porridge</td>
<td>Glucose (50gCHO)</td>
<td>“Traditional method” used in daily life of Chinese (no details)</td>
<td>Venous blood</td>
<td>Rice 83±3 SR 87±7 SR2 88±6 High AM 50±6 Porridge 70±19 83 (#513)</td>
</tr>
<tr>
<td>Gatti et al.</td>
<td>Italy</td>
<td>9M+5F</td>
<td>26</td>
<td>WR NS (90g raw): Boiled vs. boiled &amp; baked for 10min @160°C Rice</td>
<td>Glucose (70gCHO)</td>
<td>Boiled 13min vs. Boiled &amp; baked for 10min at 160°C</td>
<td>Not mentioned</td>
<td>Not mentioned 89 (#514)</td>
</tr>
<tr>
<td>Schaubinger et al.</td>
<td>Germany</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Glucose</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>53</td>
</tr>
<tr>
<td>Noriega et al.</td>
<td>France</td>
<td>3M+4F</td>
<td>38</td>
<td>WR (raw 65g, cooked 236.8g)</td>
<td>White bread (50gCHO)</td>
<td>Pressure cooked</td>
<td>Indwelling catheter</td>
<td>106±6 (WB-based GI)</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Author</td>
<td>Country</td>
<td>No. &amp; sex</td>
<td>Age (y)</td>
<td>Tested rice (g)</td>
<td>Reference food</td>
<td>Cooking method</td>
<td>Blood collection method</td>
<td>GI†</td>
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<td>------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>WR- long grain</td>
<td>Australia</td>
<td>6 M+F</td>
<td>21-34</td>
<td>WR (Mhatma long grain, unconverted) 175g(boiled)</td>
<td>Glucose (50gCHO)</td>
<td>Boiled for 15min</td>
<td>Finger prick</td>
<td>50</td>
</tr>
<tr>
<td>Brand et al. 1985</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolever et al. 2003</td>
<td>7 centres</td>
<td>28M+40F</td>
<td>25</td>
<td>WR Long grain (64.9g)</td>
<td>Glucose (50gCHO)</td>
<td>According to the manufacturer’s instruction</td>
<td>Finger prick or venepuncture</td>
<td>69</td>
</tr>
<tr>
<td>Ranawana et al. 2009</td>
<td>UK</td>
<td>10 M+F</td>
<td>38</td>
<td>Long grain rice (64.6g) Easy-cook long-grain rice (68.3g) Thai red rice (67.4g) Thai glutinous rice (64.7g)</td>
<td>Glucose (50gCHO)</td>
<td>Cooked individually in 850mL water</td>
<td>Finger prick</td>
<td>Long-grain 47±6.3 Easy-cook long grain 47±7.2 Thai red 76±8.1 Thai glutinous 92±7.6</td>
</tr>
<tr>
<td>WR- Jasmine</td>
<td>Australia</td>
<td>12 A+C</td>
<td>23</td>
<td>Jasmine (raw 63g)</td>
<td>Glucose (50gCHO)</td>
<td>Followed manufacture’s instructions Cooked in rice cooker</td>
<td>Finger prick</td>
<td>109±10</td>
</tr>
<tr>
<td>Chan et al. 2001</td>
<td></td>
<td>6A</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94±8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6C</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR – high amylose</td>
<td>Australia</td>
<td>8M+F</td>
<td>30</td>
<td>High AM (28%, Doongara)</td>
<td>White bread (50gCHO)</td>
<td>Boiled for 14-30min (according to the instruction)</td>
<td>Capillary</td>
<td>Doongara WR 64±9 (GI converted to glucose-base by x 70/100)</td>
</tr>
<tr>
<td>Author</td>
<td>Country</td>
<td>No. &amp; sex</td>
<td>Age (y)</td>
<td>Tested rice (g)</td>
<td>Referenc food</td>
<td>Cooking method</td>
<td>Blood collection method</td>
<td>GI† (when glucose=100)</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------</td>
<td>-----------</td>
<td>---------</td>
<td>-----------------------------------------</td>
<td>---------------------</td>
<td>----------------------------------------------------</td>
<td>-------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Holt &amp; Brand-Miller 1995</td>
<td>Australia</td>
<td>4M+5F</td>
<td>23</td>
<td>WR (ordinary 185g &amp; quick-cooking 178g)</td>
<td>White bread (50gCHO)</td>
<td>Boiled for 14min the day before the test (stored at 4°C)</td>
<td>Finger prick</td>
<td>Normal Doongara 64 Quick-cooking Doongara 94 (GI converted to glucose-base by x 70/100)</td>
</tr>
<tr>
<td>Yang et al. 2006</td>
<td>China</td>
<td>9-12M+F</td>
<td>20-45</td>
<td>Sticky rice (high AM)</td>
<td>Glucose (50gCHO)</td>
<td>“Traditional method” used in daily life of Chinese (no details) Boiled in 127g water for a total of 22min with controlled heat</td>
<td>Venous blood</td>
<td>High AM 50±6</td>
</tr>
<tr>
<td>Panlasigui et al. 2006</td>
<td>Canada</td>
<td>3M+7F</td>
<td>33</td>
<td>Milled rice (High AM 29.5% (IR42))</td>
<td>White bread (50gCHO)</td>
<td>Boiled for various duration</td>
<td>Finger prick</td>
<td>94±11 (WB-based GI for 60min)</td>
</tr>
<tr>
<td>WR- Basmati</td>
<td>Ashton et al. 2008</td>
<td>UK 10M+F</td>
<td>57</td>
<td>Basmati Basmati easy-cook American easy-cook rice</td>
<td>Glucose (50g CHO)</td>
<td>According to the manufacturer’s instruction</td>
<td>Capillary</td>
<td>Bas 43± 8 Bas easy-cook 68±8 American easy-cook 49±12</td>
</tr>
<tr>
<td>Henry et al. 2005</td>
<td>UK 8M+F</td>
<td>37</td>
<td>Basmati varieties boiled for: 8min (65.7g), 10min (65.7g), 12min (63.0g) easy-cook (boiled for 9min, 62.7g)</td>
<td>Glucose (50g CHO)</td>
<td>Boiled for various duration</td>
<td>Finger prick</td>
<td>Boiled for 8min; 69±6 10min; 57±10 12min; 52±11 Easy-cook 67±11</td>
<td>52 (#558) 57 (#559)</td>
</tr>
<tr>
<td>Author</td>
<td>Country</td>
<td>Sex</td>
<td>Age (y)</td>
<td>Test rice (g)</td>
<td>Referenc e food</td>
<td>Cooking method</td>
<td>Blood collection method</td>
<td>GI†</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>------</td>
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<td>--------------------------------------------------</td>
<td>----------------------</td>
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<td>-------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Ranawana et al. 2009</td>
<td>UK</td>
<td>10M+F</td>
<td>38</td>
<td>White Bas (64.8g) Brown Bas (71.9g) White+Brown Bas (67.0g) Bas+wild rice (65.0g) Easy-cook Bas (64.1g)</td>
<td>Glucose (50gCHO)</td>
<td>Cooked individually in 850mL water</td>
<td>Finger prick</td>
<td>White Bas 50±6.3 Brown Bas 75±7.8 White+brown Bas 59±9.0 Bas+wild 63±8.4 Easy-cook Bas 80±8.0</td>
</tr>
<tr>
<td>Jenkins et al. 1981</td>
<td>Canada</td>
<td>7M+F</td>
<td>29</td>
<td>BR</td>
<td>Glucose (50gCHO)</td>
<td>Boiled (in a minimum of water with 2g salt) Rice was frozen till needed</td>
<td>Finger prick</td>
<td>BR 66±5</td>
</tr>
<tr>
<td>Potter et al. 1981</td>
<td>USA</td>
<td>8M</td>
<td>22-45</td>
<td>BR (raw 97g)– 75gCHO (+ casein and/or corn oil to keep macronutrient consistent with others: all bran; pinto beans)</td>
<td>Liquid formula</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Yang et al. 2006</td>
<td>China</td>
<td>9-12</td>
<td>20-45</td>
<td>BR</td>
<td>Glucose (50gCHO)</td>
<td>“Traditional method” used in daily life of Chinese (no details)</td>
<td>Venous blood</td>
<td>BR 87±5</td>
</tr>
<tr>
<td>Miller et al. 1992</td>
<td>Australia</td>
<td>8</td>
<td>30</td>
<td>High AM (28%, Doongara) Normal AM (20%, Calrose &amp; Pelde) Quick-cooking BR</td>
<td>White bread (50gCHO)</td>
<td>Boiled for 14-30min (according to the instruction)</td>
<td>Capillary</td>
<td>Doongara BR 66±7 Calrose BR 87±8 Pelde BR 76±6 Quick-cooking BR 80±7 (GI converted to glucose-base by x 70/100)</td>
</tr>
<tr>
<td>Author</td>
<td>Country</td>
<td>No. &amp; sex</td>
<td>Age (y)</td>
<td>Tested rice (g)</td>
<td>Reference food</td>
<td>Cooking method</td>
<td>Blood collection method</td>
<td>GI† (when glucose=100) (listed number)‡</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------</td>
<td>-----------</td>
<td>---------</td>
<td>---------------------------------------------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>----------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Panlasigui et al. 2006</td>
<td>Canada</td>
<td>3M+7F</td>
<td>33</td>
<td>High AM 29.1% (IR42)</td>
<td>White bread</td>
<td>Boiled in 275g water for a total of 30min with controlled heat</td>
<td>Finger prick</td>
<td>83±11 (WB-based G, for 60min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td>101±6 (WB-based)</td>
</tr>
<tr>
<td>Noriega et al. 1993</td>
<td>France</td>
<td>3M+4F</td>
<td>38</td>
<td>Wholegrain BR (raw 64g, cooked 146.8g)</td>
<td>White bread</td>
<td>Boiled in pressure cooker</td>
<td>Indwelling catheter</td>
<td>Not listed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parboiled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glucose</td>
<td>Boiled for 20-30min</td>
<td>Veneupuncture</td>
<td>38 (#603)</td>
</tr>
<tr>
<td>Crapo et al. 1977</td>
<td>USA</td>
<td>14M &amp; 2F</td>
<td>Unkn</td>
<td>Uncle Ben’s Converted brand rice (parboiled)</td>
<td>Glucose</td>
<td>Boiled for 14-30min (according to the instruction)</td>
<td>Capillary</td>
<td>87 (#604)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61g (dry)</td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Converted rice</td>
<td>White bread</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller et al. 1992</td>
<td>Australia</td>
<td>8</td>
<td>30</td>
<td>Converted rice</td>
<td>Glucose</td>
<td>According to manufacturer’s instruction</td>
<td>Venous blood</td>
<td>56±7.1 (GI converted to glucose-base by x 70/100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td>56 (#518, listed as long grain rice)</td>
</tr>
<tr>
<td>Perry et al. 2000</td>
<td>New Zealand</td>
<td>14M+F</td>
<td>31</td>
<td>Uncle Ben’s® white long-grain parboiled rice</td>
<td>Glucose</td>
<td>According to manufacturer’s instruction</td>
<td>Venous blood</td>
<td>56±7.1 (GI converted to glucose-base by x 70/100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(50gCHO)</td>
<td></td>
<td></td>
<td>56 (#518, listed as long grain rice)</td>
</tr>
</tbody>
</table>

2.8.2 Other variability

2.8.2.1 Chewing extent

Chewing involves a mechanical disruption of food into smaller particles. This process creates a greater surface area of the chewed bolus and allows the digestive enzymes better access to the substrate contributing to an increased rate of digestion (Read NW et al., 1986, Ranawana V et al., 2010). Read et al. observed a significant reduction of glucose levels after swallowing rice compared with when the rice was well chewed (Read NW et al., 1986). Another study that compared ground and unground rice observed a significantly greater iAUC of both glucose and insulin in ground rice than in unground rice (O'Dea K et al., 1980). Thus, particle size of food is likely to be a determinant of the GI value of food. However, in these studies chewing extent, or the degree of food particle size was controlled. Ranawana et al. suggest that chewing rate and eating behaviour are different between individuals, and it may account for individual variability in postprandial glycaemic response (Ranawana V et al., 2010). Thus, they tested whether the degree of mastication affects digestion rate in vitro and in vivo. In their findings, there was a significantly higher digestion rate of the smaller chewed particles (less than 500µm) in vitro, however no such an effect in iAUC in vivo was observed. Small sample (n=15) is partly likely to explain this result.

2.8.2.2 Salivary α-amylase (sAA)

Salivary α-amylase (sAA) (1,4-α-D-glucanohydrolases, EC 3.2.1.1) is a protein component of saliva (Rohleder N and Nater UM, 2009), which is secreted from the parotid, submandibular and sublingual glands in mouth (Humphrey SP and Williamson RT, 2001). Digestion of starch begins with the action of alpha-amylase (Mandel AL et al., 2010, Butterworth PJ et al., 2011). sAA has been suggested to be a possible factor which affects glycaemic response by some studies (Wolever TMS et al., 2009, Ranawana V et al., 2010). A study found there was a significant positive correlation between the copy number of sAA gene (AMY1, salivary alpha-amylase gene) and sAA activity level (Perry GH et al., 2007a , Perry GH, 2007b, Mandel AL et al., 2010). These findings suggest that the more copies of AMY1 are likely to lead to improved starch digestion (Perry GH, 2007b) and higher glycaemic response. Populations that traditionally have high-starch diets have a greater number of the AMY1 gene copies than groups with a low-starch diet (Perry GH, 2007a). Wolever et al. suggest that this might be a factor explaining ethnic difference in iAUC and GI
for white bread between the Caucasian and non-Caucasian subjects in their study (Wolever TMS et al., 2009). However, this does not explain the difference between Caucasians and non-Caucasians, as Perry et al. did not classify European-American and Japanese subjects into different groups. Both were in a high-starch diet group. Moreover, this study showed that the mean gene copy number of European and Japanese was very similar (6.57 and 6.80, respectively) (Perry GH et al., 2007b). The number of this gene copy has large individual variation. The median number of AMY1 gene copies of the 62 subjects was four, with a range of 1 to 11 (Mandel AL et al., 2010). This variation may be because some of the tested participants might have had genes of the population with a low-starch diet (Mandel AL et al., 2010), or simply because they have neither a high- nor a low-starch diet, but an “intermediate” diet with a more evenly spread macronutrient intake. Therefore, the copy number of AMY1 gene is unlikely to be a reason of difference in glycaemic response.

However, to my knowledge, no study has examined the effect of salivary alpha-amylase activity on glycaemic response. Although the gene copy number is not different between ethnic groups, the sAA activity may be different among ethnic groups due to unknown mechanism which influences enzyme activity. And this may therefore contribute to the greater glycaemic response.
2.9 Summary and Conclusion

Rates of type 2 diabetes are increasing worldwide. Rates appear to be particularly high in some indigenous groups and Asian populations studied in their countries of origin and in countries to which they have migrated. Rapid nutrition transition and decreased physical activity in genetically predisposed individuals and populations have been suggested as likely causes. Some studies have reported ethnic differences in glycaemic response when Asian groups have been compared with Europeans. However the results have not been consistent. Most of the studies have included relatively small numbers of subjects, grouped different Asian populations together or drawn conclusions from study groups which have included both Asians and other non-Europeans.

Postprandial glycaemia is a determinant of overall glycaemia and cardiovascular risk and rice has been shown in some prospective studies to be associated with an increased risk of type 2 diabetes particularly in women. Published glycaemic index figures for the same variety of rice tend to vary, perhaps because of the small number of individuals studied. The research reported in this thesis compares glycaemic responses to glucose and different varieties of rice in people of European and Chinese ethnicity and presents the glycaemic indices of the various rice varieties available in New Zealand, based on the study of a relatively large group of individuals. The purpose of the research was to further understand the handling of dietary carbohydrate by people of different ethnicity and to facilitate ethnic specific dietary advice for people with or at risk of type 2 diabetes.
3 Methods

3.1 Experimental Design

3.1.1 Study aims

The aims of this study were: to assess whether published Glycaemic Index (GI) values are applicable to Chinese people; to assess the GI values of rice varieties and newly produced low GI sugar (“LoGiCane™”); and to determine whether the rice varieties currently recommended by dietitians are appropriate.

3.1.2 Study design

The GI value of five varieties of rice (Basmati, Brown, Doongara®, Jasmine, and Parboiled) was tested in two ethnic groups: Chinese and European (n= at least 30 each). Healthy volunteers were asked to come to the GI laboratory of the Human Nutrition department after fasting for 10 hours.

The participants were subjected to eight finger prick tests on each test day. The Jasmine rice and the glucose beverage were tested twice, and the four speciality rice varieties (Basmati, Brown, Doongara® and Parboiled) and LoGiCane™ sugar were tested once. The glucose beverage was the reference food, and all rice and LoGiCane™ sugar were test foods. Like the glucose beverage, the Jasmine rice was tested twice, because I wished to examine its possible use as an alternative reference food to glucose to ascertain whether using a starchy reference food eliminates the ethnic difference in GI reported previously using glucose as the reference (Wolever TMS, 2009). Pilot trials to standardise rice cooking were undertaken. Ethical approval for the study was obtained from the University of Otago Human Ethics Committee (Appendix 2).
3.2 Participants

3.2.1 Recruitment

Thirty Chinese and 30 European healthy volunteers were recruited. To control for any age effects on GI and to broaden generalisability, ten people from each of three different age groups, 18-30, 31-40, and 41-50 years were recruited. Participants were recruited between October 2009 and March 2010 through flyers, email and direct contact. Flyers (Appendix 4) were posted at the University of Otago libraries, departmental bulletin boards, Asian shops and Chinese restaurants in the Dunedin city area. Emails were sent to University departments and to the Otago Southland Chinese Association. Consenting participants from previous studies conducted by the Department of Human Nutrition were contacted directly.

3.2.2 Ethnic identification

The two ethnic groups were described as Chinese and European in this study. The term Asian used for the New Zealand Census (Statistics New Zealand 2011) was considered inappropriate in the present study, since Asia is a diverse region consisting of over 60 countries with different cultures, languages, and dietary habits. Rather, the more specific term, “Chinese” is used. I asked study participants what ethnic group they belong to by providing a set of ethnic groups in the questionnaire (Appendix 3).

3.2.3 Screening

People who expressed an interest in participating were contacted via telephone or email and a brief description of the study was given. Those who remained interested in the study were asked to fill out and submit the study questionnaire (Appendix 3) for further screening.
3.2.4 Eligibility

Inclusion criteria

I recruited healthy people aged 18 to 50 years of Chinese or European descent. It was a requirement that both parents of the participant and the participant themselves were of the same ethnicity.

Exclusion criteria

People were not eligible if they had been diagnosed with diabetes mellitus, cardiovascular disease, cancer or diseases of the digestive system. The other exclusion criteria were the use of medications that affect glucose metabolism, food allergies, and pregnancy.

3.2.5 Standardized protocol

The study required that a standardized protocol be followed. Full details of these requirements are given in Appendix 5.

3.2.6 Ethics

The study was approved by the Human Ethics Committee of the University of Otago, and all subjects gave written informed consent (Appendix 2, 3).
3.3 Test foods

3.3.1 Reference food

Carbotest® 50G glucose tolerance drink (Lomb scientific, Australia) was used as a reference food. A 300mL bottle contained 50 grams of glucose.

3.3.2 Rice

Five rice varieties were studied: Basmati, Brown, Doongara®, Jasmine, and Parboiled. Basmati, Brown, Doongara® and Parboiled were chosen because these are commonly recommended by dietitians. Jasmine rice is inexpensive, commonly consumed rice. All five varieties of rice were bought from the same local supermarket (Table 4).

Table 4: Description of tested rice varieties

<table>
<thead>
<tr>
<th>Rice type</th>
<th>Manufacturer</th>
<th>Marketed by</th>
<th>Country of product</th>
<th>Price** (NZ $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>Long</td>
<td>SunRice®</td>
<td>Ricegrowers Ltd.</td>
<td>4.29/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Brown</td>
<td>Medium, brown</td>
<td>SunRice®</td>
<td>Ricegrowers Ltd.</td>
<td>2.89/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Doongara®</td>
<td>Long, white</td>
<td>SunRice®</td>
<td>Ricegrowers Ltd.</td>
<td>3.99/750g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Jasmine</td>
<td>n/a*</td>
<td>SunRice®</td>
<td>Ricegrowers Ltd.</td>
<td>3.39/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Parboiled</td>
<td>Long, white</td>
<td>Uncle Ben’s®</td>
<td>MasterFoods</td>
<td>4.85/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Australia New Zealand</td>
<td></td>
</tr>
</tbody>
</table>

*not stated on packet or manufacturer’s website; **Price obtained on 29/09/2009.

The portion size of packaged rice containing 50g carbohydrate was determined using data obtained from a commercial laboratory (AsureQuality Ltd, Auckland, New Zealand) using the carbohydrate by difference method (AOAC2005). The carbohydrate content was confirmed by the direct measurement of starch carried out by the candidate in the University laboratory using the method shown in Appendix 9. Pilot studies were undertaken to determine the appropriate rice to water ratio for each rice. In practice, rice and water were weighed to the nearest gram. The rice was cooked following the same procedure using the same rice to water ratio (depending on the type of rice) throughout the study (Table 6). All rice was
cooked in the Metabolic Kitchen of the department of Human Nutrition using a rice cooker (Tefal® automatic rice cooker).

The percentages of carbohydrate in cooked rice samples are shown in Table 5. The amount of packaged rice required to provide 50g available carbohydrate was calculated by adjusting the 'solids of cooked rice' to allow for the moisture content of the dry rice (i.e. uncooked raw rice).

1. \(100 - A = B\) (i.e. wt of solids in cooked rice)
2. \(B / (100 - C) \times 100 = \) (wt of dry rice)
3. \(D / \) (wt of dry rice) = E (i.e. % CHO in dry rice)
4. \(50\)g CHO / E \(\times 100 = \) One portion of dry rice (with 50g CHO) --- (Table 6)

Table 5: Moisture and carbohydrate content of rice per 100 g

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Moisture content of cooked rice* (%) -- A</th>
<th>Solid content of cooked rice (%) -- B</th>
<th>Moisture content of dry rice** (%) -- C</th>
<th>Carbohydrate by difference* in cooked rice (%) -- D</th>
<th>Carbohydrate content of dry rice (%) -- E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>66.5</td>
<td>33.5</td>
<td>8.6</td>
<td>29.3</td>
<td>80.0</td>
</tr>
<tr>
<td>Brown</td>
<td>63.9</td>
<td>36.1</td>
<td>7.9</td>
<td>29.8</td>
<td>76.0</td>
</tr>
<tr>
<td>Doongara</td>
<td>56.9</td>
<td>43.1</td>
<td>8.6</td>
<td>38.4</td>
<td>81.4</td>
</tr>
<tr>
<td>Jasmine</td>
<td>62.4</td>
<td>37.6</td>
<td>9.4</td>
<td>33.9</td>
<td>81.7</td>
</tr>
<tr>
<td>Parboiled</td>
<td>69.2</td>
<td>30.8</td>
<td>6.7</td>
<td>27.0</td>
<td>81.8</td>
</tr>
</tbody>
</table>

*Obtained from the report of AsureQuality for cooked rice; **obtained from Moisture content test of dry rice.

Table 6: Weight of a rice portion (50g CHO), and the amount of water used in the study

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Weight of one portion of rice (dry) (g)</th>
<th>Ratio of water to rice (dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>63</td>
<td>1:1.5</td>
</tr>
<tr>
<td>Brown</td>
<td>66</td>
<td>1:2.0</td>
</tr>
<tr>
<td>Doongara</td>
<td>61</td>
<td>1:1.3</td>
</tr>
<tr>
<td>Jasmine</td>
<td>61</td>
<td>1:1.1</td>
</tr>
<tr>
<td>Parboiled</td>
<td>61</td>
<td>1:2.5</td>
</tr>
</tbody>
</table>
3.3.3 Sugar

LoGiCane™ cane sugar (Horizon Science, Australia), a non-starch food was chosen to test the hypothesis that ethnic differences in GI are due to ethnic differences in starch metabolism. The sugar is a ‘low GI’ product launched in New Zealand in November 2009. To prepare a test sample of LoGiCane™, approximately 50g (50±0.5g) was weighed using a scientific scale accurate to 0.01 (Sartorius, USA). The sugar was dissolved with a small amount of hot water, then topped up with carbonated water (kiwi blue, Coca Cola-Amatil Ltd.) to 300mL. Carbonated water was used instead of still water to make consumption of the sugary solution make palatable.
3.4 Data collection

3.4.1 Testing schedule

Study subjects visited our laboratory between 6am and 8am after a 10hr overnight fast. During the study, participants were asked not to change their diet or level of physical activity. Alcohol intake was allowed provided it was a moderate quantity (two standard drinks or 20g of alcohol for females, and three or 30g of alcohol for males) (Godley R et al., 2008). Participants were asked not to exert themselves when travelling to the clinic (i.e. to walk slowly or to be transported by vehicle).

Participants were required to attend the clinic on nine occasions, twice to test the glucose reference beverage, twice to test Jasmine rice, and once each for Basmati, Brown, Doongara® and Parboiled rice. Each participant had tests on non-consecutive days (two tests per week). Around 10–15 people were tested on each test day, with similar number of each group attending each occasion. Practical considerations precluded formal randomisation of the sequence of consumption of the different varieties of rice, glucose beverage and LoGiCane™ sugar. Groups of participants started the sequence on different days.

3.4.2 Measured parameters

3.4.2.1 Blood glucose

Capillary blood was collected by finger pricking using a sterilised disposable lancet. Blood glucose concentrations were determined from a drop (5µL) of blood using a Hemocue® Glucose 201+ Analyzer (HemoCue, Netherlands). The meters were calibrated daily before the test using three different concentrations of control solutions from the manufacturer.

During each test, a series of eight blood samples were collected over a period of 120min: -5 and 0min (fasting), then 15, 30, 45, 60, 90, and 120min after the start of ingestion of test food (Appendix 5). The baseline fasting blood glucose levels were obtained by averaging the values of the first two readings (i.e. -5 and 0mins). If those two readings had a difference greater than 0.5mmol/L, one more finger-prick was taken within five minutes and the average of three readings taken as a baseline. Following the baseline tests, a reference (i.e. glucose beverage) or test food (rice or sugar) was provided. The study subjects were instructed to consume the food at an even pace over 15 minutes. All subjects were asked to remain seated quietly during two-hour test period. The staff employed for blood glucose
measurement included an enrolled nurse and postgraduate students trained in the technique of capillary blood collection. All staff had been vaccinated for Hepatitis B.

3.4.2.2 Rice chewing

On six occasions (when rice was tested), the participants were given a teaspoonful (10g) of the cooked rice after the participant had finished all finger pricks. They were requested to put all the rice in their mouth at once, to chew for as long as usual, then instead of swallowing, to expectorate the bolus of chewed rice into a plastic container. The participants were advised not to swallow any of the rice. A sip of water was given if requested to rinse the mouth and this was also expectorated into the container. (Appendix 5)

The chewed samples were washed under running water over a stainless steel laboratory sieve (Endecotts Ltd. London, England) with a mesh aperture of 425μm. The rice particles retained on the sieve were carefully collected and placed into a metal dish for drying. The sieve was rinsed with water to ensure a complete collection. The samples were oven-dried at 70°C (for 24-48 hours). A non-expectorated duplicate sample was used for a moisture content determination. From the weight of the dried sample, the proportion of the rice sample that passed through the sieve (i.e. expectorated thoroughly) was calculated (Appendix 8).

3.4.2.3 Salivary α-amylase

Saliva samples were collected on the four test days when the study participant tested: Jasmine rice (twice), brown rice (once) and glucose beverage (once). Saliva was collected immediately after the test food (or glucose beverage) had been consumed. Fasting saliva was collected on two occasions before the Jasmine rice had been consumed. In preparation for collection, participants were instructed to clear all food debris from the mouth. Subsequently, approximately 1mL of unstimulated (passive drooling) saliva was collected (Rohleder N and Nater UM, 2009) into a snap-lock tube. The sample was stored immediately in a chilly bin with ice packs and then kept frozen at -80°C until analysis.

The analysis was conducted in the laboratory of the Department of Human Nutrition. An enzymatic colorimetric assay was performed using an AMYL® α-amylase kit (Roche/Hitachi), and a Roche/Hitachi Cobas Mira Plus® spectrophotometer (Roche,
Switzerland). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have accredited the assay. The enzymatic reaction uses 4,6-ethylidene-(G7)-2,4-nitrophenyl-(G1)-α,D-maltoheptaoside (Ethylidene Protecte Substrate) which produces p-nitrophenol (PNP) degradation products of α-amylase. PNP absorbs light at wavelength of 405nm (yellow) (Lorentz K, 1998, Roche/Hitachi, 2008, Rohleder N and Nater UM, 2009). The optical density of the assay is quantitatively related to the initial α-amylase content (Rohleder N and Nater UM, 2009). Enzymatic activity is measured in terms of enzyme units per millilitre (U/mL), defined as the amount of enzyme required to catalyse the conversion of 1µmol of substrate per minute (Rohleder N and Nater UM, 2009). A standard curve was used to convert relative activity to standardized units (Rohleder N and Nater UM, 2009). A commercial standard solution Calibrator f.a.s (Roche/Hitachi, 2008) was included in the analysis. All samples were tested in duplicate. The intraclass correlation for duplicate measures were fasting (0.9), jasmine rice (0.7), brown rice (1.0) and glucose (1.0), indicating good repeatability and reproducibility of the test.

3.4.3 Participant characteristics

3.4.3.1 Demographic characteristics

Basic demographic (age, sex, smoking habits) and anthropometric data (height and weight) were collected. Height was measured using a stadiometer. Participants were positioned without shoes, with their back and heels against the stadiometer pole. The participant’s head was placed in the Frankfort horizontal plane. They were asked to look straightforward and stand as upright as possible. Measurements were taken to the nearest millimetre. Weight was measured using a calibrated set of electronic scales (Wedderburn). Participants were instructed to remove shoes, jackets and any heavy belongings such as wallet or keys from their pockets. Their weight was recorded to the nearest 0.1kg. Height and weight measurements were duplicated and the average calculated. If duplicate measures differed by more than 10%, a third measurement was obtained and the average of the three measurements used.

3.4.3.2 Physical activity level

The physical activity of participants was assessed using a questionnaire based on the New Zealand Physical Activity Questionnaire (NZPAQ-LF). However, it was expanded to
include questions related to recreational, occupational, commuting, household, gardening, sitting and sleeping times. This questionnaire is self-reported and covers a seven-day recall period. It is designed to capture activities carried out in all contexts (SPARC, 2004). Additional questions were asked relating to the time spent in inactivity (i.e. sitting and sleeping). The participants were asked to fill out the questionnaire while they were sitting in the laboratory. A trained research assistant was present to assist participants.

Each activity was assigned to an intensity weight, Metabolic Equivalent of Task (MET) according to the Compendium of physical activities (Ainsworth BE et al., 2000). Total activity scores were derived from the questionnaire as MET-hour per week. MET is a physiological concept that represents a simple procedure for expressing the energy cost of physical activity as multiples of resting metabolic rates (Ainsworth BE et al., 2000).

One MET represents the approximate rate of oxygen consumption of a seated adult at rest, or $1 \text{ MET} = 3.5 \text{ mL O}_2/\text{kg*min}$ (Pate RR et al., 1995). The equivalent energy cost of 1MET in kcal/min is approximately 1kcal/kg/hr (Pate RR, 1995).

Classification for the MET intensity (Ainsworth BE et al., 2000):

- MET <3 for light intensity activities (e.g. 0.9 sleeping, 1.0 TV watching)
- MET 3-6 for moderate intensity activities (e.g. 3.0 bicycling with very light effort)
- MET >6 for vigorous intensity activities (e.g. jogging 7.0, push-ups 8.0)
3.5 Statistics

3.5.1 Sample size estimation

The variability of GI testing is known from previous work in the Department of Human Nutrition. A sample of 30 people per group would have 80% power to detect a difference of 10 GI units using the 5% level of significance. Thus thirty people of each ethnicity (30 Europeans and 30 Chinese) were sufficient for this study.

3.5.2 The incremental area under the blood and GI calculation

The incremental area under the blood glucose curve (iAUC) (i.e. the area above the baseline fasting glucose) was calculated as recommended by WHO for GI testing (FAO/WHO, 1998). Since the distribution of the iAUC was skewed and the variance increased as the mean increased (Williams SM et al., 2008), the data were log transformed and the results were presented as geometric means, ranges and ratios. The average iAUC of the two reference (i.e. glucose beverage) tests was used as the reference value to calculate the GI values for the test foods. The GI of each food for each participant was calculated according to the formula below and the average of the group (Chinese or European) taken as the exponent of the mean GI.

\[
\text{Participant GI} = \exp [\ln (i\text{AUC}_{\text{test food}}) - \ln (i\text{AUC}_{\text{reference}})]
\]

3.5.3 Statistical tests

Statistical analysis was performed using STATA (version 11.0). The significance of the differences in subjects’ characteristics, expectorated rice samples, and physical activity levels were compared with a two-sample t-test and one-way analysis of variance (ANOVA). P<0.05 was considered to be statistically significant.

A mixed model with participant as a random effect, which accounts for the correlation between the observations for each person was used to analyse the data from glucose and the rice meals as a single experiment. Estimates for the difference (as a ratio) between the Chinese and European participants for the iAUCs of glucose and each rice were derived from the model. The model was also used to estimate the GI and its 95% confidence interval for each rice and the ratio of the GIs for the two groups.
Univariate regression models with a random effect for participant were used to examine the association between the iAUC for rice and variables including age, sex, BMI, metabolic equivalent task (MET) score, salivary alpha-amylase activity and chewing extent. If the p-value for the univariate analysis was 0.2 or less, the variable was not included in the final (adjusted) model.
4 Results

4.1 Study participants

Sixty-three participants (31 European Caucasian and 32 Chinese) participated in this study. Table 7 shows the number of the participants in each age group. Although I tried to balance the number of males and females within each age range, I had difficulty recruiting Chinese men (age 31-50yr). Demographic characteristics of participants are shown in Table 8. Despite the over-representation of young Chinese males, the mean age of the two ethnic groups was not statistically significant (Table 8).

Of the 32, one Chinese participant withdrew from the study due to personal issues. However, we included this participant’s incomplete set of results in the dataset.

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>European (n= 31)</th>
<th>Chinese (n= 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>18-30</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>41-50</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

European participants were people of European descent from countries that were geographically dispersed (e.g. North America, UK, Europe, Australia, or New Zealand). Chinese participants also originated from a number of countries including Mainland China, Taiwan, Hong Kong, Malaysia, Singapore, Philippines, Thailand and New Zealand. The Chinese participants had self-reported that rice was their staple food.

Table 8 shows the demographic characteristics of the participants were well matched between groups. Sex, age, height distribution were not significantly different between the groups, but the body weight and BMI were significantly lower in the Chinese than in the European group (p<0.05).

There was no difference in the fasting blood glucose levels between ethnic groups. Self-reported level of physical activity recorded as metabolic equivalent task (MET) score was significantly greater in the European group than in the Chinese group.
Table 8: Characteristics of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>European</th>
<th>Chinese</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>Sex male; n (%)</td>
<td>15 (52)</td>
<td>17 (47)</td>
<td>0.71</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>34.3 (8.18)</td>
<td>33.4 (8.44)</td>
<td>0.67</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 (0.09)</td>
<td>1.68 (0.09)</td>
<td>0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.2 (15.88)</td>
<td>64.9 (11.32)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 (4.76)</td>
<td>22.9 (2.72)</td>
<td>0.005</td>
</tr>
<tr>
<td>MET hr/week</td>
<td>67.5 (51.23, 88.90)</td>
<td>43.5 (33.22, 56.91)</td>
<td>0.023</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>4.8 (0.47)</td>
<td>4.8 (0.36)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are mean (SD), unless otherwise stated; MET metabolic equivalent task score; *geometric mean (min, max).

4.2 Results for incremental Areas Under the Curve (iAUC)

For all occasions, the fasting blood glucose concentrations were not different between groups (p= 0.60). Glycaemic response following consumption of food was expressed as incremental blood glucose areas under the curve (iAUC). The iAUC of the reference food and all test foods were significantly different between the European and Chinese groups (Table 9). The iAUC of all foods were statistically greater in the Chinese group than in the European group by 39% (glucose reference beverage) to 77% (parboiled rice). The iAUC of all rice varieties were more than 60% greater in the Chinese group compared to the European group. The iAUC of glucose beverage and LoGiCane® sugar were greater in the Chinese group, too, but to a lesser extent.

In both groups, the largest iAUC was apparent after consumption of glucose beverage, followed by Jasmine rice. The smallest iAUC among rice varieties was of the Doongara® in both groups.
Table 9: Geometric mean and ratio of AUC (mmol/L *min) in the two ethnic groups

<table>
<thead>
<tr>
<th></th>
<th>European</th>
<th>Chinese</th>
<th>Ratio (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>201 (84.1, 721.9)</td>
<td>274 (86.65, 522.38)</td>
<td>1.39 (1.11, 1.74)</td>
<td>0.004</td>
</tr>
<tr>
<td>Basmati</td>
<td>116.39 (18.11, 289.88)</td>
<td>184.68 (44.79, 607.50)</td>
<td>1.61 (1.23, 2.11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Brown</td>
<td>129.30 (40.55, 285.00)</td>
<td>210.50 (74.31, 464.63)</td>
<td>1.67 (1.30, 2.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Doongara</td>
<td>109.67 (32.89, 318.75)</td>
<td>179.89 (46.35, 380.63)</td>
<td>1.68 (1.31, 2.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Jasmine</td>
<td>140.00 (42.21, 297.56)</td>
<td>220.77 (75.02, 409.50)</td>
<td>1.63 (1.31, 2.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parboiled</td>
<td>112.45 (36.78, 301.13)</td>
<td>194.00 (55.94, 401.25)</td>
<td>1.77 (1.37, 2.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LoGiCane</td>
<td>119.81 (31.49, 454.88)</td>
<td>169.41 (26.60, 325.29)</td>
<td>1.45 (1.13, 1.86)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are mean (min, max); ratio (95%CI). n=63 for all rice varieties except Basmati (n=44).

Figure 1 shows the change of the mean blood glucose for a 120min test period. The peak blood glucose levels were statistically higher in the Chinese group compared with the European group. Also, the peak blood glucose level was estimated to have occurred 4min later in Chinese than in Europeans.
Figure 1: Mean glycaemic response of reference and test foods

A. Glucose

B. Jasmine

C. Basmati

D. Brown

E. Doongara

F. Parboiled

G. LoGiCane

Chinese (dash), European (solid)
4.3 Results for Glycaemic Index (GI)

Table 10 shows the GI values of the test foods (based on glucose beverage as a reference) and the ratio of the values between groups. Doongara®, Jasmine, and Parboiled rice showed a significant difference in GI values between the two ethnic groups. The GI values of these test foods were significantly greater in the Chinese than in the European group by 21%, 18%, and 27% respectively. The difference in GI of Brown rice was borderline significance, whereas the difference in GI of Basmati rice was not significantly different between groups.

### Table 10: GI and ratio of GI (95% CI), and classification of GI (when glucose =100)

<table>
<thead>
<tr>
<th>Food</th>
<th>European</th>
<th>Chinese</th>
<th>Ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>57 (49, 67)</td>
<td>67 (58, 77)</td>
<td>1.16 (0.94, 1.43)</td>
<td>0.170</td>
</tr>
<tr>
<td>Brown</td>
<td>65 (57, 74)</td>
<td>78 (68, 89)</td>
<td>1.21 (1.00, 1.45)</td>
<td>0.054</td>
</tr>
<tr>
<td>Doongara®</td>
<td>55 (48, 63)</td>
<td>67 (58, 76)</td>
<td>1.21 (1.01, 1.37)</td>
<td>0.045</td>
</tr>
<tr>
<td>Jasmine</td>
<td>68 (61, 76)</td>
<td>80 (72, 90)</td>
<td>1.18 (1.01, 1.37)</td>
<td>0.033</td>
</tr>
<tr>
<td>Parboiled</td>
<td>57 (50, 64)</td>
<td>72 (63, 82)</td>
<td>1.27 (1.06, 1.57)</td>
<td>0.011</td>
</tr>
<tr>
<td>LoGiCane</td>
<td>60 (53, 69)</td>
<td>63 (55, 72)</td>
<td>1.04 (0.80, 1.25)</td>
<td>0.666</td>
</tr>
</tbody>
</table>

n=63 for all rice varieties except Basmati (n=44).

<table>
<thead>
<tr>
<th>Low GI 55 or under</th>
<th>Medium GI 56-69</th>
<th>High GI 70 or above</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Brand-Miller JC, 2003)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to the classification of GI: high GI (GI ≥70); medium GI (GI 56-69); or low GI (GI≤55) (Brand-Miller JC, 2003), only Basmati rice had the same classification in both groups (i.e. medium GI) (Table 10). In the European group, none of the rice varieties was classified as high GI, whereas three rice varieties (Brown, Jasmine, and Parboiled) were high GI in the Chinese group. Doongara® rice had the lowest GI in both ethnic groups. It was classified as low GI in the European and medium GI in the Chinese group. None of the rice varieties was classified as low GI in Chinese group. The GI of LoGiCane™ sugar was medium in both groups.
Table 11 shows the GI values when Jasmine rice was used as a reference. The GI values are more similar between the groups and therefore the ratio is much smaller. There was no significant difference in all the foods between the European and Chinese groups.

Table 11: GI and ratio of GI (95% CI) between ethnic groups (when Jasmine =100)

<table>
<thead>
<tr>
<th></th>
<th>European</th>
<th>Chinese</th>
<th>Ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>87 (71, 99)</td>
<td>83 (74, 96)</td>
<td>0.98 (0.80, 1.21)</td>
<td>0.877</td>
</tr>
<tr>
<td>Brown</td>
<td>95 (83, 108)</td>
<td>97 (85, 110)</td>
<td>1.02 (0.85, 1.22)</td>
<td>0.852</td>
</tr>
<tr>
<td>Doongara</td>
<td>81 (71, 92)</td>
<td>83 (73, 94)</td>
<td>1.03 (0.85, 1.23)</td>
<td>0.790</td>
</tr>
<tr>
<td>Parboiled</td>
<td>83 (73, 94)</td>
<td>89 (78, 102)</td>
<td>1.08 (0.90, 1.30)</td>
<td>0.422</td>
</tr>
<tr>
<td>LoGiCane</td>
<td>88 (77, 101)</td>
<td>78 (68, 89)</td>
<td>0.88 (0.74, 1.06)</td>
<td>0.190</td>
</tr>
</tbody>
</table>

n=63 for all rice varieties except Basmati (n=44).

4.4 Results for salivary alpha-amylase (sAA) analysis

Mean values of salivary alpha-amylase (sAA) activity level are shown in Table 12. Fasting as well as postprandial sAA activity levels were greater in the Chinese group than their European counterparts. No significant mean difference was shown in all sAA levels between the European and Chinese groups in t-test. Intra-class correlation ($\rho_I$) for fasting, post Jasmine, post Brown, and post Glucose drink were close to 1.0, which indicates no difference within a group (0.89, 0.73, 0.99 and 1.0, respectively) (data not shown). This means the assay was highly repeatable and reproducible.

Table 12: Results of the mean sAA activity level (SD)

<table>
<thead>
<tr>
<th>Time of sampling</th>
<th>European (U/mL)</th>
<th>Chinese (U/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>199.7 (244.0)</td>
<td>248.9 (198.9)</td>
<td>0.38</td>
</tr>
<tr>
<td>After Jasmine</td>
<td>199.0 (136.2)</td>
<td>285.0 (198.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>After Brown</td>
<td>192.3 (138.0)</td>
<td>246.8 (172.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>After Glucose drink</td>
<td>218.9 (146.6)</td>
<td>357.6 (440.8)</td>
<td>0.11</td>
</tr>
</tbody>
</table>
4.5 Results for expectorated rice test

Table 13 shows the proportion of the chewed rice that passed through the sieve with 425µm aperture. A greater proportion of rice passed through the sieve in the Chinese group than in the European group. However, the differences between groups only achieved statistical significance for Brown, Doongara®, and Jasmine rice.

Table 13: Proportion (%) of the chewed rice passed through the sieve (<425µm)

<table>
<thead>
<tr>
<th>Food</th>
<th>European</th>
<th>Chinese</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati</td>
<td>56.2 (16.7)</td>
<td>60.7 (20.9)</td>
<td>0.353</td>
</tr>
<tr>
<td>Brown</td>
<td>52.5 (13.9)</td>
<td>60.0 (15.6)</td>
<td>0.048</td>
</tr>
<tr>
<td>Doongara</td>
<td>46.8 (22.0)</td>
<td>62.8 (22.1)</td>
<td>0.006</td>
</tr>
<tr>
<td>Jasmine</td>
<td>46.4 (15.5)</td>
<td>55.7 (18.5)</td>
<td>0.035</td>
</tr>
<tr>
<td>Parboiled</td>
<td>59.9 (15.5)</td>
<td>66.3 (15.8)</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Values are mean (SD). n=63 for all rice varieties except Basmati (n=44).

4.6 Determinants of glycaemic response (iAUC)

Table 14 shows the results of univariate and multiple regression analyses to examine the association with iAUCs for rice, age, sex, BMI, MET, sAA activity and chewing extent. In the univariate analysis, the p-values for ethnicity, age, BMI, sAA activity and chewing extent were less than 0.2, thus these were included in the final (adjusted) model.

Ethnicity was strongly positively associated with iAUC. Chinese ethnicity significantly increases iAUC by 67% (p<0.001) in the univariate analysis, and it remained significantly higher by 45% (p<0.001) after adjustment for age, BMI, sAA and chewing extent.

Age, BMI and sAA activity level also had a significant effect on iAUC. Age and sAA activity were positively associated with iAUC. The iAUC increases by 1% for every year of age (p=0.02), and by 7% for every 100U/mL increases in sAA activity level (p=0.02). On the contrary, BMI was negatively associated with iAUC. The iAUC decreased by 3% for every
unit increase in BMI (p=0.04). However, the effect of these variables is very small by comparison with the effect of ethnicity. There was no effect of extent of chewing.

Rice variety showed a significant iAUC difference from that of Jasmine rice. The iAUCs of Basmati, Doongara® and Parboiled rice were significantly smaller than that of Jasmine, whereas iAUC of Brown rice was similar to that of Jasmine (p=0.345).

The mixed regression model also showed that overall sAA activity level was significantly higher in the Chinese subjects by 104U/mL than the European individuals (data not shown).

Table 14: Determinants of iAUC of rice expressed as unadjusted and adjusted risk ratio (95% CI) comparing Chinese with European

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk ratio (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1.67 (1.35, 2.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>1.01 (1.00, 1.03)</td>
<td>0.13</td>
</tr>
<tr>
<td>Sex</td>
<td>0.94 (0.74, 1.20)</td>
<td>0.63</td>
</tr>
<tr>
<td>BMI</td>
<td>0.96 (0.93, 0.98)</td>
<td>0.002</td>
</tr>
<tr>
<td>MET</td>
<td>1.00 (1.00, 1.00)</td>
<td>0.62</td>
</tr>
<tr>
<td>sAA activity (per 100U/mL)</td>
<td>1.11 (1.04, 1.18)</td>
<td>0.002</td>
</tr>
<tr>
<td>Chewing extent (per 10% of chewed bolus passed through the sieve)</td>
<td>1.02 (1.00, 1.05)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Jasmine (reference range) 1.00 1.00
Basmati 0.83 (0.75, 0.93) 0.001 0.83 (0.74, 0.92) <0.001
Brown 0.96 (0.88, 1.05) 0.394 0.96 (0.87, 1.05) 0.345
Doongara 0.82 (0.75, 0.90) <0.001 0.82 (0.74, 0.90) <0.001
Parboiled 0.86 (0.78, 0.94) 0.001 0.85 (0.77, 0.93) 0.001

MET: Metabolic Equivalent Task, sAA: Salivary α-amylase.
5 Discussion

The data show clear ethnic differences in glycaemic responses to glucose, rice varieties and LoGiCane sugar. Postprandial glycaemia, as measured by iAUC, was greater in Chinese than Europeans of comparable age. The magnitude of the difference was considerable, being over 60% greater for the five rice varieties, 39% for the glucose beverage and 45% for the LoGiCane sugar solution. Additionally, the Chinese group had significantly higher estimated iAUC peak than the European group. The peak was higher by 0.87mmol/L (p<0.001) higher and occurred 4min later in Chinese compared with Europeans. The calculated GI of the five rice varieties tested also tended to be higher in Chinese compared with Europeans which was in contrast to previous research showing that GI does not differ between ethnic groups (Chan HMS et al., 2001, Henry CJK et al., 2008). A novel aspect of this study was the measurement of salivary alpha-amylase enzyme activity and the effect of chewing, factors that could explain the observed ethnic differences in postprandial glycaemia. Although there was no significant difference in the measurement related to the extent of chewing, salivary alpha-amylase did appear to be a small but significant determinant of iAUC.

5.1 Postprandial glycaemia

Ethnic differences in postprandial glycaemia have been reported previously (Dickinson S et al., 2002, Wolever TMS et al., 2009, Venn BJ et al., 2010). In studies of glycaemic responses to some test foods Asian (Venn BJ et al., 2010), Chinese (Dickinson S et al., 2002) and non-Caucasian subjects (Wolever TMS et al., 2009) were shown to have a larger iAUC response in comparison with European subjects. However, the two studies which examined GI tested only a limited number of foods (Wolever TMS et al., 2009, Venn BJ et al., 2010), and the “Asian” and “non-Caucasian” groups included subjects from several different ethnic groups. In the other, only glycaemic response to white bread was measured (Dickinson S et al., 2002). The Asian group had 63% greater iAUC than their European counterparts after the consumption of the breakfast cereals (Venn BJ et al., 2010). In the glycaemic response study, 50% greater in iAUC after white bread was observed in Chinese compared with the European subjects (Dickinson S et al., 2002). However, these studies did not examine the possible explanations for the greater iAUC shown in the comparison groups, and did not examine the effects of ethnicity on rice which is the staple food of most Asian population groups. The
ethnic differences in postprandial glycaemia must be due to more glucose being available for absorption (as a result of more complete digestion of starch, or more efficient glucose absorption), or delayed clearance.

The multiple regression analysis in the present study clearly shows that ethnicity was positively associated with iAUC of rice varieties, and it was the variable which has the largest impact on iAUC compared with others (Table 14). Chinese ethnicity increases iAUC by 67% (p<0.001) when considering the unadjusted risk ratio, and it remained significantly greater by 45% after adjustment for age, BMI, salivary amylase, and chewing extent (p<0.001). Age, sex, BMI, level of physical activity, salivary amylase, and extent of chewing are some of factors which were measured and might contribute to the ethnic difference in postprandial glycaemia observed in the present study. These are discussed in the following sections.

5.1.1 Age

Postprandial glycaemia rises linearly with age (DECODE Study Group, 2003). Age was reported to be a significant determinant of iAUC in a study in which subjects 40 years or younger, or older than 40 years of Caucasian or non-Caucasian ethnicity were compared (Wolever TMS et al., 2009). The mixed regression model in the present study showed that age was significantly associated with iAUC. However, the groups were well matched for age and the effect of age was very small, and therefore unlikely to have accounted to any important extent for the difference in postprandial glycaemia observed between the Chinese and European groups.

5.1.2 Sex

Postprandial glycaemia has been shown to differ between men and women (DECODE Study Group, 2003, Basu R et al., 2006, Sicree RA et al., 2008). However, in the present study, the groups were balanced for sex and consequently sex differences could not explain ethnic differences in glycaemic response. Sex was not a significant determinant of iAUC in the regression analysis.

5.1.3 Body Mass Index

Significant differences in glycaemic response have been found between various ethnic groups despite the participants having similar BMI in previous studies (Dickinson S et al.,
In the present study, there was a significant difference in mean BMI between Chinese and European participants. The European subjects were approximately 11kg heavier than the Chinese (Table 8), which accounts for the greater BMI of the European group since height was not different between groups. BMI was inversely associated with iAUC in the multivariate regression analysis (p = 0.04) (Table 14) indicating that to some of the ethnic difference in iAUC in the present study could be attributable to differences in BMI. This result may be regarded as counterintuitive since adiposity may be expected to be associated with a greater glycaemic response. It is conceivable that the relationship between BMI and glycaemic response could be explained by the relationship between body mass and blood volume. Blood volume is estimated to be 70mL per kg of body mass (Lemmens H et al., 2006). A larger body therefore has a greater blood volume and this might have an effect on glycaemic dilution – that is the glucose in the test food is distributed in a greater volume of blood and thus is more dilute. However the proportion of lean to fat mass may be even more relevant.

Ethnic differences in the relationships between body fatness and BMI have been reported previously (Wang J et al., 1994, Deurenberg P et al., 2002, Deurenberg-Yap M et al., 2002, WHO expert consultation, 2004, Lear SA et al., 2009, Wen JYJ et al., 2010), with some Asian populations having a greater fat mass, especially abdominal fat mass, for a given BMI than Europeans (Wang J et al., 1994, Deurenberg-Yap M et al., 2002, Lear SA et al., 2009, Wen JYJ et al., 2010). Chinese men and women living in New Zealand had significantly higher abdominal fat mass, compared with NZ Europeans (Wen JYJ et al., 2010). The present study did not include an independent measure of body fatness. Thus we are unable to determine the extent to which differences in body composition between those of European and Chinese ethnicity explain the present findings. It is certainly conceivable that a greater skeletal muscle mass might in part explain the reduced glycaemic response in Europeans. It is equally possible that a greater abdominal fat mass or a higher fat and lean ratio in the Chinese (Lear SA et al., 2009, Wen JYJ et al., 2010) could explain the higher iAUC as a result of delayed glucose clearance associated with insulin resistance.

5.1.4 Physical activity

Physical activity has been shown to be negatively related to postprandial glycaemia (Healy GN et al., 2006). One effect of physical activity is to increase insulin sensitivity in skeletal muscle, which is the major site of glucose disposal (DeFronzo RA and Tripathy D,
Reduced muscle mass has been shown to be associated with reduced insulin sensitivity and greater postprandial glycaemia (Nathan DM et al., 2007). Based on the self-reported physical activity questionnaire, in the present study, the Chinese were considerably less active than the Europeans (Table 8). This was consistent with the findings in the Asian Health in Aotearoa 2006/2007 survey, in which 41% of Chinese in comparison with 54% of Europeans in New Zealand were reported as being physically active (Scragg R, 2010).

In terms of metabolic equivalence task (MET), the Chinese would be classified as inactive whilst the Europeans were moderately active (Qin L et al., 2010). The lower physical activity and body mass of the Chinese in the present study potentially suggest that the Chinese participants had a smaller skeletal muscle mass and/or reduced sensitivity to insulin compared with the Europeans. Less fat free mass and appendicular skeletal muscle mass has been shown in Chinese compared with Europeans (Wen JYJ et al., 2010).

Insulin sensitivity in European subjects was reported to be 40% greater than in Chinese subjects (Dickinson S et al., 2002). Thus, the lower physical activity levels of the Chinese group may partly explain the greater postprandial glycaemic response compared with the Europeans. However, while Chinese participants in the present study did have a low level of physical activity than the Europeans, the regression analysis did not confirm this to be an important predictor of glycaemic response.

5.1.5 Salivary alpha-amylase (sAA)

Salivary alpha-amylase (sAA) is an enzyme which breaks down starch in the oral cavity (Mandel AL et al., 2010, Butterworth PJ et al., 2011), and it has been suggested to be a potential variable which affects glycaemic response (Wolever TMS et al., 2009, Ranawana V et al., 2010). The findings of dietary related differences in sAA gene copy (AMY1) suggest that ethnic differences in postprandial glycaemia may be affected by gene copy number (Perry GH et al., 2007a). The difference is shown in a study in which the sAA activity was substantially different between two African tribes, one with a predominantly starch-based diet and the other with an almost carnivorous diet (248U/mL, 22U/mL, respectively) (Squires BT, 1953). However, European subjects in that study had intermediate levels (101U/mL), probably because Europeans had a ‘mixed-diet’ with both an intermediate carbohydrate intake and an intermediate sAA activity (Mandel AL et al., 2010). Although the results in the preliminary analysis showed only a trend towards higher fasting and postprandial enzymatic
activity of sAA in Chinese (Table 12), which was not statistically significant, the difference was more clearly shown in the multiple regression analysis. The sAA activity in the Chinese individuals was 104U/mL greater than in the European individuals (p=0.028, data not shown). This difference is not as much as shown in the difference between the African tribes, however this may have contributed to greater glycaemic response in the Chinese group. The multiple regression analysis showed a significant independent effect of sAA activities on iAUC (p=0.02) (Table 14). Every 100U/mL increase in sAA activity only increases 7% of iAUC of rice (Table 14). Therefore, although the effect is statistically significant, the impact is relatively small in comparison with the effect of rice varieties and ethnicity.

Background dietary intake of the participants was not assessed in the present study. However, the Chinese in our study were New Zealand-born, or relatively long-term New Zealand residents. Since Asian men and women living in New Zealand consume a similar proportion of energy intake from carbohydrate to New Zealand Europeans (around 50% of total energy intake) (Metcalf PA et al., 2008), it is conceivable that the diet of our Chinese participants might have been adapted (i.e. westernised) to some extent from the very high carbohydrate diet traditionally consumed in China (78% of total daily intake) (Villegas R et al., 2007). Therefore, ethnic difference in sAA, which may have been apparent with traditional eating patterns, may have slightly diminished in this study. Although the importance of the role of sAA in starch digestion and the glycaemic response to high carbohydrate foods is debatable since most of the starch is hydrolysed by pancreatic alpha-amylase in the small intestine (Moss SJ, 1995, Woolnough JW et al., 2010, Singh J et al., 2010), sAA activity is positively associated with glycaemic response.

5.1.6 Extent of chewing

Chewing reduces the size of food particles, creating a greater surface area, then allowing better accessibility for digestive enzymes (Read NW et al., 1986, Ranawana V et al., 2010). This could contribute to a more rapid and complete digestion and absorption of starch leading to a greater glycaemic response. In studies reported over 30 years ago, O’Dea and colleagues found that the consumption of ground rice resulted in greater glycaemic responses than whole rice (O’vea K et al., 1980), and chewed rice induced a greater glycaemic response compared to when the rice was swallowed without chewing (Read NW et al., 1986). In the preliminary analysis, the proportion of chewed rice passing through a fine sieve (i.e. the particles size less than 425µm) was significantly higher in Chinese compared with the
Europeans after consuming three of the rice varieties, indicating that the Chinese subjects chewed their food more thoroughly than the Europeans. For the remaining varieties, a similar non-significant trend was apparent. However, while the multiple regression analysis showed that chewing extent was not associated with iAUC (Table 14), my data cannot exclude such an effect. The ethnic difference in iAUC in our study was more apparent for a solid food than for the glucose beverages and LoGiCane™ sugar (Table 9). This provides some additional circumstantial evidence that differences in postprandial glycaemia between ethnic groups may have been partly attributable to the variable degrees of chewing.

5.1.7 Summary

Factors potentially involved in the greater postprandial glycaemic response found in the Chinese compared with the European subjects include salivary alpha-amylase activity, less physical activity and different body composition, and extent of chewing. The regression analysis did not provide confirmatory evidence for the latter. The ethnic difference in postprandial glycaemia was more evident for rice than for the liquid foods suggesting that differences in chewing may also be a relevant factor affecting glucose absorption in the Chinese. Salivary amylase activity tended to be greater in the Chinese compared with the Europeans. However, considering the minor role of the enzyme in starch digestion, any potential differences in salivary amylase activity between these ethnic groups is unlikely to have had a major influence on the observed ethnic differences in postprandial glycaemia. Indeed in the regression analysis, the effects of all measured variables (other than the rice variety) were minimal compared with the effect of ethnicity and could not have explained the ethnic differences.

These results may have considerable clinical relevance given that an increase in 2hr postprandial glycaemia is reported to increase the risk of undiagnosed diabetes and impaired glucose regulation (DECODE Study Group, 2003).
5.2 Glycaemic Index

The present data suggest a different GI value for several varieties of rice in the Chinese and European groups. The Chinese had significantly higher GIs for Jasmine, Doongara® and Parboiled rice with a tendency to higher GIs for Brown and Basmati rice (Table 10). The absolute difference was 10 to 15 GI units higher in the Chinese compared with the Europeans. These results are unexpected given that GI is purported to represent a property of the food, independent of the consumer (Wolever TM et al., 1991, Brouns F et al., 2005, Wolever TM et al., 2008). The GI of LoGiCane™ sugar was not significantly different between ethnicities.

5.2.1 Possible reasons for the ethnic difference in GI of rice

There was a tendency for salivary amylase activity to be higher in Chinese than in the Europeans (Table 12). The multiple regression analysis showed that Chinese had significantly higher sAA activity by 104U/mL than the Europeans (p=0.028). In theory, a greater amylase activity could result in a more rapid digestion of starch in the mouth. The Chinese group tended to chew the rice more thoroughly, as indicated by the amount of chewed material passing through a 425µm sieve. Smaller particle size results in a larger surface area, allowing better access to digestive enzymes (Read NW et al., 1986, Ranawana V et al., 2010). O’Dea and colleagues reported that food made from finely ground rice induces a greater postprandial blood glucose response compared with unground rice (O’Dea K et al., 1980). Following rice consumption, the Chinese iAUC was some 60–70% higher than the European iAUC in the present study. How much of that increase in iAUC was due to chewing more thoroughly and how much was due to other factors such as the Chinese having a different body composition and being less physically active could not be definitely established in this study. The regression analysis suggests that none of these factors is likely to be important determinants of glycaemic response but does not reliably exclude a small effect. In the present study, glycaemic response to Jasmine rice was tested twice for the purpose of using it as an alternative reference food to a glucose beverage. When Jasmine rice was used as the reference, the ethnic difference in GI for the other rice varieties was no longer evident. The use of a solid food as a reference was routine in GI testing for a number of years when white bread was commonly used as a standard (Wolever TM et al., 1991). It was argued that a solid food was more physiologically relevant than a beverage when testing a solid food for GI (Wolever TMS et al., 1990). In theory, any food containing a substantial amount of carbohydrate could be used as a reference (Brouns F et al., 2005). However, standardisation
and comparability among laboratories would be a problem if various laboratories were using different reference foods. Currently, GI is most commonly tested in groups of people of European descent using glucose beverage as the reference food. The GI thus obtained is regarded as being generally applicable to all consumers with some research supportive of this generalisability (Wolever TM et al., 1991, Wolever TMS et al., 2003, Brouns F et al., 2005, Wolever TM et al., 2008). For example, the GIs of several foods using glucose as a reference were not different when tested in groups of Asian Indians and UK Caucasians (Henry CJK et al., 2008). The foods tested, biscuits and ready-to-eat cereals, may not have needed such extensive chewing as the rice we used. If chewing were not as strong a factor in the Indian tests, then the lack of ethnic difference is consistent with our data. Nevertheless, despite the lack of significant difference, the Indian group tended to have higher GIs compared with the UK Caucasians. The largest numerical difference occurred for sweet biscuits for which the GIs in the Caucasian and Indian groups were 47 and 63 (p=0.052), respectively. The number of participants used was relatively small (n=10), a factor that may explain the lack of statistical difference. A difference in the GI of white bread was found in a study in which larger groups (n=40) of Caucasian and non-Caucasians were tested (Wolever TMS et al., 2009). The non-Caucasian group had a higher GI to white bread than the Caucasian group, 78 compared with 66 (p<0.05) although no ethnic difference was found for two other foods, a chocolate chip cookie and a strip of fruit leather. A difference in the GI of a ready-to-eat breakfast cereal was found between a group of 73 adults of European descent (GI=61) and a group of 27 Asians with mixed ethnicity (GI=77; p=0.012) (Venn BJ et al., 2010).

Hence, there are indications in each of these studies of differences in GI between the various comparison groups. How much of the difference is ethnic-based and how much could be attributable to normal between-group differences in GI is unclear. Certainly, the GI of food in small groups (n=10) has been found to differ (Wolever TMS et al., 2003). However, the present study had 30 participants per group and group differences in GI were found in earlier studies comprising even larger samples of people of different ethnicity (Wolever TMS et al., 2009, Venn BJ et al., 2010). Overall, these findings are suggestive of ethnic-based differences in GI. Although chewing was not measured in these earlier studies, the data from our present study indicate that the extent of chewing may be a contributing factor to the ethnic differences found in GI values of rice varieties.
5.2.2 Comparison with other GI values for rice

*The International Tables of Glycemic Index and Glycemic Load Values* (Atkinson F et al., 2008) list a number of values for the rice varieties used in our study. For each of the rice varieties, there are a range of GI values. For example, Jasmine rice has ten entries in this Table with GI values ranging from 48 to 109. The brand of rice we used (SunRice®) has a reported GI of 89 (SEM 4). The values come from an ‘unpublished’ source so the ethnic mix of the group of the presumably ten participants tested is not known. Our GI values for this rice were 68 (95% CI: 61, 76) in the European and 81 (95% CI: 72, 90) in the Chinese group. Hence the published value is closer to the GI we obtained for the Chinese group and different to that in our European group. Similarly, Basmati rice has six entries in the International Tables ranging from 43 to 69, with an average of 57 (SEM 4). The brand we used (SunRice®) has a published value of 65 (SEM 7), a value more comparable with the GI in our Chinese group (67, 95% CI: 58, 77) than with our European group (57, 95% CI: 49, 67). Although it is intended that the published values are generally applicable, it is acknowledged that foods such as rice have large variability (Foster-Powell K et al., 2002). Despite this recommendation, our data indicate that between-group differences may occur for the same food tested in groups of different ethnicity.

5.2.3 Ranking of the GI values

Rice is a staple food for Chinese providing approximately 30% of total daily energy intake (FAO, 2004). Given the high rates of diabetes in Chinese population (Yang W et al., 2010), there may be a metabolic advantage in choosing a variety of rice that gives the lower glycaemic response in the context of both treatment and prevention. The enormous variability in rice GI values reported in the *International Tables 2008* (Atkinson F et al., 2008), even within the same variety, makes ranking of GI values a rather questionable procedure. One of the strengths of the present study was having tested different rice varieties in the same subjects. Although the absolute GI values may have been different between the groups, ranking the five rice varieties according to their GI value is potentially useful even though not based on standard statistical tests. The two highest rice GI values in both ethnic groups were Jasmine and Brown, with the other three varieties (Basmati, Doongara® and Parboiled) tending to be lower. Doongara® rice has a relatively high amylose content of 24-28% that may impart lower postprandial glycaemia (Miller JB et al., 1992, Ward R and Martin M, 2009) compared with Jasmine rice that has an amylose content of 17% (Ayabe S et al., 2009) or Basmati
Starches with high amylose content tend to be either slowly digested in the small intestine or resistant to digestion until they reach the large bowel (Chung HJ et al., 2011). Parboiled rice has been generally regarded as having a relatively low GI and as such has been recommended as an alternative choice for other rice varieties in clinical practice (Canadian Diabetes Association, 2009, Harvard Medical School), and in experimental studies (Jarvi AE et al., 1999, Jenkins DJA et al., 2008, Wolever TM et al., 2008). The high temperature used in parboiling may modify the starch structure (Walter M et al., 2005) changing the pattern of cereal starch creating a helical amylose complex (Priestley RJ, 1976, Walter M et al., 2005). This complex is insoluble and resistant to digestive enzymes (Priestley RJ, 1976, Walter M et al., 2005) resulting in lower glycaemic response in people with type 2 diabetes (Larsen HN, 2000). Although parboiled rice has been considered to have a relatively low GI, our data suggest that with a mean GI of 72 (95%CI: 63, 82), it should more appropriately be considered as a medium to high GI food. This finding may have been influenced by the extent of chewing, with a high proportion of chewed rice passing through the 425µm sieve (Table 13). This might be due to a rupture of starch molecules following a cycle of parboiling/drying/rehydrating (Walter M et al., 2005), or to a greater proportion of water used when cooking compared with the other rice varieties (Table 6). Further study would be required to elucidate the mechanism whereby chewing parboiled rice tends to produce a high proportion of fine material.

5.2.4 Summary

Greater GI values of the five rice varieties (by 10-15 GI units) in the Chinese group suggest that GIs obtained from Europeans are not necessarily applicable to Chinese. Differences in chewing pattern may contribute to the ethnic differences when GI is calculated using glucose as a reference food. Ethnic differences are less striking when comparing GI of LoGiCane™ sugar. In the light of these observations, it is not surprising that the ethnic differences in GI when a solid food, such as Jasmine rice is used as a reference food is not statistically significant. These results suggest that further discussion is warranted regarding whether use of a solid rather than a liquid reference food might be more appropriate and enhance the clinical relevance of the GI concept.
5.3 Clinical implication

The range of GI in our groups for the five rice varieties tested, 67 to 80 in the Chinese and 55 to 68, in the Europeans, is relatively narrow. This range is narrower than that reported for breakfast cereals, for example All Bran (GI 44) compared with Rice Bubbles (GI 88) (Atkinson FS et al., 2008). The potential for a metabolic advantage when choosing food based on GI is small, if the range among choices is small. However, considering the high risk of diabetes in China and diasporal Chinese, the amount of rice Chinese consume (over 600g/d) (Villegas R et al., 2007) and the potential for differences in postprandial glycaemia following rice consumption, it may be prudent to advise people at risk of diabetes as well as those with the condition to choose rice with a lower GI or indeed to consider recommending that some of the rice be replaced with acceptable alternatives.

5.4 Practical considerations

The long-term effect of changing the type of rice, which has only 13-14 GI units less than another type, on overall glycaemic status, is not known. Recommending Doongara® rice just because it may give a lesser glycaemic response than Jasmine was the highest among all rice varieties tested in this study. It is equivalent to $4.85/kg, whereas the most popular Jasmine rice costs $3.39/kg (prices recorded on 29/09/2009). Furthermore, Doongara rice is only available in some supermarkets. Since many Asians buy rice from Asian shops, access to the Doongara® rice maybe limited. Although further study is required to examine the long-term effects of having lower GI rice in a population whose staple diet is rice, the accessibility as well as availability of rice needs to be considered.

Should long-term studies confirm benefit of the use of low GI rice increased demand might ultimately lead to increased production and availability and reduced cost. Further comparable strains might be developed in the future.
6 Conclusion

The findings from the present study suggest that there are ethnic differences in glycaemic response to the five varieties of rice between European and Chinese subjects. The difference was also apparent in GI, but to a lesser extent. Rice is a staple food for Chinese. An intake of 625g/d of cooked white rice has been found in Chinese females living in China (Villegas R et al., 200), and 506g/d in Chinese Singaporeans (Health Promotion Board Singapore, 2004). These amounts are large, compared with the amount given in the present study (130-150g of cooked rice, containing 50g carbohydrate). High rice intake has been reported to increase the relative risk for type 2 diabetes by 78% in Chinese women (Villegas R et al., 2007), and by 65% in Japanese women (Nanri A et al., 2010) when the highest quintile was compared with the lowest. Thus long-term studies are essential to determine whether reducing dietary GI will confer change in carbohydrate metabolism likely to translate into clinical benefit. Such information is necessary before making dietary recommendations, which involve substantial changes to long-standing eating habits.

GI is used as a guide to food choice. However, the GI of a food measured in a European group may not necessarily apply to a Chinese group. The present study does not permit definitive conclusions regarding the explanation for the observed differences between the Chinese and European groups. However, the data suggest that the GI on a food label may not be generally applicable. It is also possible that the different GIs are a consequence of the test methodology and that the use of appropriate reference foods may need to be considered. The observation that differences in GI are attenuated when using solid food as a reference warrants further investigation.
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8 Appendices

1. Literature review for GI studies & References
2. Ethics Application
3. Participant Information & Questionnaire, Consent Form and Questionnaire
4. Flyers
5. GI study protocol & Record sheet
6. Physical activity questionnaire
7. Protocol for salivary alpha-amylase analysis
8. Calculation for chewed rice passed through the sieve
9. Calculation for the amount of starch in raw rice sample
8.1 Literature review for GI studies

8.1.1 Observational studies for GI and risk of diabetes

In most observational studies, both GI and Glycaemic load (GL) have been examined with regard to the risk of diabetes. GL is a concept introduced after GI, and it describes both quality and quantity of food (Salmeron J et al., 1997a, Salmeron J et al., 1997b) while GI indicates just quality.

\[
\text{Dietary GI} = \frac{\sum [(\text{average number of servings of food per day}) \times (\text{CHO content per serving}) \times (\text{GI})]}{\text{Total CHO in diet}}
\]

\[
\text{Dietary GL} = \sum [(\text{servings of food per day}) \times (\text{CHO content of food}) \times (\text{GI})]
\]

(Salmeron J et al., 1997b)

The relationships between GI and/or GL with the risk of diabetes have been reported mixed results (Table A1, A2). Some cross-sectional studies, JMETS (Japanese Multi-centered Environmental Toxicants Study), Framingham offspring study, and Chennai Urban Rural Epidemiology Study 59 showed significant results in GI or GL and HbA1c or insulin sensitivity (Murakami K et al., 2006, McKeown et al., 2004, Mohan V et al., 2009). However, other studies could not find a significant relationship between GI and T2D risk factors (van Dam RM et al., 2000, Sahyoun et al., 2005, Du et al., 2008).

Prospective studies for GI and/or GL and T2D risk have reported even more inconsistent results. Four large prospective cohort studies (the Nurses’ Health Study, Nurses’ Health Study II, the Health Professionals’ Follow-up Study, and Melbourne Collaborative Cohort Study) showed that a higher dietary GI predicted an increased risk of diabetes in middle-aged men and women (Salmeron J et al., 1997a, Salmeron J et al., 1997b, Schulze et al., 2004, Hodge AM et al., 2004). Four other prospective cohort studies (Iowa Women’s Study; Atherosclerosis Risk in Communities (ARIC) study; Insulin Resistance Atherosclerosis Study (IRAS); and Health ABC Study), however, could not show a positive relationship between a high-GI diet and T2D risk (Meyer et al., 2000, Stevens et al., 2002, Mayer-Davis et al., 2006, Sahyoun NR, 2008).

Of those studies which showed significant results on GI, only the Nurses’ Health Study also showed that GL was associated with the incidence of diabetes (Salmeron J et al., 1997b). Indeed all other prospective studies reviewed here did not show that GL had positive association with the risk of diabetes (Table A2).
One potential reason for these inconsistencies shown in these studies relates to the use of food frequency questionnaires (FFQ), which have not been validated specifically for GI and/or GL. Some studies have used validated FFQs to assess dietary intakes and have then used these instruments to calculate dietary GI and GL – a step which has not been validated. For example, the FFQ used in two studies by Salmeron et al. had been validated only for macronutrients, not for GI and/or GL (Rimm et al., 1992, Feskanich et al., 1993), the FFQ used in the Iowa Women’s Study had been validated for total carbohydrate and crude dietary fibre (Munger et al., 1992). The FFQ used in the Nurses’ Health Study II that targeted on younger females had been validated only for older population (Schulze et al., 2004). Thus, although studies used validated FFQ, its reliability and reproducibility for estimating GI and GL for a particular population had not been assessed appropriately.

A second potential limitation is that most studies have derived GI and GL values from *The International Tables of GI and GL Values* (Foster-Powell K and Miller JB, 1995, Foster-Powell K et al., 2002, Atkinson FS et al., 2008) to obtain the mean daily GI and GL of the diets of study subjects. The tables were firstly published in 1995, and has been updated twice (in 2002 and 2008) by the GI research group of the University of Sydney, Australia. The first two editions have been used by most of observational studies to calculate daily GI values. However, the critique is that the values of particular food item in these tables are highly variable. For example, there are seven GI values (item #604, page 44-45) for boiled potato in the 2002 International Tables. Its mean GI ranges from 56 to 101 (when GI glucose =100) (Foster-Powell K et al., 2002). Most prospective studies do not mention how they selected the most appropriate GI value from the International Tables and other sources. The way of calculation for GI is therefore, not clear. There are some studies that took an average value of all GI values of a food where there was more than one GI available instead of choosing the most appropriate value (Hodge AM et al., 2004, Beulens JWJ et al., 2007, Liese AD et al., 2007). On top of this, most studies reviewed here did not mention what reference food (glucose or white bread) has been used to calculate dietary GI. Without knowing it, it makes comparison of the studies very difficult. Moreover, reliability and reproducibility of GI itself has been questioned (Williams SM et al., 2008). GI values compiled in the International Tables are usually taken from very small sample size, around ten. This is because it is recommended by the WHO/FAO (FAO/WHO, 1998), however small sample size results in large standard error of GI contributing large 95%CI. For example, GI of New Zealand potato (boiled) is 70±17 (item #604, page 44) (Foster-Powell K et al., 2002), and its 95%CI is (37,
Therefore, a larger sample size (n=30) is required to overcome large within person variability (Venn BJ and Green TJ, 2007, Williams SM et al., 2008).

A third potential limitation relates to the estimation of GI for mixed meals. Whether total GI of the mixed meal is equivalent to the sum of the GI values for individual food is still controversial (Chew et al., 1988, Hollenbeck CB and Coulston AM, 1991, Flint et al., 2004, Dodd H et al., 2011). In most prospective studies the GI values single food items are added together to obtain an estimate of the total daily GI value of the diet. However, since individuals do not eat a single food in most times, most foods are combined with other food items when consumed, and other macronutrients such as fat and protein may affect total GI values of the meal, this assumption may not be appropriate. A recent study with relatively large sample (n=30) reported that the sum of GI values to predict total GI for a mixed meal is not the same (Dodd H et al., 2011).

Despite large sample sizes, it is very difficult to compare the observational study results with regard to GI and GL values and to interpret their findings. The values were obtained using inappropriate FFQ and possibly calculated in various ways. It is not known what reference food has been used for calculation, and not taken account for the GI values in mixed meals.

A relatively recent meta-analysis of observational studies suggests that diets with a high GI or GL independently increases the risk of type 2 diabetes (T2D) (RR 1.40, and 1.27, respectively) (Barclay et al., 2008). However, this finding should also be considered with caution. Greater than 90% of the study subjects included this meta-analysis was females (Barclay et al., 2008), and these aforementioned limitations have contributed to substantial heterogeneity in methodology among the studies making the comparison and interpretation of their findings difficult.

Application of these study results to Asian population remains unknown. There are only two cross-sectional studies conducted in Asian countries in this review. Some studies have reported a relationship between GI and/or GL and risk factors for chronic disease (Amano Y et al., 2004, Kim K et al., 2008). However, different outcomes and mixed results make generalisation of their findings impossible.
Table A1: Observational studies on GI and risk of diabetes

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Subjects</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Follow up (yr)</th>
<th>Diet method</th>
<th>Outcome</th>
<th>Association</th>
<th>Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahyoun et al. 2005</td>
<td>Health ABC</td>
<td></td>
<td>70-79</td>
<td>M</td>
<td>-</td>
<td>FFQ</td>
<td>HbA1c</td>
<td>NS</td>
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<td>1169</td>
<td></td>
<td>F</td>
<td>-</td>
<td>FFQ</td>
<td>HbA1c</td>
<td>NS</td>
<td>-</td>
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<tr>
<td>Murakami et al. 2006</td>
<td>JMETS</td>
<td>1354</td>
<td>20-78</td>
<td>F</td>
<td>-</td>
<td>DHQ</td>
<td>HbA1c</td>
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<td>HbA1c (mean (SEM)): Q1: 5.0(0.1), Q5: 5.2(0.1)</td>
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</tr>
<tr>
<td>Mohan et al. 2009</td>
<td>Chennai Urban Rural Epidemiology Study 59</td>
<td>1843</td>
<td>&gt;20</td>
<td>M+F</td>
<td>-</td>
<td>FFQ</td>
<td>T2DM</td>
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<td></td>
<td>OR (95%CI) = 2.51(1.42, 4.43) in Q4 vs. Q1</td>
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<tr>
<td>McKeown et al. 2004</td>
<td>Framingham offspring study</td>
<td>2834</td>
<td>26-82</td>
<td>M+F</td>
<td>-</td>
<td>FFQ</td>
<td>HOMA-IR</td>
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<td>HOMA-IR (mean(95%CI)): Q1:6.4(6.2, 7.0) vs. Q5: 7.0(6.7, 7.2)</td>
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<tr>
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<td>Zutphen Elderly Study</td>
<td>332</td>
<td>64-84</td>
<td>M</td>
<td>-</td>
<td>FFQ</td>
<td>Fasting ins.</td>
<td>NS</td>
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<tr>
<td>Du et al. 2008</td>
<td>CoDAM Study &amp; Hoorn Study</td>
<td>974</td>
<td>42-87</td>
<td>M+F</td>
<td>-</td>
<td>FFQ &amp; 24hr recall</td>
<td>HbA1c</td>
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<td>NS increase in HbA1c per 10 GI units</td>
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<td>β (SE) = 0.09(0.03) per 10 GI units</td>
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<td>β (SE) = 0.09(0.03) per 10 GI units</td>
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<td>Subjects</td>
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<td>Study</td>
<td>No.</td>
<td>Age (y)†</td>
<td>Sex</td>
<td>Follow up (yr)</td>
<td>Diet method</td>
<td>Outcome</td>
<td>Association</td>
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<tr>
<td>Glycaemic Index</td>
<td>Longitudinal studies</td>
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<td>FFQ</td>
<td>T2DM</td>
<td>↑**</td>
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<tr>
<td></td>
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<td>40-69</td>
<td>M+F</td>
<td>4</td>
<td>FFQ</td>
<td>T2DM</td>
<td>↑*</td>
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<tr>
<td></td>
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<td>IWHS</td>
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<td>45-64</td>
<td>M+F</td>
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<td>FFQ</td>
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<td>Cross-sectional studies</td>
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<td>45-64</td>
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<td>FFQ</td>
<td>T2DM</td>
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<tr>
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<td>2722</td>
<td>45-64</td>
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<td>T2DM</td>
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<tr>
<td></td>
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<td>FFQ</td>
<td>T2DM</td>
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### Table A2: Observational studies on GL and risk of diabetes

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<th>Outcome</th>
<th>Association</th>
<th>Magnitude</th>
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<td>F</td>
<td>FFQ</td>
<td>HbA1c</td>
<td>NS</td>
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<tr>
<td>Murakami et al. 2006</td>
<td>JMETS</td>
<td>1354</td>
<td>F</td>
<td>DHQ</td>
<td>HbA1c</td>
<td>NS (↑)</td>
</tr>
<tr>
<td>Mohan et al. 2009</td>
<td>Chennai Urban Rural Epidemiology Study 59</td>
<td>1843</td>
<td>M+F</td>
<td>FFQ</td>
<td>T2DM</td>
<td>↑***  OR (95%CI) = 4.25 (2.33, 7.77) in Q4 vs. Q1</td>
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<td>M+F</td>
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<td>M+F</td>
<td>FFQ &amp; 24hr recall</td>
<td>HbA1c</td>
<td>NS (↓)</td>
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<tr>
<td><strong>Glycaemic Load</strong></td>
<td><strong>Longitudinal studies</strong></td>
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<td>Salmeron et al. 1997</td>
<td>HPFS</td>
<td>42754</td>
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<td>FFQ</td>
<td>T2DM</td>
<td>NS</td>
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<tr>
<td>Salmeron et al. 1997</td>
<td>NHS</td>
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<td>F</td>
<td>FFQ</td>
<td>T2DM</td>
<td>↑**</td>
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<tr>
<td>Schulze et al. 2004</td>
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<td>91249</td>
<td>F</td>
<td>FFQ</td>
<td>T2DM</td>
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<tr>
<td>Hodge et al. 2004</td>
<td>Melbourne Collaborative Cohort Study</td>
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<td>M+F</td>
<td>FFQ</td>
<td>T2DM</td>
<td>NS (↓)</td>
</tr>
<tr>
<td>Meyer et al. 2000</td>
<td>IWHS</td>
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<td>M+F</td>
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<td>T2DM</td>
<td>NS</td>
</tr>
<tr>
<td>Study</td>
<td>Study Name</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Sex</td>
<td>FFQ</td>
<td>Disease</td>
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<td>Stevens et al. 2002</td>
<td>ARIC study</td>
<td>9529</td>
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<td>M+F (Wh)</td>
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<td>2722</td>
<td>45-64</td>
<td>M+F (AA)</td>
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<td>Mayer-Davis et al. 2006</td>
<td>IRAS</td>
<td>1255</td>
<td>40-69</td>
<td>M+F</td>
<td>5</td>
<td>FFQ</td>
</tr>
<tr>
<td>Sahyoun et al. 2008</td>
<td>Health ABC</td>
<td>1898</td>
<td>70-79</td>
<td>M+F</td>
<td>4</td>
<td>FFQ</td>
</tr>
</tbody>
</table>

†Age at baseline (range), Health ABC: Health Aging and Body Composition study, JMETS: Japanese Multi-centered Environmental Toxicants Study, CoDAM (Cohort study Diabetes and Atherosclerosis Maastricht), HPFS (Health Professionals Follow-up Study), NHS (Nurses’ Health Study), NHSII (Nurses’ Health Study II), IWHS (Iowa Women’s Health Study), ARIC (Atherosclerosis Risk in Communities), IRAS (Insulin Resistance Atherosclerosis Study), M: male, F: female, Wh: white, AA: African American, FFQ: Food Frequency Questionnaire, DHQ: Diet history questionnaire, NS: non significant, *p<0.05, **p<0.01, ***p<0.001
8.1.2 Experimental studies for GI and HbA1c

Inconsistent results have also been shown in experimental studies. Fourteen randomised controlled studies were examined in this review (Table A3). Most studies had free-living subjects with T2DM, and compared low GI (LGI) diet with high GI (HGI) diet. A primary outcome of the studies is the change in HbA1c or fructosamine. Only seven studies showed a significant difference in improvement of HbA1c or fructosamine between the diets (Brand et al., 1991, Wolever et al., 1992, Jimenez-Cruz et al., 2003, Rizkalla et al., 2004, Jenkins DJA et al., 2008, Gutschall et al., 2009, Nisak et al., 2010). It should be noted that six of the seven studies had short study duration (i.e. 12 weeks or less), and more than half studies had small sample size (less than 40) (Table A3).

The critique to LGI diet is that the reduction of HbA1c has shown only in the studies with short period. Majority of the seven studies which showed a significant improvement in glycaemic control had study duration 3-9 weeks (Wolever et al., 1992, Jarvi AE et al., 1999, Rizkalla et al., 2004, Jimenez-Cruz et al., 2003, Gutschall et al., 2009). The level, however, appears to have decreased after 8-9 weeks (Heilbronn et al., 2002, Gutschall et al., 2009). Gutschall et al reported a significant difference in fructosamine between the groups at the end of the 9week intervention, but it disappeared after the intervention (Gutschall et al., 2009). In another study, a significant reduction in fructosamine was observed in week4, however it became non significant in HbA1c between the groups in week12 (Yusof et al., 2009). A similar trend has been shown in long-term studies. In these studies, a significant reduction in HbA1c occurred in the first six months, however the effect attenuated by the end of the 12month intervention returning to the baseline level in 12 months, and thus no difference between the groups (Wolever TM et al., 2008, Ma et al., 2008). The glycaemic control even deteriorated in the well designed, 12 months Canadian Trial of Carbohydrate in Diabetes (CCD) (Wolever TM et al., 2008). In which 162 subjects were given up to 21 key foods of either high or low GI, which was equivalent to 20-25% of total energy (Wolever TM et al., 2008).

A meta-analysis that examined 14 randomised controlled trials (with study period from 12day to 12month) showed that LGI diet improves HbA1c modestly, but clinically
and significantly by 0.40 percentage points (CI -0.66 to -0.14) (Brand-Miller J et al., 2003). However, in this meta-analysis, majority of the nine studies with people with T2DM had small sample size between six and 21, and short intervention duration from two to six weeks (Brand-Miller J et al., 2003). Therefore, their results may not be able to be generalised for long term. A recent Cochrane review for LGI for diabetes supported the results of this meta-analysis, however recognized a limitation. This review concluded a significant decrease in the HbA1c (-0.5%) in LGI diet (Thomas D and Elliott E, 2009). It should be noted that the aforementioned two long-term studies were excluded (Wolever TM et al., 2008, Ma et al., 2008) from this review for various reasons (i.e. the people in LGI group had significantly less medication (Ma et al., 2008), and baseline HbA1c level was already optimal (Wolever TM et al., 2008). The limitation of the Cochrane review is that the study duration of the most of 11 randomised controlled trials was short (less than 12 weeks). Therefore, it concluded that the LGI diets, compared with the HGI diets, are likely to improve HbA1c or fructosamine to small extent in “short period”, and the effect may not continue for longer period (Thomas D and Elliott E, 2009).

Compliance to LGI diet is another issue. High dropout rates (nearly 20%) seen in several studies (Wolever et al., 1992, Luscombe et al., 1999, Tsihlias et al., 2000, Jimenez-Cruz et al., 2003, Rizkalla et al., 2004, Wolever TM et al., 2008) indicate that LGI diet is hard to adhere for some people. This suggests impracticality of the diet. Especially in the long-term studies (Tsihlias et al., 2000, Wolever TM et al., 2008), the high dropout rates (20% and 21%, respectively) also suggest the difficulty of following LGI diet for longer period, which is indispensable for lifestyle change. This was partly because of impractical advice, for example, people in LGI diet group were not allowed to eat potatoes for 12 months (Wolever TM et al., 2008). This unrealistic dietary advice is not useful for the population who consume potatoes most days, and accounts for the higher dropout rates.

Another limitation of the experimental studies is wide range of the dietary advice for LGI (Table A4). Less than half studies stated a definition of LGI and HGI diets (Luscombe et al., 1999, Tsihlias et al., 2000, Kabir et al., 2002, Heilbronn et al., 2002, Jimenez-Cruz et al., 2003, Rizkalla et al., 2004, Wolever TM et al., 2008). Some studies
even classify fruit into LGI and HGI (Heilbronn et al., 2002, Jimenez-Cruz et al., 2003, Jenkins DJA et al., 2008), but some did not (Jarvi AE et al., 1999, Tsihlias et al., 2000, Kabir et al., 2002, Rizkalla et al., 2004, Wolever TM et al., 2008).

In those studies which separated fruit into different groups, further definition varies. For example while orange was classified as LGI fruit in two studies (Jimenez-Cruz et al., 2003, Jenkins DJA et al., 2008), it was classified as HGI food in one study (Heilbronn et al., 2002).

The mean GI values of LGI and HGI (or alternative) diets in each study are not consistent among the studies, too. Figure A1 shows the mean GI of the LGI diet and that of the HGI (or alternative) diet of each study. The lower-end of the scales is the mean value of LGI diet, whereas the higher-end is that of HGI diet. For example, the mean HGI value of Jenkins et al. is 84. Since they used white bread as a reference food, if this is converted to glucose-based GI value by dividing by 1.4, the value becomes 61. This is now similar to the mean LGI value of Amano et al (i.e. 62). Since nine studies did not mention what reference food they used, their GI values cannot be compared, however this inconsistency among the studies might be a result of lack of definition of LGI diet.
Table A3: Randomised controlled studies on GI and fructosamine and/or HbA1c

<table>
<thead>
<tr>
<th>Author &amp; country</th>
<th>Study type &amp; Duration</th>
<th>No.</th>
<th>Mean age</th>
<th>Sex</th>
<th>Ref food</th>
<th>GI @ end (LGI vs other)</th>
<th>Outcome</th>
<th>Change b/w diets</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolever et al. 1992 Canada</td>
<td>X 6wk</td>
<td>6</td>
<td>63</td>
<td>M+F</td>
<td>Unstated</td>
<td>58 vs. 86</td>
<td>FA</td>
<td>↓*</td>
<td>LGI: 513→456 mmol/L HGI: 506→512</td>
</tr>
<tr>
<td>Jarvi et al. 1999 Sweden</td>
<td>X 3wk</td>
<td>20</td>
<td>66</td>
<td>M+F</td>
<td>WB</td>
<td>57 vs. 83</td>
<td>FA</td>
<td>NS (↓)</td>
<td>HbA1c NS (↓) LGI: 7.2→6.7 (p&lt;0.01 time) HGI: 7.2→6.9 (NS)</td>
</tr>
<tr>
<td>Luscombe et al. 1999 Australia</td>
<td>X 4wk</td>
<td>28</td>
<td>57.4</td>
<td>M+F</td>
<td>Unstated</td>
<td>43 vs. 63</td>
<td>FA</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Jimenez-Cruz et al. 2003 Mexico</td>
<td>X 6wk</td>
<td>36 (14 completed)</td>
<td>53</td>
<td>M+F</td>
<td>Unstated</td>
<td>44 vs. 56</td>
<td>HbA1c</td>
<td>↓**</td>
<td>LGI: 8.5→8.1 (p=0.04 time) HGI: 8.6→8.6 (NS)</td>
</tr>
<tr>
<td>Rizkalla et al. 2004 France</td>
<td>X 4wk</td>
<td>12</td>
<td>54</td>
<td>M</td>
<td>Glucose</td>
<td>39 vs. 71</td>
<td>HbA1c</td>
<td>↓*</td>
<td>LGI: 7.56→7.17 HGI: 7.45→7.57</td>
</tr>
<tr>
<td>Kabir et al. 2002 France</td>
<td>X 4wk</td>
<td>13</td>
<td>59</td>
<td>M</td>
<td>Unstated</td>
<td>49 vs. 64</td>
<td>HbA1c</td>
<td>NS (↓)</td>
<td>LGI: 8.3→7.8 HGI: 8.1→7.9</td>
</tr>
<tr>
<td>Gutschall et al. 2009 US</td>
<td>II 9wk</td>
<td>109</td>
<td>40-70</td>
<td>M+F</td>
<td>Unstated</td>
<td>54 vs. 58</td>
<td>FA</td>
<td>↓*</td>
<td>INT: 258.5→253.5 mmol/L CON: 267.4→278</td>
</tr>
<tr>
<td>Nisak et al. 2010 &amp; Yusof et al. 2009 Malaysia</td>
<td>II 12wk</td>
<td>104</td>
<td>58 vs. 55</td>
<td>M+F</td>
<td>Unstated</td>
<td>GI 57 vs. CCE 64</td>
<td>FA</td>
<td>↓**</td>
<td>↓0.2 vs. ↓0.08 mmol/L @wk4</td>
</tr>
<tr>
<td>Brand et al. 1991 Australia</td>
<td>X 12wk</td>
<td>16</td>
<td>62</td>
<td>M+F</td>
<td>Unstated</td>
<td>77 vs. 91</td>
<td>HbA1c</td>
<td>↓*</td>
<td>LGI: 7.7→7.0 HGI: 7.7→7.9</td>
</tr>
<tr>
<td>Heilbronn et al. 2002 Australia</td>
<td>II 8wk</td>
<td>45</td>
<td>56.7</td>
<td>M+F</td>
<td>Glucose</td>
<td>43 vs. 75</td>
<td>HbA1c</td>
<td>NS (↓)</td>
<td>LGI: 6.65→6.04 HGI: 6.35→6.06</td>
</tr>
<tr>
<td>Author &amp; country</td>
<td>Study type &amp; Duration</td>
<td>No.</td>
<td>Mean age</td>
<td>Sex</td>
<td>Ref food</td>
<td>GI @ end (LGI vs other)</td>
<td>Outcome</td>
<td>Change b/w diets</td>
<td>Results</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
<td>-----</td>
<td>----------</td>
<td>-----</td>
<td>----------</td>
<td>-------------------------</td>
<td>---------</td>
<td>------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Amano et al. 2007</td>
<td>II 3mo</td>
<td>40</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Glucose</td>
<td>GI 62 vs CNE 68</td>
<td>HbA1c</td>
<td>NS (↓)</td>
<td>GI: 6.51→6.05 (p&lt;0.001 for time) CNE: 6.29→6.06</td>
</tr>
<tr>
<td>Jenkins et al. 2008</td>
<td>II 6mo</td>
<td>210</td>
<td>60 vs. 61</td>
<td>M+F</td>
<td>WB</td>
<td>LGI 70 vs. HCF 84</td>
<td>HbA1c</td>
<td>↓***</td>
<td>LGI: 7.14→6.64 HCF: 7.07→6.89</td>
</tr>
<tr>
<td>Wolever et al. 2008</td>
<td>II 12mo</td>
<td>162</td>
<td>61 vs. 60</td>
<td>M+F</td>
<td>Unstated</td>
<td>55 vs. 63</td>
<td>HbA1c</td>
<td>NS (↑)</td>
<td>LGI: 6.2→6.34 HCF: 6.2→6.34</td>
</tr>
<tr>
<td>Ma et al. 2008</td>
<td>II 12mo</td>
<td>40</td>
<td>53.5</td>
<td>M+F</td>
<td>WB</td>
<td>LGI 77 vs. ADA 86</td>
<td>HbA1c</td>
<td>NS (↓)</td>
<td>LGI: 8.7→8.4 (p&lt;0.001 for time) ADA 8.1→7.</td>
</tr>
</tbody>
</table>

X: cross-over, II: parallel, M: male, F: female, WB: white bread, FA: fructosamine, NS: non significant, LGI: low GI diet, HGI: high GI diet, INT: intervention, CON: control, CCE: conventional carbohydrate exchange, CNE: conventional nutritional education, HCF: high cereal fibre, ADA: American Diabetes Association, *p<0.05, **p<0.01, ***p<0.001
<table>
<thead>
<tr>
<th>Author &amp; Country</th>
<th>Low GI diet</th>
<th>High GI or alternative diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolever et al. 1992 Canada</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jarvi et al. 1999 Sweden</td>
<td>Wholegrain barley bread, white durum pasta, parboiled rice, wholegrain barley porridge</td>
<td>Wholemeal bread, white durum bread, sticky rice, wholemeal barley porridge</td>
</tr>
<tr>
<td>Luscombe et al. 1999 Australia</td>
<td>Wholegrain bread, SpecialK, LGI fruit (apples, grapes) &amp; vegetables (sweet potato, peas)</td>
<td>Wholemeal bread, cornflakes, HGI fruit (bananas, tropical fruit) &amp; vegetables (potato, carrot)</td>
</tr>
<tr>
<td>Jimenez-Cruz et al. 2003 Mexico</td>
<td>Oranges, beans (legumes), yoghurt, pasta, corn tortillas</td>
<td>Corn flakes, white bread, potatoes, ripe bananas</td>
</tr>
<tr>
<td>Rizkalla et al. 2004 France</td>
<td>GI&lt;45 (pumpernickel, pasta, lentils, haricot beans, chickpeas, mug beans)</td>
<td>GI&gt;60 (wholemeal bread, French baguettes, potatoes, white rice)</td>
</tr>
<tr>
<td>Kabir et al. 2002 France</td>
<td>Only breakfast (Oat bran concentrate, apple and muesli, pumpernickel)</td>
<td>Only breakfast (wholewheat grains (Weetabix), wholemeal bread)</td>
</tr>
<tr>
<td>Gutschall et al. 2009 US</td>
<td>Lower GI substitutions according to an arbitrary classification (low ≤55, medium 56-69, high ≥70)</td>
<td>-</td>
</tr>
<tr>
<td>Nisaket al. 2010 &amp; Yusof et al. 2009 Malaysia</td>
<td>Rice (basmati, parboiled), tubers (sweet potatoes)</td>
<td>CCE: 1 exchange of CHO = 15g CHO. Eat a set of number of exchange</td>
</tr>
<tr>
<td>Brand et al. 1991 Australia</td>
<td>Oatmeal, porridge, all bran, pasta, legumes. Restrict potatoes, bananas and other HGI fruits &amp; veges. (*Bread was not strictly controlled)</td>
<td>Weet-Bix, other processed BF cereals</td>
</tr>
<tr>
<td>Heilbronn et al. 2002 Australia</td>
<td>LGI cereal, fruits (apples, pears, grapes, peaches, cherries, plums, grapefruit, dried apricots), wholegrain bread, pasta, wheat meal biscuits.</td>
<td>HGI cereals, fruits (bananas, oranges, pineapple, pawpaw, mango, kiwifruit, water melon), wholemeal bread, potato flakes, plain sweet biscuits.</td>
</tr>
<tr>
<td>Amano et al. 2007 Japan</td>
<td>Combine HGI staple foods with vinegar, dairy products or fermented foods (e.g. pickles)</td>
<td>CNE: Based on Japan Diabetes Society Guidelines</td>
</tr>
<tr>
<td>Tsililas et al. 2000 Canada</td>
<td>Targeted BF cereals only. Bran buds with psyllium, proto-type oat-loop cereal enriched with psyllium</td>
<td>Cornflakes, puffed rice, crispy rice</td>
</tr>
<tr>
<td>Jenkins et al. 2008 Canada</td>
<td>Bread (pumpernickel, rye, pita, quinoa, flaxseed), hot cereal (made of bulgur and flaxseed), pasta, parboiled rice, beans, peas, lentils, nuts, temperate fruit (apples, pears oranges, peaches, cherries &amp; berries) “Brown” option (wholegrain breads, wholegrain BF cereals, brown rice, potatoes with skins, tropical fruit (bananas, mangos, guavas, grapes)</td>
<td></td>
</tr>
<tr>
<td>Ma et al. 2008 US</td>
<td>Choose CHO foods according to GI ranking. Goal = daily GI &lt;55</td>
<td>ADA: All CHO are treated the same. CHO =55% TE</td>
</tr>
</tbody>
</table>

**Figure A1: Scales of GI value for LGI vs HGI (or other) diets**

<table>
<thead>
<tr>
<th>Reference food</th>
<th>GI scales</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unstated</strong></td>
<td></td>
</tr>
<tr>
<td>Brand 1991</td>
<td>39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 …… 90 … 93</td>
</tr>
<tr>
<td>Wolever 1992</td>
<td>43 ------ 63</td>
</tr>
<tr>
<td>Luscombe 1999</td>
<td>44 ------ 56</td>
</tr>
<tr>
<td>Tsihlias 2000</td>
<td>49 ------ 64</td>
</tr>
<tr>
<td>Kabir 2002</td>
<td>54 ------ 63</td>
</tr>
<tr>
<td>Jimenez-Cruz 2003</td>
<td>55 ------ 63</td>
</tr>
<tr>
<td>Wolever 2008</td>
<td>57 ------ 64</td>
</tr>
<tr>
<td>Gutchall 2009</td>
<td>77 ------------------ 91</td>
</tr>
<tr>
<td>Yusof 2009</td>
<td>76 ------------------ 86</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
</tr>
<tr>
<td>Heilbronn 2002</td>
<td>43 ------------------ 75</td>
</tr>
<tr>
<td>Rizkalla 2004</td>
<td>39 ------------------ 71</td>
</tr>
<tr>
<td>Amano 2007</td>
<td>62 ------------------ 68</td>
</tr>
<tr>
<td><strong>White bread</strong></td>
<td></td>
</tr>
<tr>
<td>Jarvi 1999</td>
<td>57 ------------------ 83</td>
</tr>
<tr>
<td>Jenkins 2008</td>
<td>70 ------------------ 84</td>
</tr>
<tr>
<td>Ma 2008</td>
<td>77 ------------------ 86</td>
</tr>
</tbody>
</table>

GI for LGI is in bold.
8.1.3 Observational studies on rice and risk of chronic disease

There are a few observational studies that examined an association between rice consumption and chronic disease (Table A5). Two Asian studies reported that rice intake is positively associated with T2DM risk in women (Villegas R et al., 2007, Nanri A et al., 2010). The highest rice intake group had T2DM risk increased by 65%, compared with the lowest intake group, however non significant association has been found in men (Nanri A et al., 2010). On the contrary, the risk for mortality from cardiovascular disease is negatively associated with rice intake in men (Nanri A et al., 2010).

In a cross-sectional study, the pooled group of three US prospective study cohorts shows positive relationship between white rice consumption and T2DM risk, but the magnitude of the risk ratio is a lot smaller (i.e. 17%) (Sun Q et al., 2010) than the aforementioned Asian studies. Since there are only a few studies, it is hard to interpret these findings. However, it is obvious that the rice intake is much larger in Asian population than American cohorts. Therefore, the relationship between rice intake and T2DM risk may be different between Asians and Europeans.
Table A5: Observational studies on rice consumption and T2DM

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Author &amp; Country</th>
<th>Study</th>
<th>Rice intake (g/d)</th>
<th>Outcome</th>
<th>Association</th>
<th>Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>197228 US</td>
<td>Sun et al. 2010</td>
<td>NHS I &amp; II, HPFS</td>
<td>WR: &lt;1serv/mo, 1-3serv/mo, 1serv/wk, 2-4serv/wk, ≥5serv/wk (i.e. ≥107g/d)</td>
<td>T2DM</td>
<td>↑***</td>
<td>RR(95%CI) = 1.17 (1.102, 1.136)</td>
</tr>
<tr>
<td>64227 China</td>
<td>Villegas et al. 2007</td>
<td>Women's Shanghai Study</td>
<td>&lt;500, 500-624, 625-749, ≥750g/d</td>
<td>T2DM</td>
<td>↑</td>
<td>RR(95%CI) = 1.78 (1.48, 2.15) in Q4 vs. Q1</td>
</tr>
<tr>
<td>125666 Japan</td>
<td>Nanri et al. 2010</td>
<td>125666</td>
<td>280, 420, 560g/d</td>
<td>T2DM</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>33622 Japan</td>
<td>Eshak et al. 2011</td>
<td>125666</td>
<td>&lt;500, 500-624, 625-749, ≥750g/d</td>
<td>Mortality from CVD</td>
<td>↑</td>
<td>CHD: HR(95%CI) = 0.70 (0.49, 0.99) in Q5 vs. Q1, Total CVD: HR(95%CI) = 0.82 (0.70, 0.97) in Q5 vs. Q1</td>
</tr>
</tbody>
</table>

Longitudinal studies

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Author &amp; Country</th>
<th>Study</th>
<th>Follow up (y)</th>
<th>Rice intake (g/d)</th>
<th>Outcome</th>
<th>Association</th>
<th>Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>26872 F</td>
<td>Sun et al. 2010</td>
<td>NHS I &amp; II, HPFS</td>
<td>WR: &lt;1serv/mo, 1-3serv/mo, 1serv/wk, 2-4serv/wk, ≥5serv/wk (i.e. ≥107g/d)</td>
<td>T2DM</td>
<td>↑</td>
<td>RR(95%CI) = 1.17 (1.102, 1.136)</td>
<td></td>
</tr>
<tr>
<td>40-70 M</td>
<td>Villegas et al. 2007</td>
<td>Women's Shanghai Study</td>
<td>&lt;500, 500-624, 625-749, ≥750g/d</td>
<td>T2DM</td>
<td>↑</td>
<td>RR(95%CI) = 1.78 (1.48, 2.15) in Q4 vs. Q1</td>
<td></td>
</tr>
<tr>
<td>40-79 M</td>
<td>Nanri et al. 2010</td>
<td>125666</td>
<td>280, 420, 560g/d</td>
<td>T2DM</td>
<td>NS</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>40-79 M</td>
<td>Eshak et al. 2011</td>
<td>125666</td>
<td>&lt;500, 500-624, 625-749, ≥750g/d</td>
<td>Mortality from CVD</td>
<td>↑</td>
<td>CHD: HR(95%CI) = 0.70 (0.49, 0.99) in Q5 vs. Q1, Total CVD: HR(95%CI) = 0.82 (0.70, 0.97) in Q5 vs. Q1</td>
<td></td>
</tr>
</tbody>
</table>

† Age at baseline (range), NHS: Nurses' Health Study, HPFS: Health Professionals Follow-up Study, M: male, F: female, WR: white rice, BR: brown rice, serv: serving(s), T2DM: Type 2 diabetes mellitus, NS: non significant, RR: relative risk, HR: hazard ratio, CHD: coronary heart disease, CVD: cardiovascular disease, *p<0.05, **p<0.01, ***p<0.001
8.1.4 References


THOMAS D & ELLIOTT E 2009. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd.


8.2 Ethics Application

APPLICATION TO THE UNIVERSITY OF OTAGO HUMAN
ETHICS COMMITTEE FOR ETHICAL APPROVAL OF A
RESEARCH OR TEACHING PROPOSAL INVOLVING HUMAN
PARTICIPANTS

1. University of Otago staff member responsible for project:
   (surname) (first name) (title)
   Venn Bernard Dr

2. Department: Human Nutrition

3. Contact details of staff member responsible:
   ph. 479 5068
   email: bernard.venn@otago.ac.nz

4. Title of project: Glycaemic Index Study – GI of rice

5. Brief description in lay terms of the purpose of the project:
   The Glycaemic Index (GI) provides a measure of a person’s rise in blood glucose following
   consumption of a test food relative to a reference food. Data from some observational studies
   suggest that consuming high GI food is a risk factor for obesity and type II diabetes. Although
more than 60% of the world’s population with diabetes will come from Asia, studies of GI in Asian people are scarce. White rice is a staple food in Asia and a major determinant of GI in the Asian diet. In the clinical setting in New Zealand, dietitians recommend patients consume varieties of rice shown to have a low GI value. However, published GI values are largely obtained in other countries using non-Asian test subjects. The relevance of GI values determined in Caucasian people for Asian consumers is questionable because we have found ethnic differences in GI. The reason for the ethnic difference is unknown but one possible explanation is a genetic propensity for people whose diets are predominantly starch-based to express more salivary amylase (the enzyme that digests starch). Another possibility is an ethnic difference in the extent of chewing before swallowing. In addition to the ethnic difference in GI, studies show that the GI of rice is highly variable and accordingly it has been recommended that rice should be tested locally, brand by brand. The GI of one rice has been tested previously in New Zealand in a small number of Caucasian people some 10 years ago. Small numbers of people yield GI values with wide confidence intervals. A larger sample of 30 as proposed here will give greater confidence in the GI values obtained and a greater ability to discriminate differences in GI among the rice varieties. The purpose of this study is to test the GI of five brands of rice available in New Zealand in Asian and Caucasian people. The study will yield ethnically appropriate GI values for common rice varieties, information that will be available to practising dietitians to help guide nutritional advice.


6. Indicate type of project and names of other investigators and students:

Staff Research
Department of Human Nutrition
Prof Jim Mann

Department of Preventive and Social Medicine, Dunedin School of Medicine
Assoc. Prof. Sheila Williams, Research Associate Professor

Student Research
This will be Minako Kataoka’s Masters project in 2009/2010.

7. Is this a repeated class teaching activity?
No
8. **Intended start date of project:**

   November 2009

   **Projected end date of project:**
   November 2010

9. **Funding of project.**

   Grant from the Riddett Centre and Bernard Venn’s start-up research fund

10. **Aim and description of project:**

   The aim of this study is to test the GI of five types of rice available in New Zealand using Asian and Caucasian participants. This will have practical application in that the information will be useful for dietitians in guiding nutritional advice.

   For GI determinations, capillary blood is collected by finger pricking using a sterilised disposable lancet. During each test, a series of eight blood samples are collected over a period of two hours following the consumption of the rice. To determine the GI of the rice varieties, the participants must attend the clinic on eight occasions (twice glucose reference beverage, twice white rice reference, once each for parboiled, Basmati, Doongara and brown rice). The test days are non-consecutive. The Department of Human Nutrition will use trained personnel to do the finger pricking.

11. **Researcher or instructor experience and qualifications in this research area:**

    The method for conducting GI testing is well established at the University of Otago. The University has an accredited GI testing laboratory. Prof Mann and Dr. Venn are experienced in conducting research trials involving human participants. GI testing will be carried out according to our standard procedure in the Department of Human Nutrition Undergraduate Laboratories.
12. **Participants**

12(a) **Population from which participants are drawn:**

Participants will be members of the public voluntarily recruited through advertisement.

12(b) **Specify inclusion and exclusion criteria:**

Inclusion: Asian group - Chinese men and women in the age range of 18 - 60 y inclusive (n = 30).

Caucasian group – People of European descent age range 18 – 60 y inclusive (n = 30).

Exclusions: People diagnosed with chronic disease including diabetes mellitus, cardiovascular disease, cancer, and diseases of the digestive system; who are taking any medications that affect glucose tolerance; that suffer from food allergies; and women who are pregnant.

12(c) **Number of participants:**

The clinical utility of dietary GI has been used to calculate the necessary sample size. In population studies, the range of dietary GI is up to 15 GI units. Data from 30 people would have 80% power to detect a difference of 10 GI units using the 5% level of significance. It would be underpowered to detect a smaller difference, but a difference of less than 10 GI units is of limited clinical significance.

12(d) **Age range of participants:**

18 - 60 years.

12(e) **Method of recruitment:**

Recruitment will be by advertisement in local newspapers and flyers posted around the University of Otago.

12(f) **Please specify any payment or reward to be offered:**

Participants will be reimbursed for their time at a rate of $35 per test. Those who do not complete all tests will be paid pro-rata.
13. **Methods and Procedures:**

When volunteers first make contact in response to the advertisement an information sheet and participant questionnaire will be sent out (documents attached). The participants will return the completed questionnaire and if interested and eligible, will be booked in for their eight appointments. At the first appointment, research staff will be available to answer questions regarding the study. If respondents are willing to continue, a consent form (attached) will be given to them. Participants will have their height and weight measured in a screened-off area to ensure the participants privacy. A medical questionnaire will be administered to ensure that eligibility criteria are met.

Participants will attend the glycaemic index facility after an overnight fast of at least 10 hours on eight occasions (2 glucose tests + 2 white rice reference tests + 1 test for each type of four rices). On the evenings preceding each of these test days, participants will be advised not to exercise and to ensure that their evening meal contains a carbohydrate-rich food. On each of the test days, two finger-prick blood samples will be taken five minutes apart as a baseline blood glucose concentration. This method of collecting blood for analysis causes minimal discomfort to the participant. Human Nutrition Department personnel who are experienced in this method of blood sampling will perform the finger pricking. Blood glucose concentrations will be determined from a drop of blood using a Hemocue Glucose 201 Analyzer. Following this, a reference or test food will be consumed over a fifteen minute period and a series of six more finger-pricks will be undertaken at 15, 30, 45, 60, 90 and 120 min. In the event of an abnormal result, a repeat fingerprick may be required. Adhesive plasters will be provided to hold in place a cotton wool swab covering the small incision. The total volume of blood extracted from the finger-pricks will be less than one millilitre. Participants will be asked to remain seated for the duration of the tests. Duplicate saliva samples will be taken on two of the test days to analyse for salivary alpha-amylase. On two occasions, participants will also be asked to chew the rice as they would normally and to expectorate into a container. The samples will be rinsed over a sieve to determine the proportion of solids. At the end of two hours the participants will be offered a light breakfast before leaving. After testing, volunteers will be asked
if they would like their blood and saliva samples disposed of using standard methods or with a karakia (prayer). These options are included in the participant questionnaire.

14. **Compliance with The Privacy Act 1993 and the Health Information Privacy Code 1994** imposes strict requirements concerning the collection, use and disclosure of personal information. These questions allow the Committee to assess compliance.

14(a) **Are you collecting personal information directly from the individual concerned?**

We will be collecting contact details comprising name, mailing address, email and telephone numbers. Basic demographic and anthropometric data will be collected to enable us to describe the population groups. This will involve collecting data on age, smoking habits and gender and measuring height and weight. We will include a question on birthplace. Through a medical questionnaire we will confirm that the participants have not been diagnosed with diabetes mellitus, cardiovascular disease, cancer, and diseases of the digestive system and that they are not on medication that would affect glucose metabolism.

14(b) **If you are collecting personal information directly from the individual concerned, specify the steps taken to make participants aware of the following points:**

- **the fact that you are collecting the information:**
  Participants will receive the information sheet and questionnaires (both are attached). Research staff will be available to answer any questions.

- **the purpose for which you are collecting the information and the uses you propose to make of it:**
  Participants will receive the information sheet and will be asked to confirm that they understand what is required of them. Research staff will be available to answer questions. All data and information will be kept in a locked room, with access limited to the researchers.
• who will receive the information:

No information containing a person’s identity will be distributed. Anonymous group demographics and statistical results may be published and/or used in future studies.

• the consequences, if any, of not supplying the information:

If the participant chooses not to supply any information, it may exclude them from the study.

• the individual’s rights of access to and correction of personal information:

The participant will have rights to access the personal information they have provided may also correct or change this information. They will be advised they can request a copy of the results of the project if they wish.

14(c) If you are not making participants aware of any of the points in (b), please explain why:

N/A

14(d) Does the research or teaching project involve any form of deception?

No.

14(e) Please outline your storage and security procedures to guard against unauthorised access, use or disclosure and how long you propose to keep personal information:

The information will remain confidential to the study investigators. Paper copies will be kept in a lockable office and electronic data stored on departmental computers. The results of this study may be published but no individual’s identity will be revealed.

At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any
raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

14(f) Please explain how you will ensure that the personal information you collect is accurate, up to date, complete, relevant and not misleading:
Participants will fill out their own personal details onto a participant questionnaire (attached). Height and weight will be measured by research staff, recorded and checked in the presence of the participant. The blood samples will be collected directly from the participants.

14(g) Who do you propose will have access to personal information, under what conditions, and subject to what safeguards against unauthorised disclosure?

Only study personnel directly involved in the testing will have access to personal information. The paper versions will be kept in a filing cabinet in a secure office. Electronic versions will be maintained on staff computers. The statistician will be given anonymous data.

14(h) Do you intend to publish any personal information and in what form do you intend to do this?

A person’s identity will remain anonymous in any form of published data. Demographic and anthropometric data will be presented only as group means.

14(i) Do you propose to collect information on ethnicity?

Yes, ethnicity data and birthplace will be collected.

15. Potential problems:

There will be minimal discomfort to participants from the fingerprick blood glucose test. The Department of Human Nutrition staff involved will be available throughout the test should any problems arise.

16. Informed consent

Please refer to consent form (attached).
17. Fast-Track procedure  Do you request fast-track consideration? No

18. Other committees  
N/A

19. Applicant's Signature: ..................................................  Date: ................................

20. Departmental approval: I have read this application and believe it to be scientifically and ethically sound. I approve the research design. The Research proposed in this application is compatible with the University of Otago policies and I give my consent for the application to be forwarded to the University of Otago Human Ethics Committee with my recommendation that it be approved.

Signature of *Head of Department:.................................................................

Date: ........................................

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8.3 Participant Information & Questionnaire, Consent Form and Questionnaire

Glycaemic Index Study- GI of rice

INFORMATION SHEET

Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?
The aim of the project is to compare the glycaemic index (GI) of five types of rice available in New Zealand in Asian and Caucasian groups.

Project Design and Methods

The project requires attending the Department of Human Nutrition on eight occasions. During the first visit you will be provided with information about the study. If you agree to participate and sign a consent form, we will collect some personal information from you comprising demographics, height and weight. Following this, the first GI test will be conducted. GI testing is conducted in the morning with a start time of between 7-9 am. You will be required to fast, ie: to have no food or drinks except water after 10 pm on the night before the test. We would prefer that you did not walk to the University. If you do walk or cycle we would like you to arrive 20 minutes early so that your heart rate and blood glucose have a chance to settle down before you start the test. On arrival and five minutes after, a finger-prick blood sample will be taken in the fasting state. You will then be given a glucose drink or a small meal to eat. After this, additional finger-prick blood samples will be taken at 15, 30, 45, 60, 90, and 120 min. In the event of an abnormal result, a repeat finger-prick may be required. The total volume of blood collected will amount to less than half a teaspoon. During this two hours we would like you to remain seated in the room with the exception of
toilet visits if necessary. You are free to read or talk and there will be some magazines available. We will ask you to provide duplicate saliva samples on two of the test days to analyse for salivary alpha-amylase. On two occasions, you will also be asked to chew the rice as you would normally and to expectorate into a container. The samples will be rinsed over a sieve to determine the proportion of solids. At the end of two hours there will be a light breakfast available for you to eat on the premises or to take away.

Can Participants Change their Mind and Withdraw from the Project?

You may decide not to participate or withdraw from participation in the project without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

We will collect data on your age, ethnicity, smoking habits and gender and we will be measuring your height and weight. The purpose of collecting this information is to describe the overall characteristics of the study population. We will also ask you to fill in a medical questionnaire to ensure you meet the study eligibility criteria (no diagnosis of diabetes mellitus, cardiovascular disease, cancer, diseases of the digestive system, you are not pregnant, you do not suffer from food allergies or take medication that affects glucose absorption and metabolism). From your blood samples we will be testing glucose concentration. The information will remain confidential to the study investigators. Paper copies will be kept in a lockable office and electronic data stored on departmental computers. The results of this study may be published but no individual’s identity will be revealed. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed. If you choose not to supply information this may exclude you from taking part in the study. You have rights of access to the personal information that you have given to us and you may correct or change this information.

Reimbursement

There will be reimbursement for your time with $35 per test paid at the end of the study.

If you have questions about this project, either now or in the future, please contact:
Dr. Bernard Venn  Tel: 479-5068  Email: bernard.venn@otago.ac.nz

This project has been reviewed & approved by the University of Otago Human Ethics Committee.
Glycaemic index study- GI of rice

CONSENT FORM

I have read the Information Sheet and understand the procedures. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I consent to:
• Attending the glycaemic index facility on eight days following an overnight fast
• Consuming a test food, meal, or beverage on twelve occasions
• Providing eight blood samples obtained by finger pricking over two hours on each glycaemic index test day.
• Providing a sample of saliva and a chewed sample of rice on two test days.

I know that:
• The data may be published but my name will not be disclosed
• My participation is voluntary
• I am free to withdraw from the project at any time without any disadvantage
• I will be reimbursed at the end of the study

I agree to take part in this project. Date …………………….

Name ……………………………….. Signature…………………………..

This project has been reviewed & approved by the University of Otago Human Ethics Committee.
Glycaemic index study- GI of rice

PARTICIPANT QUESTIONNAIRE

Name:

Are you male or female?

Postal address:

Email address: (if applicable)

Telephone numbers: (Work/Home/Mobile)

Date of birth:

Are you a non-smoker, past smoker, current cigarette smoker, cigar smoker or pipe smoker?

Frequency of smoking (if applicable)
Have you been diagnosed with diabetes mellitus, heart disease, stroke, cardiovascular disease, cancer, diseases of the digestive system?

Please list current medicines, dose and frequency:

Please list current supplements, brand and frequency:

Are you pregnant?

Please list any food allergies:

Please indicate to which ethnic group you belong:
Caucasian group: New Zealand European; Other (please specify)

Asian group:
Chinese from mainland China; New Zealand born Chinese; Hong Kong Chinese; Taiwan Chinese; Malaysian Chinese; Singaporean Chinese

Other. Please state:

Please circle whether you would like your blood samples to be disposed of using:
a) standard methods
b) with a karakia (prayer)
30 Chinese and 30 New Zealand European volunteers required

If you are of Chinese ethnicity or of European descent, the Department of Human Nutrition is looking for healthy volunteers aged 18 to 50 y to test the Glycaemic Index (GI) of five types of cooked rice.

The project involves attending the Department of Human Nutrition on nine mornings. Each visit will take approximately two hours with a start time of between 7:00 to 9:00 am. You will consume a sugary drink or a bowl of rice after which your blood glucose will be monitored by taking eight fingerprick samples over two hours. On completion, a light breakfast will be available.

There will be reimbursement for your time at a rate of $35 per test day ($315 in total) paid at the end of the study.

If you will be in Dunedin over the next 5 - 6 weeks (Nov to mid-way through Dec) and are interested in participating, please contact Minako on 479-5690 or by emailing minako.kataoka@otago.ac.nz
# Procedure Sheet

## 1. Sample overview

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saliva collection</th>
<th>Chewed sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 1</td>
<td>‘After’ only</td>
<td>-</td>
</tr>
<tr>
<td>Glucose 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jasmine 1</td>
<td>‘Before’ &amp; ‘After’</td>
<td>after 120</td>
</tr>
<tr>
<td>Jasmine 2</td>
<td>‘Before’ &amp; ‘After’</td>
<td>after 120</td>
</tr>
<tr>
<td>Basmati</td>
<td>-</td>
<td>after 120</td>
</tr>
<tr>
<td>Brown</td>
<td>‘After’ only</td>
<td>after 120</td>
</tr>
<tr>
<td>Doongara</td>
<td>-</td>
<td>after 120</td>
</tr>
<tr>
<td>Parboiled</td>
<td>-</td>
<td>after 120</td>
</tr>
<tr>
<td>Sugar</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

## 2. Time schedule

### Saliva & Glucose-drink: Rice1+2:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Bloodglucose test in minutes</th>
<th>Time of Bloodglucose test in minutes</th>
<th>Time of Bloodglucose test in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>Saliva sample ‘before’</td>
<td>0</td>
<td>Rice + 250ml water</td>
<td>Rice + 250ml water</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rice drink</td>
<td>0</td>
<td>Rice + 250ml water</td>
<td>Rice + 250ml water</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Saliva sample ‘after’</td>
<td>30</td>
<td>Saliva sample ‘after’</td>
<td>Saliva sample ‘after’</td>
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<tr>
<td>30</td>
<td>30</td>
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<tr>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Chewing &amp; spitting</td>
<td>Chewing &amp; spitting</td>
<td>Chewing &amp; spitting</td>
<td>Chewing &amp; spitting</td>
</tr>
</tbody>
</table>
3. Flow chart GI testing

**Saliva step 1 (only when Jasmine rice is tested)**
- Check the label of collecting tube, ‘B’ for ‘BEFORE’ consumption of rice
- Advice people to think about food or try it several times if trouble collecting enough saliva
- Collect about 1/3 of the tube
- Place the tube immediately into cool chilly bin after collection

**Saliva step 2**
- Make sure participants have no rice leftovers in their mouth (Don’t rinse the mouth)
- Check the label of collecting tube, ‘A’ for ‘AFTER’ consumption of rice
- Collect about 1/3 of the tube
- Place the tube immediately into cool chilly bin after collection

**Chewed sample**
- Give participant approximately 10g of RICE
- Make sure they put it in their mouth all at once
- Let them chew as much/ long as they normally do
- Let them expectorate it into a labeled container just before they want to swallow
- Make sure they do not swallow!!
- Ask participant to expel all particles of chewed rice
- Participant can flush mouth with a little water (max. one sip) to help rice-leftovers to be expectorated into the container
- Tell participant that if some rice is inadvertently swallowed, we would like to retest

**GI step 1**
- Finger prick @ -5 min

**GI step 2**
- Finger prick @ 0 min

**GI step 3**
- Finger prick @ 15 min

**GI step 4**
- More finger pricks @ 30, 45, 60, 90, and 120 min

- Make sure and check that all the saliva tubes are collected with ice packs in the chilly-bin. Take them upstairs & store in the fridge
GI Rice test - Record sheet

Name: __________________________
Date: __________________________
Sample: __________________________

Please record your blood glucose levels
-5min: __________________________
0min: __________________________
15min: __________________________
30min: __________________________
45min: __________________________
60min: __________________________
90min: __________________________
120min: __________________________

- Please finish the sample & water within 15min
- Please stay on your seat quietly until you finish the test
- Please feel free to tell us if you feel sick

HemoCue No. ______

Name: __________________________
Date: __________________________
Sample: __________________________

Please record your blood glucose levels
-5min: __________________________
0min: __________________________
15min: __________________________
30min: __________________________
45min: __________________________
60min: __________________________
90min: __________________________
120min: __________________________

- Please finish the sample & water within 15min
- Please stay on your seat quietly until you finish the test
- Please feel free to tell us if you feel sick
8.6 Physical activity questionnaire

Physical Activity Questionnaire

Please fill in this questionnaire as honestly and accurately as possible.

➡️ We want to characterize your physical activity over the last 7 days, which means any activity up until yesterday. Do not include activity undertaken today.

➡️ By ‘activity’ we mean doing anything using your muscles.

Sporting activities
Please list all sporting activities you were involved in and list the total hours spent on each activity.
(e.g. cycling/ running/ gym/ tramping/ judo/ tennis/ cricket/ rugby/ golf....)

<table>
<thead>
<tr>
<th>Sport</th>
<th>Hours</th>
<th>Sport</th>
<th>Hours</th>
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</thead>
<tbody>
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<td>Sun</td>
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</tbody>
</table>
**Travelling**

Please list all the *active* travelling (to and from work, friends, supermarket...) over the last 7 days and state whether you walk, cycle, run, skateboard or other (no driving a car or taking a bus!) and the total time spent on each.

<table>
<thead>
<tr>
<th>Active travelling</th>
<th>Minutes</th>
<th>Active travelling</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
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<td>Mon</td>
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<tr>
<td>Sun</td>
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</tbody>
</table>

**Other activities during the day**

Please list any other physical activities you have been involved in and the total time spent for each activity.

What were these activities exactly (e.g. brisk walking/ taking the stairs / lifting light-heavy things/ voluntary work/ walking the dog/ household chores/ gardening/ hobbies/ work ...)?

<table>
<thead>
<tr>
<th>Type of activity</th>
<th>Minutes</th>
<th>Type of activity</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
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<td>Mon</td>
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<tr>
<td>Sun</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
**Non-active hours**

How many **hours** did you **sleep** last week in **total**?

........................ hours

How many **hours in total** are you **inactive** each day? *(e.g. car driving, eating, computer, watching TV, reading, ...)*

<table>
<thead>
<tr>
<th></th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mon</td>
<td></td>
</tr>
<tr>
<td>Tue</td>
<td></td>
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<tr>
<td>Wed</td>
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<td>Sat</td>
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<tr>
<td>Sun</td>
<td></td>
</tr>
</tbody>
</table>
8.7 Protocol for salivary alpha-amylase analysis

Protocol
Analysis salivary-alpha-amylase

Important points

- **Calibrate** the Cobas Mira Plus spectrophotometer machine every week before you start analyzing samples!!
- Make a new AMYL-reagent solution every week before you start!!
- Make a new Phosphate Buffered Saline solution every week before you start!!
- Ratio of saliva in solution = 1: 300

Calibration

- Turn on the Cobas Mira Plus spectrophotometer machine and enter username and the password
- Check if there are enough empty cuvettes in the analyse-circle of the machine, otherwise put new ones in and press until you here the ‘click’.
- Check if there is enough distilled water for the machine to rinse the needle (big white reservoir on the left side of the machine and in the little tube in the ‘CL rack’)
- Make sure there is enough AMYL reagent (white cardboard-box in the fridge)/ make new by adding 5 x a pipette white and 1x a pipette black into the tube (e.g. P1000, 1.00) = just enough for 12 samples! Or make a whole lot, use the same ratio!
- Take out of the freezer: the controls CFAS, Precinorm U and Precipath U and let them thaw on room temperature (takes about 20 min.)
- Put in the racks for calibration: - New CFAS in ‘CL rack’ position 1
  - New Precinorm U in ‘CL rack’ position 2
  - New Precipath U in ‘Sample rack’ position 1

  Enter in the machine: Routine, F3 (=action), CA (=calibrate), D (= α-amylase) , ENTER, 1, ENTER, D(=α-amylase), ENTER and press START to start the analysis (takes about 20 min).

- Check if all values are within range!

  STD-1, STD 11 and STD 21: ~175 U/L
  CFAS = CS-1: ~65-75 U/L (depends on earlier measured values)
  Precinorm U = value ~79.8 U/L, range 65.4-94.2 U/L (LOT nr:175650)
  Precipath U = ~191 U/L, range 158-224 U/L (LOT nr:181948)
  Pooled sample = ~ 46-50 U/L (depends on earlier measured values)

- If all values are **within range**; start analysis of saliva-samples
• If some values are **out of range**; you can try different things: ask Ashley or Michelle, put controls in sample rack, use new reagent etc.

**Preparation**

• Take saliva samples (of max. 7 persons!) and 2 pooled controls **out of the freezers** and let them thaw on room-temperature.
• Turn the **Cobas Mira Plus spectrophotometer** machine on and enter ASH and the password required.
• Check if there are enough **empty cuvettes** in the analyse-circle of the machine
• Check if there is enough **water** for the machine to rinse the needle (big box on the left side and in the little tube in the ‘CL rack’)
• Make sure there is enough **AMYL reagent** (white cardboard-box in the fridge)/ make new by adding 5x a pipette white and 1x a pipette black into the tube (e.g. P1000, 1.00) = just enough for 12 samples!
• **Label** all the **mixing-tubes** (6*2=12 pp) and blue **analyse tubes** (6*2=12 pp) that are going to be used and **2 pooled sample** tubes.
• Put the blue analyse tubes in the ‘Sample Rack’ and the mixing-tubes in a ‘Working rack’.
• Make the solution for the saliva dilution **every week** by first making a **Phosphate Buffered Saline (PBS) solution** (dissolve 1 tablet in 100 mL of distilled water) and let the tablet(s) dissolve. (takes about 20 min.)
  You will need 3 mL*6*2 = 36 mL per person, max: 36 mL*7 = 252 mL!

**Saliva analysis**

• Put **6 mL** (P5000 2.99) **PBS Solution** into all mixing- tubes (12 for all saliva samples for each participants including duplicates)
• Label of the 12 samples

<table>
<thead>
<tr>
<th>Test tube label</th>
<th>Saliva samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Jasmine1 -B</td>
</tr>
<tr>
<td>2</td>
<td>Jasmine1 –B (duplicate)</td>
</tr>
<tr>
<td>3</td>
<td>Jasmine1 -A</td>
</tr>
<tr>
<td>4</td>
<td>Jasmine1 –A (duplicate)</td>
</tr>
<tr>
<td>5</td>
<td>Jasmine2 -B</td>
</tr>
<tr>
<td>6</td>
<td>Jasmine2 –B (duplicate)</td>
</tr>
<tr>
<td>7</td>
<td>Jasmine2 –A</td>
</tr>
<tr>
<td>8</td>
<td>Jasmine2 –A (duplicate)</td>
</tr>
<tr>
<td></td>
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<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>Brown</td>
</tr>
<tr>
<td>10</td>
<td>Brown (duplicate)</td>
</tr>
<tr>
<td>11</td>
<td>Glucose</td>
</tr>
<tr>
<td>12</td>
<td>Glucose (duplicate)</td>
</tr>
</tbody>
</table>

B, before rice consumption (i.e. fasting sample); A, after rice consumption

- **Vortex** the Saliva-tubes after they are thawed completely, with the lid still on!
- Take 20 µL (P20 20.0) **saliva** out of a Saliva-tube and dip the disposable top of the pipette into the **PBS solution** in a mixing-tube, releasing the saliva, and pulling it back against the wall. Repeat this for the same saliva-sample in another mixing-tube as a duplicate.
  → Repeat above mentioned for all saliva samples, with using a **new** disposable pipette-top for each **saliva-sample**!
- Put the lids onto the mixing-tubes and **vortex** them for a few seconds.
- Take 0.3 mL (P1000 0.30) of the **saliva-saline solution** with a **new** pipette out of every mixing-tube and put into the rightly labeled little blue analyse-tube, close the lids.
- **Vortex** the 2 **pooled samples** after thawing and put 0.3 mL (P1000 0.30) of each pooled sample into one blue analyse-tube.
- Put the analyse-tubes in the following **racks** and order:

  **Reagent rack 10:** 1. AMYL reagent (explanation above)

  **Sample rack 1:** 1. Precipath U 2. Pooled sample 1 3. Sample 1 4. Sample 2 etc. .......... 29. Sample ... 30. Pooled sample2

- Make sure all analyse-tubes are **closed** and in the racks properly.
- **Enter in the machine:** ROUTINE, 1, ENTER, F2 (=to), 30 ENTER, D, ENTER, and press START to start the analyzing!
Cleaning

- Pr8ess PAPER on the Cobas Mira machine to get the results on paper
- Put9 Parafilm on the reagent tube and the Phosphate Buffered Saline solution and store0 in the fridge
- Throw the CFAS and Precinorm U away after calibration
- Throw the pooled samples away, put Parafilm on Precipath U and store in the Freezer
- Remove the filled cuvettes from the circle in the machine and replace with new ones.
- Throw away all used mixing tubes and analyse-tubes in the yellow-bins and rinse the saline-solution beaker
- Store the used saliva-samples back in the -80 °C freezer.
- Clean the bench and pipettes
- Turn off the machine
8.8 Calculation for chewed rice passed through the sieve

Calculation for chewed rice sample

1. Record the wt of a cooked rice sample for chewing (about 10g) ------ A
2. Dry both a duplicate & chewed rice samples for at least 24 hours
3. Obtain the moisture content (%) of the duplicate sample (20-30g) ---- B
   \[ B = \frac{\text{wt (g) before drying} - \text{wt (g) after drying}}{\text{wt before drying}} \]
4. Calculate the dry content (%) of the duplicate sample -------------- C = 100 – B%
5. Calculate the dry wt (g) of the chewed sample supposed to be ------ D = A x C
6. Record the wt of a dried chewed rice sample --------------------- E
7. Subtract the calculated dry wt of the chewed sample from the actual wt of the sample
   ----------------------------------------------------------------------------- F = (D - E)
8. Obtain proportion (%) of chewed rice sample lost through the sieve
   ----------------------------------------------------------------------------------------------- G = D/F x 100

(e.g.)
Cooked rice sample for chewing = 10.56g --- A
Moisture content of the duplicate = 68% ----- B
Dry content of the duplicate = 100 -68% = 32% --- C
Dry wt of the chewed sample = 10.56g x 32%
   = 3.38g --- D
Weight of the dried chewed sample = 2.7g --- E
Subtract the dry weight of the chewed sample from dry weight supposed to be:
   3.38g – 2.7g = 0.68g --- F
   0.68 / 3.38 x 100 = 20.1% --- G
8.9 Sample calculation for the amount of starch in dry (raw) rice sample

e.g.) Basmati rice

1. Weight of ground dry rice sample = 0.1634g (for Basmati)
2. Glucose concentration (µg/mL) of the sample (from the standard curve) = 14.5µg/mL
3. Work backwards through dilution factors (1/100 dilution twice):
   14.5µg/mL x 100 x 100 = 145000
4. Convert micro gram (µg) to gram by dividing by 1000 x1000:
   145000 / 10000000 = 0.145g
5. Glucose concentration / weight of ground raw rice = % of glucose of the sample:
   0.1545g/0.1634g x 100 = 88.7%