THE ETHNICITY ENIGMA: UNRAVELLING THE DIETARY, METABOLIC AND PHYSIOLOGICAL DIFFERENCES

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information, which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

VERENA TAN MING HUI

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"We know that all things work together for good for those who love God, who are called according to his purpose."

Romans 8:28

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TABLE OF CONTENT

Contents

SUMMARY

Background

Within the Asian population, there are ethnic differences in susceptibility to and prevalence of obesity and Type 2 Diabetes Mellitus (T2DM). We aimed to understand the dietary habits of individuals with T2DM in Singapore, examine ethnic differences in glucose homeostasis, glycemic response and insulin kinetics, as well as physiological digestive factors amongst the ethnic groups to provide insights into the varying susceptibilities to T2DM.

Objectives

1. To examine the dietary habits (carbohydrate sources and dietary GI) of individuals with T2DM in Singapore.
2. To study beta-cell function in relation to insulin sensitivity and understand glucose homeostasis amongst the ethnic groups.
3. To measure physiological factors involved in digestion amongst the ethnic groups.

Methods

1. Three hundred and six participants (99 Chinese, 96 Malays, and 111 Asian-Indians) were recruited. Blood samples, anthropometric and clinical

measurements, and two 24-hour dietary recalls were obtained. Nutrient intake, dietary glycemic index (GI) and glycemic load (GL) were computed.

2. Fifty nine normoglycemic lean males (14 Chinese, 21 Malay and 24 Asian-Indians) underwent a euglycemic-hyperinsulinemic clamp procedure and a liquid mixed meal tolerance test (LMMTT). Insulin sensitivity, beta-cell function, postprandial glucose and insulin responses were determined.
3. Seventy five healthy males (25 Chinese, 25 Malay, and 25 Asian-Indians) were recruited. Subjects were served glucose reference, Jasmine rice (JR) and Basmati rice (BR) on separate occasions. Postprandial blood glucose and plasma insulin concentrations were measured over 180 min. Mastication parameters, saliva α-amylase activity, *AMY1* gene copy numbers and gastric emptying rate were also determined to investigate their relationships with glycemic response.

Results

1. The Malays had both poorer metabolic (higher BMI, poorer lipids profile) and dietary profiles (high GL, GI, intake of saturated fat and sugars) compared to Asian-Indians and Chinese.
2. Asian-Indians were the least insulin sensitive among the ethnic groups. The glycemic response during LMMTT was significantly greater in Malays compared to Asian-Indians and Chinese. However, there were no significant differences in insulin response between ethnic groups. The oral disposition index, a measure of beta-cell function relative to insulin sensitivity during the LMMTT, was the lowest in Malays, followed by Asian-Indians and Chinese.

Results

3. The GI and Insulinemic Index (II) of JR and BR did not differ significantly between ethnicities. We found no effect of ethnicity, salivary α-amylase activity, particle size distribution, gastric emptying rate and *AMY1* gene copy numbers on glycemic response (GR). Mastication parameters such as the number of chews at each mouthful and the chewing time per mouthful showed a significant relationship with GR.

Conclusion

We found ethnic differences in the diet of individuals with T2DM in terms of the quantity and quality of the carbohydrates consumed. The understanding of such dietary differences can aid ethnic-specific nutrition recommendations to achieve better glycemic control and metabolic profile. Through understanding glucose homeostasis and insulin kinetics among the ethnic groups, we demonstrated that the pathways leading to T2DM might differ between Malay and Asian-Indians. In the final study, we showed that digestive function such as mastication rate, salivary α-amylase activity and gastric emptying rate modulates carbohydrate metabolism and influenced the glycemic response.

LIST OF ABBREVIATIONS

AUC	Area under curve
BR	Basmati rice
DI	Disposition index
GI	Glycemic index
GL	Glycemic load
GR	Glycemic response
HbA1c	Glycosylated haemoglobin
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment – insulin resistance
HOMA-B	Homeostatic model assessment – beta-cell function
II	Insulinemic index
IR	Insulin response
ISI-clamp	Insulin sensitivity index derived from euglycemic-hyperinsulinemic clamp
ISI-Mat	Matsuda insulin sensitivity index
JR	Jasmine rice
LDL-C	Low-density lipoprotein cholesterol
LMMTT	Liquid mixed meal tolerance test
PAL	Physical activity level
T2DM	Type 2 diabetes mellitus

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 Introduction

1.1 Background

The prevalence of type 2 diabetes mellitus (T2DM) worldwide has reached pandemic proportions with the number of individuals with diabetes set to increase by up to 60% by 2025 (Chan et al., 2009). This increase reflects the unfortunate global shift towards unhealthy diets and physical inactivity, resulting in obesity. With obesity being a prominent risk factor for T2DM, the ensuing burden of T2DM looks set to worsen.

Asia will make up more than 60% of the world's population with type 2 diabetes (Chan et al., 2009) and the rates of increase show no signs of slowing. For the same BMI, Asian populations have a higher prevalence of diabetes than people of European descent due to a higher predisposition to insulin resistance (Yoon et al., 2006). Thus, there is a cogent need to understand the factors that lead to the development of T2DM amongst Asians.

Dietary advice, with or without an increase in physical activity was shown to improve glycemic control in patients with early diagnosed individuals T2DM (Andrews et al., 2011). This highlights the importance of dietary education to achieve optimal diabetes management. In a multi-ethnic Asian society like Singapore, with different ethnic groups of diverse cultural habits sharing a common environment, generic population nutrition advice may not be effective in the prevention and management of diabetes. We must first understand the dietary habits of the major ethnicities in order to develop targeted nutrition advice for diabetes management. Additionally, we seek to unravel the metabolic and physiological factors possibly contributing to the pathogenesis of T2DM.

The scope of this thesis encompasses findings of studies relating to the dietary habits of individuals with T2DM in Singapore, comparing the postprandial responses following a liquid mixed-meal and rice in healthy individuals from the three main ethnic groups, and examining the physiological digestive factors that influence glycemic and insulin responses. The final chapter summarizes and integrates the important conclusions resulting from this thesis and provides suggestions for future work.

1.2 Ethnic differences in obesity, diabetes and cardiovascular disease

The rising incidence of obesity and T2DM are escalating problems for Singapore. Despite sharing a common environment with overlapping dietary habits and social behaviour, the three main ethnic groups differ in the prevalence of obesity, T2DM and cardiovascular disease (Ministry of Health Singapore, 2010a). The Malays have the highest rates of obesity (24.0%), followed by Asian-Indians (16.9%) and then Chinese (7.9%). For T2DM, Asian-Indians have the highest prevalence (17.2%), followed by Malays (16.6%) and Chinese (9.7%) (Ministry of Health Singapore, 2010a). There is thus an ethnic disparity in the prevalence of obesity that does not correspond with the prevalence of diabetes. In terms of cardiovascular risk, high total blood cholesterol was most prevalent among Malays (22.6%) compared with Chinese (17.1%) and Asian-Indians (12.6%). The age-standardised event rates of myocardial infarction (MI) are 2- and 3-fold higher for Malays and Asian-Indians respectively compared to the Chinese (Mak et al., 2003).

It is evident that there are ethnic variations and significant differences in susceptibility to obesity, T2DM and cardiovascular disease. How dietary habits, metabolic phenotype and physiological diversity impact the varying susceptibilities among the ethnic groups are currently unclear. This leads us to study some possible

reasons (diet, glucose homeostasis and physiological digestive functions) for these observed differences and through which, subsequently develop management strategies for preventative medicine.

1.3 Dietary differences between ethnic groups

The National Nutrition Survey in 2010 (Ministry of Health Singapore, 2010b) highlighted distinct ethnic differences in dietary practices of the normal population. For example, Malays (353.8g) and Asian-Indians (355.6g) consumed more carbohydrates than Chinese (331.7g). In terms of percent energy contribution from macronutrients, the Asian-Indians consumed the highest proportion of carbohydrate (54.6%), the Chinese consumed the most protein (15.8%), while the Malays consumed the most amount of fat (31.7%).

In essence, there were substantial dietary differences among the various ethnic groups in Singapore. There is however a paucity of information concerning the diet in individuals with Type 2 diabetes mellitus (T2DM). Lifestyle modifications, in particular through dietary management are accepted as the cornerstone for the management of T2DM. However, many studies and international evidence-based nutritional recommendations (Bantle et al., 2008; Franz et al., 2008; Franz et al., 2010; Mann et al., 2004) were principally based on the Caucasian population consuming fairly typical "Western" diets. For populations consuming rice-based diet, there were few dietary studies that had comprehensively described the dietary habits of a multi-ethnic Asian population (Amano et al., 2007; Hu et al., 2009). With T2DM becoming an escalating epidemic in some Asian populations (Wild et al., 2004), and Asian diets being markedly different from a western diet, there lies a need to comprehensively profile the dietary habits of Asians with T2DM. Through understanding of the dietary habits of a multi-ethnic Asian population with T2DM,

we can thus facilitate the development of targeted ethnic-specific nutrition recommendations.

1.4 The Glycemic Index and its clinical significance

1.4.1 Physiological importance of carbohydrates

Carbohydrate is the main dietary component affecting blood glucose levels. Both the amount and type of carbohydrate have an effect on both postprandial glycemic and insulinemia. Postprandial glycemia is known to contribute to the aetiology of chronic metabolic diseases such as T2DM and cardiovascular disease (CVD) (Blaak et al., 2012).

Early studies showed that starchy carbohydrate foods have very different effects on postprandial blood glucose and insulin responses (Brand et al., 1985; Jenkins et al., 1982; Wolever and Bolognesi, 1996). As carbohydrates differ in their ability to increase blood glucose, the concept of glycemic index (GI) was introduced in the early 1980s to classify and rank carbohydrates according to their effect on postprandial glycemia (Jenkins et al., 1981). This was calculated by dividing the incremental area under the curve of blood glucose concentrations measured after the ingestion of a portion of test food containing 50g available carbohydrate by the incremental blood glucose area achieved with a portion of a reference food (typically glucose) containing the same amount (50g) of available carbohydrate and expressed as a percentage (Jenkins et al., 1981). Foods with a GI value of above 70 are classified as high GI, foods with a GI of 56-69 as medium GI, and foods that have a GI of 55 and less are classified as low GI (Standards Australia, 2007).

In order to account for the amount of carbohydrates ingested, the concept of glycemic load (GL) was developed to represent both the quantity and the quality (GI) of the carbohydrate consumed. GL is calculated as follows: GI of a specific food

multiplied by the amount of available carbohydrate contained in an average portion of the food consumed.

1.4.2 Role of glycemic index (GI) and glycemic load (GL) and risk of obesity and Type 2 diabetes mellitus (T2DM)

The effectiveness of low GI and GL diets in the prevention of obesity and diabetes has been examined in epidemiological, clinical trials and meta-analysis (Bhupathiraju et al., 2014; Dong et al., 2011; Greenwood et al., 2013; Lagerpusch et al., 2013; Livesey et al., 2013; Riccardi et al., 2008; Schwingshackl and Hoffmann, 2013; Solomon et al., 2010).

Obesity

Obesity, an imbalance of energy intake and energy expenditure is a global challenge and a major concern in Singapore, where there are marked differences in prevalence among the ethnic groups. Raised body mass index (BMI) is a major risk factor for chronic diseases such as CVD and T2DM, musculoskeletal disorders (especially osteoarthritis) and some cancers (endometrial, breast and colon) (Pi-Sunyer, 2002)

Recent evidence has illustrated the beneficial effect of long-term low-GI/GL diets with respect to improvements in C-reactive protein and fasting insulin, both of which were implicated in the development of obesity and obesity-related complications like T2DM (Schwingshackl and Hoffmann, 2013). Following the consumption of a high GI meal, the decline in blood glucose levels, often below fasting concentrations (Ludwig, 2002; Ludwig et al., 1999) may lead to excessive hunger, overeating and a preference for foods that rapidly restore blood glucose to

normal (such as high GI foods) (Campfield et al., 1996; Strachan et al., 2004). This propagates cycles of overeating and subsequently leads to problems like overweight and obesity. A recent study found that the consumption of a high- compared with a low-GI meal increased brain activity in regions related to food intake, reward and craving in the late postprandial period. This coincided with lower blood glucose levels and greater hunger (Lennerz et al., 2013). This recent neurophysiologic finding, adds to the body of evidence to suggest that a reduced consumption of high-GI carbohydrates may ameliorate overeating and facilitate the prevention of obesity and its related metabolic consequences.

Diabetes

In recent years, meta-analysis of prospective cohort studies (Barclay et al., 2008; Bhupathiraju et al., 2014; Dong and Qin, 2011; Greenwood et al., 2013; Livesey et al., 2013) found a positive association between GI, GL and a higher risk of T2DM. Despite the existence of the GI and GL for many years, their roles in preventing T2DM remain controversial. A recent updated meta-analysis of prospective cohort studies found a 19% and 13% higher risk of T2DM with higher dietary GI and GL respectively (Bhupathiraju et al., 2014).

Several physiologic mechanisms were proposed to explain the positive association of GI and GL with T2DM (Barclay et al., 2008; Ludwig, 2002; Willett et al., 2002) (Figure 1-1). First, high GI and GL diets produce higher blood glucose concentrations and stimulate increased production of insulin, resulting in a state of hyperinsulinemia. The chronically increased insulin secretion may eventually lead to pancreatic beta-cell failure and consequently, impaired glucose tolerance (Pawlak et al., 2004). Second, high-GI diets may directly increase insulin resistance by modulating glycemia, increasing free fatty acids concentrations and elevating

counter-regulatory hormone secretion (Jenkins et al., 1981; Willett et al., 2002), thereby inducing beta-cell failure and the development of diabetes.

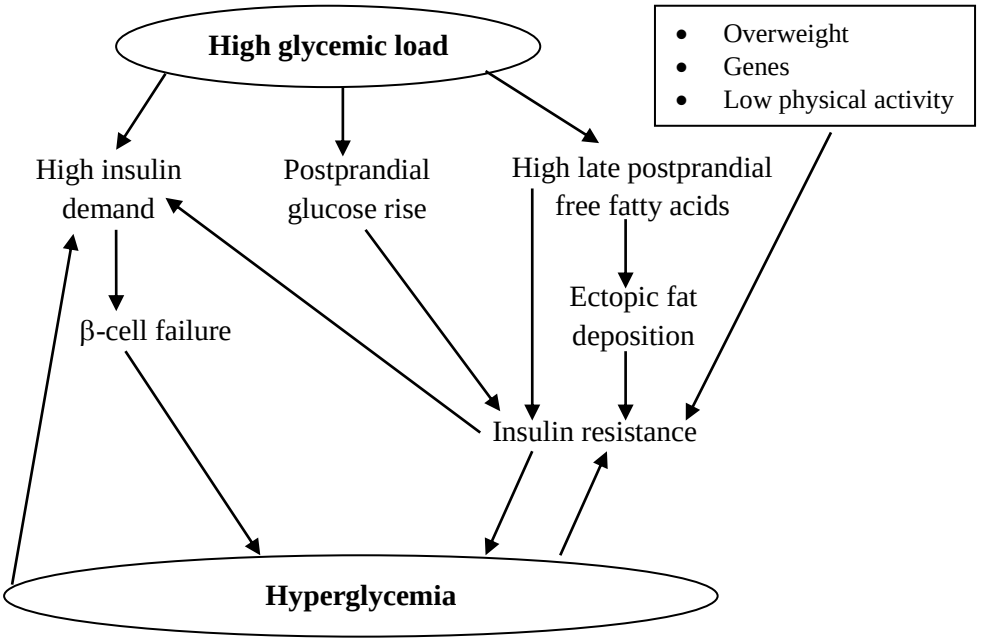


Figure 1-1 Potential mechanisms linking a high glycemic load with the development of T2DM [adapted from Riccardi et al (2008)].

The biological plausibility of an association between GI and T2DM is evident from results of metabolic and intervention studies. In a meta-analysis of 12 randomized controlled trials, low GI diets reduced glycated haemoglobin by 0.4% (95% CI: -0.7%, -0.2%) compared to the control diets (Thomas and Elliott, 2010). Furthermore, the Study to Prevent NonInsulin Dependent Diabetes Mellitus (STOP-NIDDM) trial, which showed that acarbose, an oral α-glucosidase inhibitor, which effectively converts the diet to a low GI/GL diet, reduced T2DM risk by 25% over a mean follow-up of 3 year provides a proof-of-concept for the beneficial effect of low GI diets (Chiasson et al., 2002)

In summary, studies have found that low GI or GL diets may result in reduced insulin release, improved glycemic control, and increased insulin sensitivity, and ultimately might help prevent T2DM (Brand-Miller et al., 2003; McClenaghan,

2005). On the other hand, high intakes of high GI and GL carbohydrates over short-term and long-term periods may produce greater insulin resistance and increase the risk for developing T2DM (Bhupathiraju et al., 2014).

1.5 Ethnic differences in metabolic phenotype

Insulin insensitivity and progressive pancreatic beta-cell dysfunction have been established as the fundamental features in the pathogenesis of T2DM (DeFronzo, 1981; Goldstein, 2002; Kahn, 2003; Saad et al., 1991). However, most studies of diabetes pathogenesis were conducted in Caucasian populations. Local studies have previously shown that differences in insulin resistance among Asian ethnicities are more prominent amongst the lean individuals (Khoo et al., 2014; Liew et al., 2003; Tai et al., 2000). However, insulin resistance alone does not lead to the development of T2DM. Several prospective studies have shown that both insulin resistance and defective insulin secretion are already present before the onset of dysglycemia (Chiasson and Rabasa-Lhoret, 2004). It has been suggested that insulin secretory defect is more important in the pathogenesis of T2DM, particularly in East Asians (Fukushima et al., 2004; Kodama et al., 2013).

There is a paucity of information regarding insulin secretion in a multi-ethnic Asian population where individuals from different ethnic groups have shown to exhibit different levels of insulin resistance (Khoo et al., 2011; Tai et al., 2000). The presence of three major Asian ethnic groups residing in Singapore offers a unique opportunity to study ethnic variability in pancreatic beta-cell function in relation to insulin sensitivity.

1.6 Physiological diversity between ethnic groups

There is extensive evidence that biological digestive factors vary between individuals and influence postprandial glycemic and insulin response (Lassauzay et al., 2000; Mandel et al., 2010; Ranawana et al., 2011; Schwartz et al., 1995).

1.6.1 Glycemic and insulin response

Ethnic differences in postprandial glycemic response (GR) and insulin (IR) have been reported previously (Chan et al., 2001; Dickinson et al., 2002; Henry et al., 2008; Venn et al., 2010) with Asians, Chinese and non-Caucasians consistently showing a 2-3 fold higher glycemic and insulin response than the Caucasians.

Rice provides about one fifth of the world's dietary energy and more than one third of daily dietary energy in China and many Asian countries (IRRI, 2001). White rice, in general has a higher GI than whole grain rice and thus, it is a major contributor to the overall GL of most Asian diets. In Chinese and Japanese adults, there are positive associations between white rice consumption and risk of T2DM (Nanri et al., 2010; Villegas et al., 2007). A recent meta-analysis found a 11% increase in the risk of T2DM with each increment serving of white rice per day, with a significantly higher risk among Asian than Western populations (Hu et al., 2012). These findings agree with studies showing that Asian subjects exhibit greater glycemic responses to the same foods compared to Caucasian subjects (Henry et al., 2008; Kataoka et al., 2013).

The question whether the GI of a food varies between ethnicities is currently still debatable. Some studies showed no ethnic disparities in GI values (Chan et al., 2001; Henry et al., 2008) while other studies demonstrated a significant effect of ethnicity on the GI value of white bread (Wolever et al., 2009) and rice (Kataoka et al., 2013). The latter study showed that the GI of some commonly consumed rice

varieties were approximately 20% greater amongst Chinese compared with Europeans (Kataoka et al., 2013). This implies that the standard classification of low, medium and high GI may differ between Asians and Caucasians. This has important implications for dietary recommendations for healthy and individuals with T2DM in an Asian population. To our understanding, no studies have examined the effect of ethnicity on the GI values of rice in a multi-ethnic Asian population.

1.6.2 Physiological digestive factors

Mastication

Mastication, or chewing, is the first step in the digestion process with studies showing large variation between individuals (Lassauzay et al., 2000; Woda et al., 2006). Recent evidence has also indicated that mastication plays a crucial role in determining the postprandial GR (Ranawana et al., 2010a; Suzuki et al., 2005). However, no studies have compared the rates of mastication between individuals of different ethnic groups and evaluated how this may influence postprandial GR and IR.

Salivary amylase activity and AMY1 gene copy number

A recent study demonstrated that the quantity and enzymatic activity of salivary α-amylase vary considerably among individuals (Mandel et al., 2010). Genetically, salivary amylase levels are influenced by individual copy number variation (CNV) of the *AMY1* gene on chromosome 1p21 (Bank et al., 1992). An individual can carry between 2 to 15 diploid copies of the *AMY1* gene in their genome (Mandel et al., 2010). Recent evidence suggests that populations who consume a traditional high-starch diet have higher copy numbers of the *AMY1* gene, with correspondingly higher concentrations of salivary amylase, than populations who

consumed a low-starch diet (Perry et al., 2007). This is suggestive of a nutritional evolution in response to the dramatic increase in the availability of carbohydrate foods in many populations since the agricultural revolution. The presence of highly refined processed carbohydrates has increased the number of *AMY1* copies in selected populations to facilitate the digestion and metabolism of carbohydrates. This leads to higher postprandial glycemic and corresponding insulin response (Perry et al., 2007). From anthropological and ecological data (Noor, 2002; Norimah, 2008), we understand that the early Chinese and Asian-Indian immigrants came from populations in China and India with agriculturally based diets and would generally consume a high carbohydrate diet. Thus, we postulate that the Chinese and Asian-Indians have higher numbers of *AMY1* gene copy numbers and corresponding higher concentrations of salivary α-amylase compared to the Malays. How this relates to postprandial glycemic and insulin response still remains unclear, which we sought out to investigate in this study.

Gastric emptying

Gastric emptying is a physiological response to the presence of food and is a major determinant of postprandial glycemia (Berry et al., 2003; Horowitz et al., 1993; Rayner et al., 2001; Schwartz et al., 1995). Few gastric emptying studies have been performed in ethnic populations that are at high risk of developing T2DM. In healthy populations, existing evidence suggests that the gastric emptying of nutrients varies between ethnic groups (Schwartz et al., 1995). To our knowledge, there are no studies that have examined the gastric emptying rates in a multi-ethnic Asian population.

1.7 Rationale of studies in this thesis

It is evident from the extant literature that even within the Asian population, there are ethnic differences in susceptibility to obesity and T2DM. We intend to examine the dietary habits of individuals with T2DM in Singapore, focusing on carbohydrate sources and as well as the dietary GI. With Asian diets being markedly different from a western diet, the comprehensive profiling of dietary intake of Asians with T2DM will allow more targeted nutrition intervention in diabetes management. Furthermore, we intend to understand pancreatic beta-cell function through measuring glucose homeostasis and insulin kinetics amongst the ethnic groups to provide insights into varying susceptibilities to T2DM. Lastly, we seek to derive physiological explanations for ethnic variability in glycemic and insulin responses through measuring factors that are involved in food digestion. We have found no studies to date that have systematically measured and compared physiological parameters in a multi-ethnic Asian population. Findings from this study will provide deeper insights into how biological variations between individuals can explain for the varying prevalence of obesity and T2DM among the three main ethnic groups in Singapore. The understanding of the early pathogenesis of T2DM among the susceptible ethnic groups is critical to designing effective prevention health-care strategies, which can be translated into better health outcomes, improve quality of life and reduce the chronic disease burden for the country.

1.7 Main hypotheses

There are ethnic differences in dietary carbohydrate intake, dietary glycemic index (GI) as well as postprandial glycemic, insulin and metabolic responses in our population. The differences in glycemic and insulin responses may in part be due to differences in digestive function. These differences may contribute to the ethnic differences in the prevalence of obesity and T2DM in our population.

Study 1 – Ethnic dietary differences in individuals with T2DM

Hypothesis

There are ethnic differences in carbohydrate sources, dietary GI and metabolic profile among individuals with T2DM in Singapore

Aims

1. To profile the dietary habits and metabolic characteristics of Chinese, Malay and Asian-Indian individuals with T2DM in Singapore
2. To determine the dietary GI, glycemic load (GL) and examine the main sources of carbohydrates in each of the ethnic diets

Study 2 – Ethnic differences in insulin sensitivity and beta-cell function among Asian men

Hypothesis

Ethnic differences in glycemic and insulin responses to liquid mixed meal are due to differences in pancreatic beta-cell function after accounting for differences in insulin resistance.

Aims

1. To measure insulin sensitivity in lean and healthy individuals among the three main ethnic groups in Singapore.
2. To measure postprandial glycemic and insulin responses following a liquid mixed meal tolerance test.
3. To examine pancreatic beta-cell function between ethnic groups, after accounting for differences in insulin sensitivity

<u>Study 3</u> – Investigating inter-individual differences in glycemic and insulin responses

Hypotheses

i) There are ethnic differences in postprandial glycemic and insulin responses among Chinese, Malay and Asian-Indian lean, young and healthy males

ii) There are ethnic differences in the GI value to the same food between the ethnic groups

iii) Physiological digestive functions and determinants, namely mastication rate, particle size distribution, salivary amylase activity, *AMY1* gene copy numbers and gastric emptying rate influences the postprandial glycemic response

iv) The ethnic differences in mastication rate, particle size distribution, salivary amylase activity, *AMY1* gene copy numbers and gastric emptying rate influences the glycemic response

Aims

1) To investigate ethnic differences in postprandial glycemic and insulin responses following rice consumption
2) To investigate ethnic differences in the GI classification of a specific rice type
3) To measure physiological digestive factors such as mastication rate, particle size distribution, salivary amylase activity, *AMY1* gene copy numbers and gastric emptying rate between the ethnic groups
4) To examine the relationships between digestive factors and glycemic response between the ethnic groups

CHAPTER 2 Ethnic Dietary Differences in Individuals with Type 2 Diabetes Mellitus

2.1 Abstract

Objectives – To profile the dietary habits of individuals with Type 2 diabetes mellitus (T2DM) in Singapore and examine the main sources of carbohydrates in each of the ethnic diets

Methods – Three hundred and six participants (99 Chinese, 96 Malays, and 111 Asian-Indians) were recruited. Fasting blood samples, clinical history, physical examination and two 24-hour dietary recalls were obtained. Energy, macronutrient intake, dietary glycemic index (GI) and glycemic load (GL) were computed.

Results – The Malays had higher BMI (*P<0.001*), higher total cholesterol (*P=0.030*), higher LDL-C (*P=0.020*) and higher triglycerides (*P=0.001*). In terms of diet, the Malay group had the highest GL (110.5±5.3, *P=0.003*), GI (59.7±0.8, *P=0.025*), intake of saturated fat (M: 22.5±1.6, *P=0.001*) and sugars (M: 55.7±3.7, *P=0.001*) after adjusting for age, gender and medication. Qualitative differences were also observed in the preferred choice of carbohydrates, with the Malays favouring flavoured rice as compared to other ethnic groups.

Conclusions –There are important ethnic differences in the diet of individuals with T2DM in terms of the quantity and quality of the carbohydrates consumed. The understanding of such dietary differences will therefore aid ethnic-specific nutrition recommendations to achieve better glycemic control and metabolic profile.

2.2 Introduction

The prevalence of type 2 diabetes mellitus (T2DM) worldwide has reached an alarming proportion with a higher prevalence of T2DM occurring at a younger age in Asians compared with Caucasians (Ramachandran et al., 2010). In Singapore, the prevalence of T2DM has increased though the years and in all three ethnic groups, reaching a prevalence of 17.2% among the Asian-Indians, followed by 16.6% in Malays and 9.7% in Chinese (National Health Survey, 2010).

Despite the availability of a wide range of pharmacological therapy, lifestyle modification and dietary adjustments are mainstay in the management of T2DM. Many studies and international nutritional recommendations (Bantle et al., 2008; Franz et al., 2008; Franz et al., 2010; Mann et al., 2004) were principally based on the Caucasian populations consuming typical "western" diets. For Asian populations consuming a rice-based diet, there were few dietary studies (Amano et al., 2007; Hu et al., 2009) that evaluated appropriate dietary patterns for the prevention and management of diabetes.

Foods containing carbohydrates are the primary determinants of postprandial blood glucose levels. Managing carbohydrate intake is, therefore a primary strategy for achieving glycemic control in T2DM. In nutrition therapy, the portions of meal and snack carbohydrate quantity should be consistently distributed throughout the day on a day-to-day basis for optimal glycemic control (Franz et al., 2010). As carbohydrates differ in their ability to increase blood glucose, the concept of the glycemic index (GI) was introduced in the 1980s to rank carbohydrate foods by their overall effect on blood glucose levels (Jenkins et al., 1981). The glycemic load (GL), on the other hand takes into consideration both the GI and the available carbohydrate content of the food (Salmeron et al., 1997a). Numerous studies have shown that low-GI diets improve glycemic control (Amano et al., 2007; Brand-Miller et al., 2003; Mann et al., 2004; Wolever et al., 1991), lipids profile (Rizkalla et al., 2004) and

reduce systemic inflammation (Wolever et al., 2008b) in T2DM individuals. A recent Cochrane review concluded that low glycemic index (GI) diets were beneficial in glycemic control for patients with T2DM (Thomas and Elliott, 2010).

Despite much evidence advocating the benefits of low GI diets in dietary management of T2DM, the dietary GI and GL, as well as major CHO food sources, have yet to be examined among individuals with T2DM in a multi-ethnic Asian population. Most epidemiological studies, including the Nurses' Health Study (Liu et al., 2000; Salmeron et al., 1997b) and Health Professionals'' Follow-up Study (Salmeron et al., 1997a) have characterized Whites or African Americans (Stevens et al., 2002). There remains a paucity of information concerning the diet of individuals with T2DM and its influence on glycemic and metabolic parameters. The National Nutrition Survey in 2010 (Ministry of Health Singapore, 2010b) highlighted distinct ethnic differences in dietary practices. For example, the Asian-Indians consumed the highest proportion of carbohydrate as percent energy contribution from macronutrients (54.6%); the Chinese consumed the most protein (15.8%), while the Malays consumed the most amount of fat (31.7%). However, this survey was not specific to individuals with diabetes and did not investigate the glycemic index (GI) or glycemic load (GL) of the ethnic diets.

As there were substantial dietary differences among the various ethnic populations and a paucity of information about the eating habits of a multi-ethnic Asian population with T2DM, our aims were to determine dietary patterns of Chinese, Malay and Asian-Indian adults with T2DM in Singapore, focusing on the dietary glycemic measures in the Asian diet, and to investigate the associations between dietary glycemic measures with glycemic control and cardiovascular risk factors. This was the first study that characterized and compared the dietary and metabolic profiles of T2DM individuals of each ethnic group. This study will eventually guide us to conduct further intervention studies to modify the ethnic-

specific diet and manage the glycemic control of individuals with T2DM in Singapore.

2.3 Methods

2.3.1 Participants

Individuals with T2DM aged between 45-64 years old were recruited from the Singapore Consortium of Cohort Studies (SCCS) – Diabetic Cohort (DC) (NUS IRB 06-128). The SCCS – DC was a nationwide, prospective cohort study to determine the interactions between the genetic, lifestyle and environment factors and the occurrence of diabetic complications among individuals with T2DM. Recruitment took place at polyclinics and outpatient diabetes clinics in public sector hospitals. After obtaining written informed consent, participants were interviewed by experienced interviewers using questionnaires that included questions on social demography, weight history, eating patterns, medical and drug history, presence of diabetes-associated complications, family history of common chronic illnesses and cancer, alcohol consumption, smoking and environmental tobacco smoke exposure to ascertain their eligibility.

For this study, 309 participants were recruited. The inclusion criteria included established diagnosis of T2DM of more than 2 years, on diet, oral or insulin therapy, aged 45 to 64 years old inclusive of Chinese, Malay or Asian-Indian ethnicity. Eligible participants were free of significant cardiovascular, renal or liver disease. This study was approved by the National University of Singapore Institutional Review Board (NUS-IRB) and the National Healthcare Group Domain Specific Review Board (NHG DSRB). All participants provided written informed consent.

2.3.2 Study design

Eligible participants were invited to attend a health screening on a separate occasion after an overnight 10-hour fast. Anthropometric and clinical measurements were obtained. Height was measured without shoes using a stadiometer (SECA 213, Germany). Weight was measured in light clothing, using a digital scale (SECA 780 and SECA 703, Germany). An automatic blood pressure monitor (Dinamap DPC 100X/ProCare 100, Germany) was used to take 2 blood pressure readings from participants at rest. A third reading was performed if the difference between the 2 readings of systolic blood pressure was greater than 10 mmHg or if diastolic blood pressure was greater than 5 mmHg. The mean values of the closest 2 readings were calculated.

Blood samples were collected and analysed for glycemic control measures and lipids profile. All biochemical analyses of blood were conducted at the National University Hospital Referral Laboratory, which is accredited by the College of American Pathologists. Serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using an automated analyser (ADVIA 2400; Bayer Diagnostics, New York, USA). Plasma glucose was assayed using enzymatic methods (ADVIA 2400). Glycosylated haemoglobin (HbA1c) was measured using high performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA), an assay that was accredited by the National Glycohaemoglobin Standardization Program with controls traceable to the Diabetes Control and Complications Trial. The inter-day and intra-day coefficients of variation were as follows: total cholesterol (1.00-1.33% and 0.53-1.45%), triglycerides (0.00-1.29% and 0.00-0.94%), HDL-C (1.18-3.53% and 0.56-0.65%), LDL-C (2.54-4.17% and 1.14-1.38%), plasma glucose (0.72-1.96% and 0.00-0.75%) and HbA1c (0.96-1.15% and 0.66-1.06%).

Two 24-hour dietary recall questionnaires (1 weekend and 1 weekday) were obtained by trained research staff. A food picture guide was used as a visual aid to help the participants estimate the correct portion of food. Each food picture was assigned a code, which translated into the weight (in grams) of the food portion. The coding and corresponding food weights were checked and implausible values were verified with the participants' recalls and rectified where appropriate. Dietary information was analyzed by three qualified dietitians using locally developed nutrient analysis software (E-food Nutrient System, 2010, National University of Singapore) that was linked with the Singapore Health Promotion Board's local foods database. Food intake data of 306 subjects (99 Chinese, 96 Malays and 111 Asian-Indians) were included in the final nutrient analysis. In order to determine specific carbohydrate sources, we grouped similar foods into the same food sub-groups and weighted the carbohydrate intake in each ethnic group.

Physical activity data was determined for each participant through an interviewer-administered validated physical activity questionnaire (Khaing Nang et al., 2010). The total metabolic equivalent (MET hours per week) for each participant was calculated by multiplying the frequency, duration, and intensity of physical activity and converted to physical activity level (PAL).

Pharmacological therapy for T2DM was reclassified into diet only (7.2%); oral hypoglycemic agents – full dose prandial (16.3%), half dose prandial (37.9%), metformin only (19.0%); prandial insulin only (11.8%), basal insulin only (0.7%), oral hypoglycemic agents and prandial insulin (4.6%) and oral hypoglycemic agents and basal insulin (2.5%). Oral hypoglycemic agents include biguanides, sulphonylureas, DPP-4 inhibitors, alpha-glucosidase inhibitors and thiazolidinediones. Insulin included fast-acting, intermediate-acting and long-acting insulin.

2.3.3 Assigning GI values to 24-hour dietary recalls

All the food items in the dataset were classified into food groups, which were further divided into smaller food sub-groups based on the energy and nutrient composition of foods from the Health Promotion Board, Singapore.

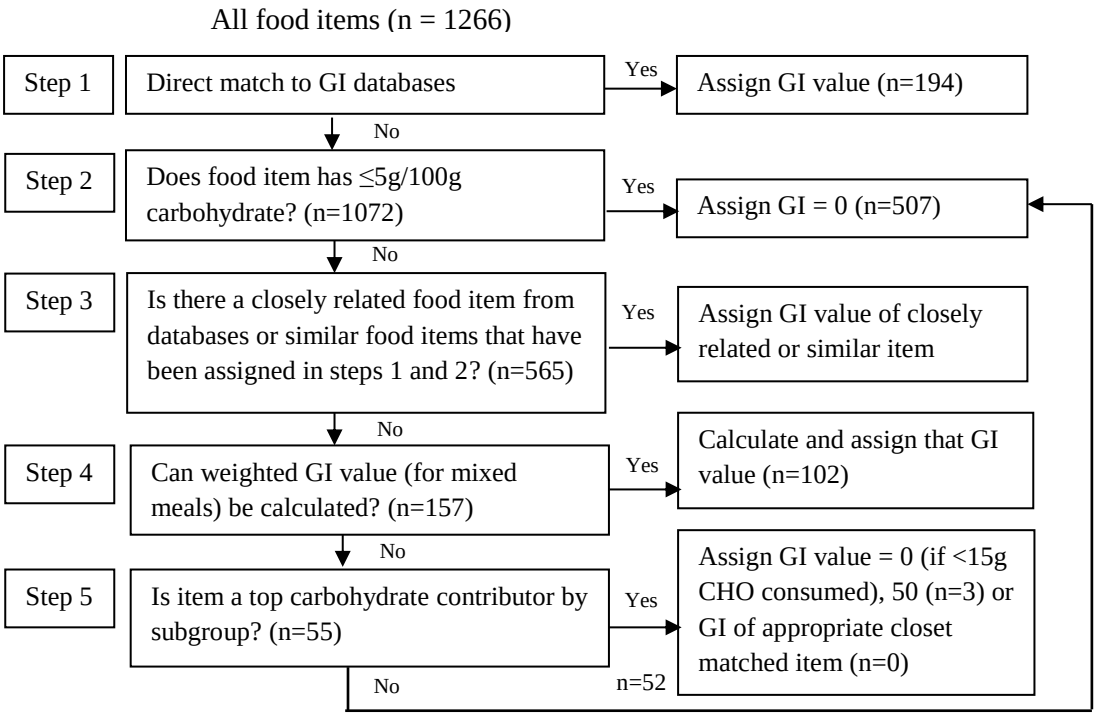
Within each food sub-groups, the food items were ranked according to their carbohydrate contribution in grams per 100g. Food items contributing to the top 90% of carbohydrate intake of that sub-group will subsequently be assigned GI values. Individual food items were assigned GI values using methods adapted from other studies (Louie et al., 2011; van Bakel et al., 2009). The source of GI values for foods came from the following: publications reporting measured GI values (Atkinson et al., 2008; Chen et al., 2010; Foster-Powell et al., 2002; Louie et al., 2011; Robert and Ismail, 2012), data from locally tested foods (unpublished data) and GI values from University of Sydney's website (www.glycemicindex.com) (S.U.G.I.R.S., 2011).

Figure 2-1 outlined the methodology for assigning GI values to individual food items in the dataset. Only food items with a direct match to an item in a GI database with GI values obtained from standard testing protocol (Standards Australia, 2007) were assigned an immediate GI value. Food items containing ≤5g carbohydrate per 100g were excluded from the assignment of GI values on the basis that normal consumption of these food items would not contribute significantly to the glycemic response unless eaten in very large quantities (Louie et al., 2011; Neuhouser et al., 2006). Thus, foods like non-starchy vegetables, oils and meats did not have a GI value. Some foods that contain less than 5g carbohydrate per 100g are also major contributors to dietary carbohydrate intake, and these were included where a GI value was available (eg. milk). When possible, the GI values of tests with normal subjects were chosen. When large variations in GI for the same food item were found, we chose to use the mean values of the most relevant studies for the particular food item or the closest variation of the particular food item that was consumed in our local

culture. We also took into considering the fat content, fibre content, protein content, preparation methods and other nutrient composition information in the decision-making process in the assignment of GI values.

In total, 1266 carbohydrate-containing foods were identified from the 24-hour recalls. Using a decision algorithm (Figure 2-1), 194 foods (15.3%) were assigned GI values from direct matches with various GI databases (Step 1), 507 foods (40.1%) contained less than 5g carbohydrate per 100g and were assigned GI values of 0 (Step 2), 408 foods (32.2%) were assigned the GI value of a closely related food item or similar food items that had been assigned GI values in Steps 1 and 2 (Step 3). For 102 foods (8.1%), which were mixed meals, calculated weighted GI values were assigned. For the remaining foods, 52 foods (4.1%) were assigned GI of zero because they were not the top carbohydrate contributors, while 3 foods (0.2%) were assigned the GI value of 50 (Step 5).

Figure 2-1 Decision flowchart for the assignment of GI values to food items recorded in 24-hour dietary recalls



The GI values for 102 composite foods were estimated as the weighted mean of the GI values of the available carbohydrate-containing components (FAO/WHO, 1998; Wolever and Jenkins, 1986). For foods that contained none or negligible amounts of carbohydrate (mainly protein or fat-containing foods, such as meats and oil), the GI values were set to zero (n=559). The dietary GL, which represents both the quality and quantity of carbohydrate intake, was first calculated by multiplying the GI of each food (divided by 100) by its available carbohydrate content and summing up all food items consumed. Consequently, each participant's overall dietary GI, a variable that represents the overall quality of carbohydrate in the diet, was calculated by dividing the total dietary GL by the total amount of available carbohydrate consumed that day and multiplied by 100 (Salmeron et al, 1997a; Wolever et al, 1991; Wolever et al., 1994). The mean daily dietary GI and GL for each subject were obtained by averaging the dietary GI and GL of the 2 diet recall days respectively.

Dietary GL = Σ (GI of food x amount of available carbohydrate provided by food)

Dietary GI = Σ[(GI of food x amount of available carbohydrate provided by food)/ total dietary available carbohydrate]

2.3.4. Statistical analyses

Data are presented as means ± standard error (SE). Categorical variables were expressed as counts and percentages. Ethnic differences in subject characteristics, metabolic and glycemic parameters, nutrient intakes and dietary glycemic measures were assessed using one-way ANOVA, with *post-hoc* Bonferroni corrections applied for pair-wise comparisons. Chi-square tests were used to compare categorical variables. Pearson correlation analysis was used to assess relationships between dietary GI and GL with other metabolic variables. Multiple linear regression models

were used to investigate relationships between all parameters and were adjusted for the following potential confounding variables: age, gender, BMI, energy intake, dietary fibre and dosage of medications, where appropriate. All analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL). Differences were considered significant if $P<0.05$.

2.4 Results

The anthropometric and metabolic characteristics of individuals with T2DM of different ethnic groups were summarized in Table 2-1. Of the 309 participants that completed the study, 306 participants had complete nutritional, clinical, socio-demographic and lifestyle data and were included in the final analysis. Reasons for exclusion were having only one 24-hr dietary recall (n=1), missing anthropometric measurement (n=1) and an elevated creatinine level (n=1).

In this study, 32.4% were Chinese, 31.4% Malays, and 36.2% Asian-Indians, with a predominance of women (64.7%, $P=0.002$). The Malays were significantly younger ($P<0.001$) compared with Chinese and Asian-Indians. Among the ethnic groups, there were no significant differences in the type of glucose-lowering therapy (diet, oral hypoglycemic agents, or a combination of oral hypoglycemic agents and insulin) ($P=0.565$) or cholesterol-lowering therapy (diet, statins/omega-3 only or statins/omega-3 and fibrate combination) ($P=0.777$). The subsequent analysis and data presented were adjusted for age, gender, diabetes and cholesterol-lowering medications where appropriate.

Table 2-1 Anthropometric and metabolic characteristics by gender and ethnicity

	Chinese (n= 99)	**Malay** (n= 96)	**Asian-Indians** (n= 111)	**Total** (n=306)	***P-value*$^{\phi}$**
Gender (% within race)					
Males	48 (48.5%)	32 (33.3%)	28 (25.2%)	108 (35.3%)	
Females	51 (51.5%)	64 (66.7%)	83 (74.8%)	198 (64.7%)	***0.002***
Age (years)	58.6 (0.5)^{a,c}	54.2 (0.5)^{a,b}	56.0 (0.5)^{b,c}	56.3 (0.3)	***<0.001***
Duration of diabetes (years)	11.6 (0.6)^{a}	9.1 (0.6)§^{a,b}	11.7 (0.5)^{b}	10.9 (0.3)	***0.002***
BMI (kg/m^2)	26.1 (0.5)^{a}	29.0 (0.5)^{a,b}	27.5 (0.5)^{b}	27.5 (0.3)	***<0.001***
Waist circumference (cm)	87.7 (1.1)^{a}	90.6 (1.1)	91.8 (1.0)^{a}	90.0 (0.6)	***0.019***
Systolic BP (mmHg)	137.0 (1.9)	140.1 (1.9)	135.0 (1.7)	137.4 (1.0)	*0.147*
Diastolic BP (mmHg)	75.4 (0.9)	76.4 (0.9)	76.3 (0.8)	76.0 (0.5)	*0.688*
Total cholesterol (mmol/L)	4.24 (0.09)	4.48 (0.09)^{a}	4.16 (0.08)^{a}	4.30 (0.05)	***0.029***
LDL-C (mmol/L)	2.66 (0.08)^{a}	2.99 (0.08)^{a}	2.88 (0.08)	2.84 (0.05)	***0.021***
HDL-C (mmol/L)	1.31 (0.03)^{a}	1.23 (0.03)	1.14 (0.03)^{a}	1.23 (0.02)	***<0.001***
Triglycerides (mmol/L)	1.21 (0.08)^{a}	1.56 (0.08)^{a,b}	1.23 (0.07)^{b}	1.33 (0.04)	***0.002***
Fasting glucose (mmol/L)	7.64 (0.24)	7.04 (0.24)	7.41 (0.22)	7.36 (0.13)	*0.201*
HbA1c (%)	7.84 (0.13)	8.07 (0.13)	8.00 (0.12)	7.98 (0.07)	*0.450*

Abbreviations: BMI, body mass index; BP blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated haemoglobin. ϕAdjusted for age, gender, diabetes medications and cholesterol-lowering medications, where appropriate. §n=94
Data are presented as mean (SE) (all such values). *P-value* represents overall significant differences across groups. Values in the same row with same superscript letters differ significantly, $P<0.05$ (Bonferroni *post hoc* test). *P-values* that were significant were highlighted in bold.

Compared with the Chinese and Asian-Indians, Malays had the shortest duration of diabetes, the most overweight, higher levels of total cholesterol, LDL-C and triglycerides. The Asian-Indians had the highest waist circumference. There were no significant differences among the three ethnic groups in terms of HbA1c, fasting glucose, systolic and diastolic blood pressure.

Table 2-2 shows the energy, nutrient intakes and glycemic measures of each of the ethnic group. There was no significant difference in energy intake among the

ethnic groups. In terms of macronutrients, the Chinese had the lowest intake of carbohydrates but they had the highest intake of protein as a percentage of total energy intake. The Malays consumed the most saturated fat, sugars and sodium while the Asian-Indians consumed the most dietary fibre compared with the other two ethnic groups. There were no significant differences in total fat intake or reported physical activity level (PAL). Dietary glycemic load (GL) was highest in Malays and lowest amongst the Chinese. Similarly, dietary glycemic index (GI) was highest among the Malays.

We have used the Goldberg equation (Goldberg et al., 1991), using self-reported PAL to calculate the confidence limits (lower and upper cut-offs) to determine whether the mean reported energy intake for our study population was plausible as a valid measure of their habitual food intake. The mean ratio of energy intake to calculated basal metabolic rate (EI: BMR) values of 0.87, 0.93 and 0.89 for the Chinese, Malays and Asian-Indians respectively, were all well below the accepted ranges for accurate recording of dietary intake when compared to their self-reported PAL (Black, 2000; Goldberg et al., 1991). This suggests that our cohort of T2DM participants had collectively under-reported their food intake. The mean PAL of 1.56 also seemed to suggest that our subjects may possibly have over-reported their physical activity level while under-reporting their food intake.

Table 2-2 Energy, nutrient intakes and glycemic measures of the three ethnic groups

	Chinese (n= 99)	**Malay (n= 96)**	**Asian-Indians (n= 111)**	**Total (n=306)**	***P-value*$^{\phi}$**
Energy (kcal)	1388.0 (47.6)	1520.5 (47.4)	1443.2 (43.4)	1450.6 (25.7)	*0.158*
Carbohydrate (g)	169.8 (6.2)[a]	197.9 (6.2)[a]	189.3 (5.7)	185.7 (3.4)	***0.007***
Carbohydrate (percent energy)	50.2 (0.8)[a]	52.7 (0.8)	53.5 (0.7)[a]	52.1 (0.4)	***0.008***
Protein (g)	59.3 (2.1)	55.5 (2.0)	54.3 (1.9)	56.3 (1.1)	*0.198*
Protein (percent energy)	17.0 (0.4)[a,b]	14.8 (0.4)[a]	15.3 (0.3)[b]	15.7 (0.2)	***<0.001***
Total fat (g)	49.3 (2.5)	55.3 (2.5)	51.1 (2.2)	51.9 (1.3)	*0.218*
Total Fat (percent energy)	31.1 (0.7)	31.8 (0.7)	30.5 (0.7)	31.1 (0.4)	*0.434*
Saturated fat (g)	17.1 (1.1)[a]	22.9 (1.1)[a]	19.8 (1.0)	20.0 (0.6)	***0.001***
Saturated fat (percent energy)	11.0 (0.4)[a]	13.3 (0.4)[a]	12.0 (0.4)	12.1 (0.2)	***0.001***
Sugars (g)	38.3 (2.5)[a]	52.1 (2.5)[a,b]	43.9 (2.3)[b]	44.8 (1.3)	***0.001***
Sugars (percent energy)	11.1 (0.5)[a]	13.9 (0.5)[a]	12.2 (0.5)	12.4 (0.3)	***0.002***
Sugars (percent carbohydrate)	22.5 (1.0)[a]	26.2 (1.0)[a]	23.0 (0.9)	23.9 (0.5)	***0.019***
Dietary fibre (g)	13.1 (0.9)[a]	15.1 (0.9)[b]	19.4 (0.8)[a,b]	15.9 (0.5)	***<0.001***
Sodium (mg)	2487 (108)	2650 (108)[a]	2255 (99)[a]	2464 (59)	***0.025***
Mean Dietary GL	91.5 (3.6)[a]	109.6 (3.5)[a]	99.0 (3.2)	100.0 (1.9)	***0.002***
Mean Dietary GI	58.1 (0.5)	59.8 (0.5)[a]	58.1 (0.5)[a]	58.6 (0.3)	***0.029***
PAL	1.52 (0.03)	1.58 (0.03)	1.57 (0.03)	1.56 (0.02)	*0.349*
EI: BMR	0.87 (0.03)	0.93 (0.03)	0.89 (0.03)	0.90 (0.02)	*0.475*
Goldberg confidence limits (lower – upper)	1.42-1.56	1.52-1.68	1.51-1.65	1.49-1.63	-

Abbreviations: GL, glycemic load; GI, glycemic index; PAL, physical activity level; EI: BMR, ratio of energy intake to calculated basal metabolic rate. ϕAdjusted for age (covariate), gender, diabetes medications and cholesterol-lowering medications (fixed factors)

Date are presented as mean (SE). *P-value* represents overall significant differences across groups. Values in the same row with same superscript letters differ significantly, $P<0.05$ (Bonferroni *post hoc* test). *P-values* that were significant were highlighted in bold.

Table 2-3 shows the mean carbohydrate consumption of each ethnic group by food sub-groups, ranked in decreasing order of amount consumed. Rice and rice-based dishes were the main staple food for all three ethnic groups. However, subsequent carbohydrate foods showed ethnic-specific food preferences. We observed that the Malays consumed almost twice as much beverages and sweetened drinks compared with the Chinese and Asian-Indians; the Chinese consumed twice as much noodles and noodles-based dishes compared with the other two groups; the Asian-Indians consumed up to three times as much processed grain products compared with the other two groups. The specific food items (the top 3) within each food subgroups are presented in Table 2-4. Even though rice and rice-based dishes were the common staple food among ethnic groups with white rice (Jasmine) being the universally preferred carbohydrate staple, we observed obvious ethnic differences in cooking practices. The Chinese favoured brown rice and rice porridge while the Malays and Asian-Indians preferred flavoured rice for example, coconut-flavoured rice (nasi lemak) and fried rice.

Table 2-3 Mean carbohydrate consumption of each ethnic group by food sub-groups, ranked in decreasing order

Chinese (n=99)		**Malays (n=96)**		**Asian-Indians (n=111)**	
Food sub-groups	**Mean intake (g)**	**Food sub-groups**	**Mean intake (g)**	**Food sub-groups**	**Mean intake (g)**
Rice and rice based dishes	51.73	Rice and rice based dishes	70.13	Rice and rice based dishes	59.25
Noodles and noodles based dishes	35.74	Beverages and sweetened drinks	22.32	Processed grain products	28.88
Local cakes, desserts and snacks	13.08	Noodles and noodles based dishes	18.87	Noodles and noodles based dishes	15.86
Beverages and sweetened drinks	10.29	Bread, white	14.60	Fresh fruits	12.48
Fresh fruits	9.89	Local cakes, desserts and snacks	13.92	Beverages and sweetened drinks	12.01
Bread, white	8.61	Processed grain products	9.12	Bread, wholemeal	11.73
Processed grain products	6.88	Fresh fruits	6.95	Local cakes, desserts and snacks	11.72
Leafy and non-leafy veg based dishes	6.35	Other breads	6.71	Bread, white	8.47
Bread, wholemeal	5.88	Bread, wholemeal	6.54	Leafy and non-leafy veg based dishes	6.33
Biscuits	3.46	Biscuits	6.43	Biscuits	5.36
Other breads	2.95	Leafy and non-leafy veg based dishes	5.10	Milk and dairy products	5.08

Table 2-3. Continued

Chinese (n=99)		Malays (n=96)		Asian-Indians (n=111)	
Food sub-groups	**Mean intake (g)**	**Food sub-groups**	**Mean intake (g)**	**Food sub-groups**	**Mean intake (g)**
Starchy veg and starchy veg based dishes	2.66	Sugars and syrups	3.82	Starchy veg and starchy veg based dishes	4.99
Milk and dairy products	2.38	Sauces, gravies and miscellaneous	3.15	Pulses, beans and seeds	4.75
Poultry and poultry dishes (duck, chicken, turkey)	1.76	Starchy veg and starchy veg based dishes	3.10	Other breads	3.49
Sugars and syrups	1.56	Seafood, shellfish and roe	2.50	Sugars and syrups	2.49
Meat and meat dishes (pork, beef, veal)	1.44	Milk and dairy products	2.09	Fish and fish based dishes	1.61
Juices and juice drinks	1.26	Juices and juice drinks	1.95	Herbs, spices, condiments	1.33
Fish and fish based dishes	1.21	Herbs, spices, condiments	1.61	Sauces, gravies and miscellaneous	1.32
Herbs, spices, condiments	0.95	Fish and fish based dishes	1.17	Poultry and poultry dishes (duck, chicken, turkey)	1.15
Processed meats (frankfurters/ham/ sausage)	0.77	Pulses, beans and seeds	1.11	Nuts and products	0.91
Alcoholic beverages	0.58	Poultry and poultry dishes (duck, chicken, turkey)	0.87	Juices and juice drinks	0.83
Sauces, gravies and miscellaneous	0.56	Meat and meat dishes (pork, beef, veal)	0.44	Processed meats (frankfurters/ham/ sausage)	0.39
Pulses, beans and seeds	0.56	Jam, jellies, preserves	0.37	Non-dairy creamers/whiteners	0.33
Jam, jellies, preserves	0.44	Processed meats (frankfurters/ham/ sausage)	0.23	Jam, jellies, preserves	0.32
Non-dairy creamers/whiteners	0.36	Egg based dishes	0.23	Seafood, shellfish and roe	0.29
Confectionery	0.36	Processed fruits	0.22	Processed fruits	0.18
Egg based dishes	0.34	Soups (clear and cream)	0.19	Soups (clear and cream)	0.18
Nuts and products	0.34	Nuts and products	0.18	Meat and meat dishes (pork, beef, veal)	0.16
Seafood, shellfish and roe	0.26	Non-dairy creamers/whiteners	0.07	Confectionery	0.14
Soups (clear and cream)	0.20	Stocks	0.03	Alcoholic beverages	0.03

All values are weighted mean carbohydrate intake per day

Table 2-4 Top 3 food items within each food subgroups

Food subgroups	Chinese	Malays	Asian-Indians
Rice and rice based dishes	White rice	White rice	White rice
	Brown rice	Nasi lemak	Parboiled rice
	Rice porridge	Fried rice	Fried rice
Beverages and sweetened drinks	Coffee (no sugar)	Milo™	Coffee (no sugar)
	Sweetened coffee	Sweetened coffee	Sweetened tea
	Milo™	Sweetened tea	Sweetened coffee
Noodles and noodles based dishes	Yellow noodles	Yellow noodles	Yellow noodles
	Beehoon	Beehoon	Beehoon
	Kway teow	Instant noodles	Instant noodles
Fresh fruits	Apple	Apple	Apple
	Papaya	Banana	Papaya
	Banana	Pear	Banana
Processed grain products	Oats	Roti prata	Thosai
	Wheat/wheat germ	Thosai	Chapatti
	Roti prata (plain)	Chapatti	Roti prata
Local cakes, desserts, snacks	Chee cheong fun	Banana fritter	Banana fritter
	Pow	Curry puff	Malay kuih
	Dim sum	Kaya bun	Vadai

Description of local foods: nasi lemak, rice cooked in coconut milk; roti prata, flaky Indian bread and pan-fried in ghee; thosai, thin Indian pancake made with fermented rice and gram batter; chapatti, grilled Indian flat bread made with ghee; chee cheong fun, steamed rice sheets served with sweet sauce; pow, steamed bun with pork/chicken/red bean/lotus paste filling; dim sum, assortment of steamed dumplings; banana fritter, banana coated in flour and deep-fried; curry puff, deep-fried pastry filled with chicken and curry potatoes; kaya bun, bun filled with coconut jam; malay kuih; vadai, deep-fried dough made with yellow gram and green onion

Correlation analyses between mean dietary GL (Table 2-5) and GI (Table 2-6) with energy and nutrients intake were performed. Across each ethnic group, a higher dietary GL was positively associated with energy intake ($P<0.001$), a higher carbohydrate (percent energy) intake ($P<0.01$), lower protein (percent energy) (only among the Malays and Asian-Indians) ($P<0.05$), higher intake of saturated fat ($P<0.001$), higher absolute intake of sugars ($P<0.001$) and a higher consumption of dietary fibre ($P<0.001$). A lower GI diet, on the other hand was associated with higher sugar intake (percent energy and percent carbohydrate) in all three groups.

Table 2-5 Correlations between nutrient intake and dietary glycemic load (GL) by ethnicity

	Mean dietary GL					
	Chinese (n=99)		**Malays (n=96)**		**Asian-Indians (n=111)**	
	R	*P-value*	R	*P-value*	R	*P-value*
Energy (kcal)	0.703	***<0.001***	0.867	***<0.001***	0.840	***<0.001***
Carbohydrate (percent energy)	0.329	***0.001***	0.270	***0.008***	0.247	***0.009***
Protein (percent energy)	-0.151	*0.137*	-0.251	***0.013***	-0.224	***0.018***
Total fat (percent energy)	-0.256	***0.011***	-0.140	*0.173*	-0.097	*0.311*
Saturated fat (g)	0.413	***<0.001***	0.537	***<0.001***	0.502	***<0.001***
Sugar (g)	0.378	***<0.001***	0.564	***<0.001***	0.679	***<0.001***
Sugar (percent energy)	-0.076	*0.455*	-0.020	*0.849*	0.134	*0.162*
Sugar (percent carbohydrate)	-0.169	*0.095*	-0.094	*0.363*	0.055	*0.567*
Dietary fibre (g)	0.371	***<0.001***	0.282	***0.005***	0.465	***<0.001***

R, Pearson correlation coefficient. P-values that were significant (*p<0.05*) are highlighted in bold.

Table 2-6 Correlations between nutrient intake and dietary glycemic index (GI) by ethnicity

	Mean dietary GI					
	Chinese (n=99)		**Malays (n=96)**		**Asian-Indians (n=111)**	
	R	*P-value*	R	*P-value*	R	*P-value*
Energy (kcal)	-0.094	*0.356*	0.000	*0.994*	-0.047	*0.621*
Carbohydrate (percent energy)	0.256	***0.010***	0.051	*0.625*	0.024	*0.801*
Protein (percent energy)	0.011	*0.915*	-0.141	*0.170*	-0.018	*0.851*
Total fat (percent energy)	-0.116	*0.253*	0.018	*0.859*	-0.044	*0.647*
Saturated fat (g)	-0.125	*0.217*	-0.014	*0.892*	-0.040	*0.674*
Sugar (g)	-0.260	***0.009***	-0.209	***0.041***	-0.113	*0.238*
Sugar (percent energy)	-0.299	***0.003***	-0.280	***0.006***	-0.194	***0.041***
Sugar (percent carbohydrate)	-0.402	***<0.001***	-0.350	***<0.001***	-0.231	***0.015***
Dietary fibre (g)	-0.052	*0.606*	-0.196	*0.055*	-0.161	*0.092*

R, Pearson correlation coefficient. P-values that were significant (*p<0.05*) are highlighted in bold.

Further analysis of the results using regression models did not reveal any associations between dietary GI and GL with measures of glycemic control and lipids profile (Table 2-7). Additional analyses with intake of saturated fat or dietary fibre with glycemic control and lipid markers did not reveal any associations (data not shown).

Table 2-7 Associations between mean dietary GI and GL with glycemic control measures and metabolic markers by ethnicity

	Mean dietary GI						**Mean dietary GL**					
	Chinese (n=99)		**Malays (n=96)**		**Asian-Indians (n=111)**		**Chinese (n=99)**		**Malays (n=96)**		**Asian-Indians (n=111)**	
	B (SE)	*P-value*	B (SE)	*P-value*	B (SE)	*P-value*	B (SE)	*P-value*	B (SE)	*P-value*	B (SE)	*P-value*
HbA1c (%)	-0.001 (0.001)	0.297	-0.003 (0.002)	0.103	-0.002 (0.001)	0.119	<0.001 (<0.001)	0.335	<0.001 (<0.001)	0.820	<0.001 (<0.001)	0.362
FBG (mmol/L)	0.001 (0.003)	0.764	-0.004 (0.003)	0.196	<0.001 (0.002)	0.768	<0.001 (0.001)	0.788	<0.001 (0.001)	0.850	<0.001 (0.001)	0.780
TC (mmol/L)	0.010 (0.014)	0.494	-0.017 (0.024)	0.474	0.015 (0.017)	0.391	0.002 (0.004)	0.656	-0.007 (0.005)	0.218	-0.002 (0.004)	0.638
LDL-C (mmol/L)	<0.001 (0.013)	0.973	-0.026 (0.020)	0.184	0.017 (0.018)	0.325	-0.001 (0.003)	0.817	-0.005 (-0.004)	0.260	-0.002 (0.005)	0.661
HDL-C (mmol/L)	0.005 (0.006)	0.464	-0.002 (0.006)	0.683	0.002 (0.005)	0.683	0.001 (0.002)	0.478	-0.002 (0.001)	0.095	<0.001 (0.001)	0.914
TG (mmol/L)	-0.001 (0.004)	0.770	0.003 (0.005)	0.581	0.002 (0.003)	0.531	<0.001 (0.001)	0.458	<0.001 (0.001)	0.886	<0.001 (0.001)	0.952

Abbreviations: B, regression coefficient; SE, standard error; HbA1c, glycated haemoglobin; FBG, fasting blood glucose; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Multiple linear regression analysis was performed with HbA1c, FBG and TG, with the remaining metabolic parameters as dependent variables and GI as the main independent variable. Adjustments were made for age, gender, BMI and medications (OHA, insulin, statins and fibrates), dietary fibre and mean energy intake.

2.5 Discussion

This was the first study that fully characterized the dietary profiles of individuals with Type 2 Diabetes Mellitus (T2DM) in a multi-ethnic Asian population. We found interesting ethnic differences in metabolic profiles, which may explain for the differences in the rates of coronary heart disease in the ethnic groups, particularly so amongst the Malays and Asian-Indians (Mak et al., 2003). In our study, Malays were more obese and had higher total and LDL-cholesterol than Chinese and Asian-Indians. In contrast, Asian-Indians had lower levels of HDL-cholesterol, a well-known finding in this ethnic group (Tai et al., 2010; Tan et al., 1999).

We observed important differences in nutrients intake, even though there was clearly under-reporting. Malays and Asian-Indians consumed a greater proportion of energy from carbohydrates while Chinese consumed more protein. These findings were similar to those of the National Nutrition Survey 2010 (Ministry of Health Singapore, 2010b). Although the total fat intake was similar across ethnic groups, Malays consumed the greatest proportion of energy in the form of saturated fat and Chinese the least. Asian-Indians consumed the most dietary fibre and Malays consumed more sugar and sodium than other ethnic groups.

Our findings provide some evidence that dietary factors contribute to the observed differences in metabolic measures amongst the three ethnic groups. In particular, the higher intakes of saturated fats and sugars amongst the Malays, whether expressed as a percentage of total energy or in absolute amounts, seem likely to explain, at least to some extent, the higher total cholesterol, LDL-C and triglycerides levels observed. This finding is particularly noteworthy as among the three ethnic groups, there were no significant differences on the use of lipid-lowering drugs.

The Singapore Dietary Guidelines recommend that total fat should be limited to 25-30% of total calorie intake, of which less than 10% is from saturated fat (Health Promotion Board, 2012). In our study, all three ethnic groups exceeded their total fat as well as saturated fat recommendations. Reduction of saturated fat and refined sugars are universally recommended for people with T2DM (Franz et al., 2010), who also have higher risk of cardiovascular disease (Buse et al., 2007; Mirrahimi et al., 2012). In our study, despite Malays having the highest intake of saturated fat, all three ethnic groups would need to reduce their intake of saturated fat to improve and optimize their lipids profile. For added sugars, it should contribute to no more than 10% of dietary energy (Health Promotion Board, 2012). However, we were unable to differentiate total sugars from added sugars in our database and thus even though all three ethnic groups exceeded the sugars recommendations, this might be due to combination of natural sugars present in food and added sugars that contributed to the higher values observed. Our subsequent food-based analyses revealed that Malays in particular, had higher intakes of sugars from added sugars, which will be described in details below.

Carbohydrate intake, in particular its quantity and quality were a focus in this study. Food-based dietary recommendations regarding the quantity of carbohydrate consumed are relevant especially for T2DM management (Franz et al., 2010). Advice regarding reducing intake of beverages and sweetened drinks is paramount for the Malays who obtain about twice as much carbohydrate from these sources as the other two groups. It is noteworthy that Milo™, coffee and tea sweetened with condensed milk were the main beverages frequently consumed by the Malays rather than sodas and carbonated drinks, as would be the case in other countries (Du et al., 2008; Malik et al., 2010). In contrast, noodles and processed grain products were more frequently consumed by the Chinese and Asian-Indians respectively. These findings were broadly comparable with the findings from the National Nutrition Survey (Ministry of

Health Singapore, 2010b), which were conducted in people without a history of T2DM. The Chinese consumed more rice, porridge and noodles dishes; the Asian-Indians consumed more bread and cereals (termed processed grain products in this study) compared to the other ethnic groups. Food-based interventions would need to focus on the quantity of these carbohydrate sources, especially amongst the Malays where high intakes of sugar was common.

Rice is the main staple for most Asian populations and Singapore is of no exception. The main carbohydrate source for all three ethnic groups was white rice. Further evaluation of the food data revealed that the cooking method of rice differed between ethnicities; Malays and Asian-Indians preferred flavoured rice in the form of coconut-flavoured rice and fried rice, which are higher in fat, whilst the Chinese favoured plain brown rice and rice porridge. This information has implications for nutrition advice that needs to also focus on healthier cooking methods to improve on the quality of the carbohydrate staple.

In investigating the quality of carbohydrate consumed, we found that although the dietary GI among Malays was significantly higher than in the other ethnic groups, the absolute difference is probably of little clinical significance. All three groups had dietary GI values classified within the medium category of between GI 56 to 69 (Standards Australia, 2007). Our findings were comparable to numerous studies reporting that people with T2DM already consume a moderate GI diet, ranging from dietary GI 57 in the Longitudinal Assessment of Ageing in Women (LAW) study (O'Sullivan et al., 2010) to dietary GI 60 in patients with T2DM (Wolever et al., 1994). In an Asian study, the dietary GI calculated among individuals with T2DM was 64 (Yusof et al., 2009), which was also comparable with our findings. Our findings also indicate a correlation between higher sugar intakes with a low GI diet, which may be contrary to what most people perceive. In this study, we recognize that one of the limitations of the food database was the inability to

differentiate between the types of sugar consumed. This was because all common sugars, with the exception of glucose, are of moderate (sucrose) or low GI (fructose and lactose). Thus, we postulate that the positive correlation with a low GI diet could have been due to a higher consumption of fruits and dairy products.

We did not find any significant associations between dietary GI or GL with diabetes control measures and lipids profile in this cross-sectional study, which was similar to a larger cross-sectional analyses of data from 2 joint observational studies by Du et al. (Du et al., 2008). However, this does not preclude the possibility that a low GI diet intervention will produce changes. One possibility was that this study might not have spread of foods with varying GI and GL to show significant associations. Additionally, we did not find any associations with intake of saturated fat or dietary fibre with glycemic control and cardiovascular risk markers. Strong evidence from clinical trials found that low GI diets improve glycemic control in people with diabetes, improve serum lipids and other cardiovascular risk factors and possibly promote weight loss (Jenkins et al., 2014; Augustin et al., 2015) Future research must therefore focus on alternative objective measures such as continuous glucose monitoring that may better reflect postprandial glycemia and capture effects of diet quality on glycemic control. The risk of complications associated with elevated blood glucose and insulin concentrations may be more dependent on the magnitude of postprandial excursions in blood glucose per meal, described as hyperglycemic spikes (Le Devehat, 1997), rather than the average daily glycemic response. If the current results are typical of individuals with T2DM, our data thus suggest that more education needs to be focused on the Malay population, as it appeared that this ethnic group had poorer dietary and metabolic profiles.

The strengths of this study are that we have a significant number of participants with a comprehensive clinical, anthropometric, metabolic and dietary assessment of individuals with T2DM of different ethnicities. We used a locally

available national food database as a source reference and conducted separate GI analysis on a proportion of the some locally consumed carbohydrates (data not published). In addition, we used two interviewer-assisted 24-hour food recalls to determine the quantity, type and quality of the foods consumed.

This study has some limitations. There remains contention and controversy in the determination of dietary GI and GL mainly due to several factors, one of which is the lack of a comprehensive database for measured GI values for commonly consumed local foods. This may lead to errors in assigning GI values to food items (Flood et al., 2006). We sought to overcome this through the use of a local food database developed and updated by the Singapore Health Promotion Board and our method of assigning GI values were consistent with those described in the literature (Flood et al., 2006; Louie et al., 2011; Neuhouser et al., 2006; Olendzki et al., 2006) either through direct matches or through the calculation of the weighted GI value. We also acknowledge that there is global under-reporting in food intake amongst all the participants and is a common finding in studies of this nature (Yusof et al., 2009). We have confirmed this in a separate study locally using 24-hour food recalls, in which the average energy intake is in the same order of magnitude as this study (sub-study in the National Nutrition Survey 2010, Health Promotion Board, Singapore, data unpublished). Based on the Goldberg cut-off for the ratio of energy intake (EI): basal metabolic rate (BMR), the under-reporting was similar in all 3 ethnic groups and as such, we feel that our observations remain valid.

In conclusion, there were ethnic differences in the diet, anthropometric and metabolic profile of individuals with T2DM. To our knowledge, this was the first study that examined the dietary habits of T2DM in a multi-ethnic Asian population, and the only study that investigated the associations between dietary GI and GL with dietary intake, glycemic control measures and metabolic parameters. Population and individual level interventions targeting changes in carbohydrate quality and quantity

in the dietary management of people with T2DM need to keep cultural food preferences in mind when relating to different ethnic groups. In particular, more education needs to focus on the Malay population, as it appeared that this ethnic group had both poorer dietary and metabolic profiles. Dietary advice to lower GI foods is clearly of critical relevance to all three ethnic groups for optimal glycemic control. Further prospective interventional studies are required to determine optimal dietary habits for individuals in the management of individuals with T2DM in a multi-ethnic Asian population.

2.6 Acknowledgement

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The candidate was responsible for data cleaning, assigning GI values to individual foods, computing GI values for composite food items and all statistical data analyses.

CHAPTER 3 Ethnic Differences in Insulin Sensitivity and Beta-Cell Function among Asian Men

3.1 Abstract

Introduction – Lean Asian-Indians are less insulin sensitive compared with Chinese and Malays, but the pancreatic beta-cell function among these ethnic groups has yet to be studied in-depth. We aimed to study beta-cell function in relation to insulin sensitivity among individuals of Chinese, Malay and Asian-Indian ethnicity living in Singapore.

Methods – This is a sub-group analysis of 59 normoglycemic lean (BMI<23 kg/m^2) adult males (14 Chinese, 21 Malays and 24 Asian-Indians) from the Singapore Adults Metabolism Study. Insulin sensitivity was determined using fasting state indices (HOMA-IR), the euglycemic-hyperinsulinemic clamp (ISI-clamp) and a liquid mixed meal tolerance test (LMMTT) [Matsuda insulin sensitivity index (ISI-Mat)]. Beta-cell function was assessed using fasting state indices (HOMA-B) and from the LMMTT (insulinogenic index and insulin secretion index). The oral disposition index (DI), a measure of beta-cell function relative to insulin sensitivity during the LMMTT, was calculated as a product of ISI-Mat and insulin secretion index.

Results – Asian-Indians had higher waist circumference and percent body fat than Chinese and Malays despite similar BMI. Overall, Asian-Indians were the least insulin sensitive while the Chinese were most insulin sensitive. Asian-Indians had higher beta-cell function compared with Chinese or Malays but these were not statistically different. Malays had the highest incremental area-under-the-curve

(AUC) for glucose during LMMTT compared with Asian Indians and Chinese. However, there were no significant ethnic differences in the incremental insulin AUC. The oral DI was the lowest in Malays, followed by Asian-Indians and Chinese.

Conclusion – Among lean Asians, Chinese are the most insulin sensitive while Asian-Indians are the least insulin sensitive. However, Malays demonstrate higher postprandial glucose excursion with lower beta-cell response compare with Chinese or Asian-Indians. The paths leading to type 2 diabetes mellitus might differ between these Asian ethnic groups.

3.2 Introduction

Type 2 diabetes mellitus (T2DM) is projected to affect nearly 600 million people worldwide by 2035 (Chan et al., 2009), with China and India projected to contribute more cases of T2DM than any other country in the world. The population of Singapore comprises three major ethnic groups; Chinese, Malays and Asian-Indians. The prevalence of T2DM in Singapore has increased from 8.2% in 2004 to 11.3% based on the Singapore National Health Survey 2010 (Ministry of Health Singapore, 2010a). The highest prevalence of T2DM has been seen in Asian-Indians (17.2%), followed by Malays (16.6%) and Chinese (9.7%) (Ministry of Health Singapore, 2010a).

Much of the work to understand ethnic differences in susceptibility to T2DM has focused on insulin resistance (Gujral et al., 2014; Misra and Vikram, 2004; Staimez et al., 2013). Recently, one study showed that the degree of insulin sensitivity differs between Asian ethnic groups and this ethnic difference is more prominent amongst lean individuals (Khoo et al., 2014). Among lean and young Singaporean males, Chinese and Malays are more insulin sensitive compared with Asian-Indians (Khoo et al., 2014; Tan et al., 2015).

The pathogenesis of T2DM involves both decreased insulin sensitivity and impaired beta-cell function (Bergman et al., 2002; Kahn et al., 1993). Initially, decreased insulin sensitivity may be compensated by increased beta-cell response, and progression to T2DM is thought to occur when beta-cells fail to compensate to a sufficient degree (Stumvoll et al., 2005). Accumulating evidence demonstrates that insulin secretory defect plays a more important role than insulin sensitivity in the pathogenesis of T2DM, particularly in East Asians (Fukushima et al., 2004; Kahn, 2003; Kodama et al., 2013). However, studies comparing beta-cell function between Asian ethnic groups are limited. It is not clear whether Chinese, Malays and Asian-

Indians exhibit differences in beta-cell function, in addition to the differences in insulin sensitivity.

To gain further insights into the ethnic susceptibility to T2DM, the present study aimed to examine ethnic differences in beta-cell function among lean individuals of Chinese, Malay and Asian-Indian ethnicity, after accounting for differences in insulin sensitivity.

3.3 Methods

3.3.1 Subjects

This was a sub-group analysis of the Singapore Adults Metabolism Study (SAMS) (Khoo et al., 2014). SAMS was a cross-sectional study that examined the associations between ethnicity, obesity and insulin resistance in 3 ethnic groups involving 100 Chinese, 80 Malays and 78 Asian-Indian males. A more detailed account of the selection procedure and the study participants was elsewhere (Khoo et al., 2014). The main study (SAMS) was registered at clinicaltrials.gov as NCT00988819.

Fifty-nine healthy lean (BMI less than 23 kg/m^2) adult males, comprising 14 Chinese, 21 Malays and 24 Asian-Indians who had full data from the euglycemic-hyperinsulinemic clamp procedure and the liquid mixed meal tolerance test were included in this study. We selected lean individuals as we have shown previously that ethnic differences in insulin sensitivity was observed among these individuals in the lean but not in the overweight or obese individuals (Khoo et al., 2014). All subjects had fasting blood glucose of less than 7.0 mmol/L and had no prior history of hypertension or dyslipidemia. We excluded those with significant changes in diet or weight loss of more than 5%, a history of heart disease, epilepsy, insulin allergy, current smoking, a history of ingesting any drug known to alter insulin sensitivity

(e.g. corticosteroids), or any hospitalization or surgery during the 6 months before enrollment in the study. Ethics approval was obtained from the National Healthcare Group Domain Specific Review Board (Singapore) (approval code number C/2009/00022). All subjects provided written informed consent.

3.3.2 Clinical measurements

Demographic data, medical and drug history, and data on lifestyle factors were collected using interviewer-administered questionnaires. Height was measured using a wall-mounted stadiometer, and weight using a digital scale (SECA, model 803; Vogel & Halke, Hamburg, Germany). BMI was calculated using the weight (kg) divided by the square of height (m). Waist circumference was measured at the midpoint between the lower costal margin and iliac crest at mid-respiration. Body composition (percent body fat and lean body mass) was measured using a dual-energy X-ray absorptiometry (DXA) scanner (Hologic Discovery Wi, Hologic, Bedford, MA). All DXA measurements were performed within one week of the euglycemic-hyperinsulinemic clamp procedure and the liquid mixed meal tolerance test.

3.3.3 Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was assessed using the euglycemic-hyperinsulinemic clamp technique, after an overnight fast (10 hours) (Bergman et al., 1985). Insulin was infused at a fixed rate of 40m U/m^2 body surface area/min for the duration of the clamp (120 min). Blood glucose was measured every 5 min using the glucose oxidase method (Yellow Spring Glucose Analyzer; YSI Life Sciences, Yellow Spring, OH). The infusion rate of the dextrose 20% solution was adjusted to maintain a constant blood glucose level at about 90 mg/dL (5 mmol/L) throughout the clamp. The insulin sensitivity index (ISI-clamp) was calculated using the mean glucose infusion rate and

steady state insulin concentrations (SSSI) during the final 30 min of the clamp, adjusted for the lean body mass.

3.3.4 Liquid mixed meal tolerance test (LMMTT) procedure

On a separate day after the clamp procedure, the LMMTT was conducted after an overnight 10-hour fast. As a mixed-nutrient load, the liquid mixed meal provides a more physiologic stimulus than glucose alone for assessing postprandial glucose and insulin responses (Maki et al., 2010), dynamically reflects beta-cell function (Brodovicz et al., 2011) and provides an appropriate stimulus for assessing insulin sensitivity (Caumo et al., 2000; Steil et al., 2004). Subjects were provided a liquid meal that consisted of two 200 ml servings of Ensure Plus (Abbott Laboratories, Columbus, OH), each providing 300 kcal, 40.4 g carbohydrate, 9.8 g fat, and 12.5 g protein. A single intravenous catheter was placed in the antecubital space for collection of venous blood. Blood samples were obtained from the indwelling catheter for plasma glucose and insulin concentrations at 0, 30, 60, 90, 120 and 240 min.

3.3.5 Biochemical analyses

Biochemical analyses were conducted at the National University Hospital Referral Laboratory, which is accredited by the College of American Pathologists. Plasma glucose concentrations obtained during LMMTT were analyzed using enzymatic methods (ADVIA 2400, Bayer Diagnostics), and plasma insulin concentrations using a chemiluminescence assay (ADVIA Centaur Analyzer, Siemens Healthcare Diagnostics).

3.3.6 Derivatives of insulin sensitivity and beta-cell function

Using fasting indices, we computed HOMA-IR using the formula: [fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5]. HOMA B, a measure of beta-cell

function was computed using the formula: [20 x fasting insulin (mU/L)] / [fasting glucose (mmol/L) – 3.5].

From the LMMTT, we calculated total (total AUC) and incremental (IAUC) area under the curve for glucose and insulin responses using the trapezoidal rule (Brouns et al., 2005; FAO/WHO, 1998). The insulin secretion index, calculated as the ratio of total AUC insulin to total AUC glucose, provides information on the total insulin response following the LMMTT. The Matsuda insulin sensitivity index (ISI-Mat) was calculated as follows: 10,000 / square root of (fasting glucose x fasting insulin) x ($IAUC_{glucose240}$ x $IAUC_{insulin240}$) (DeFronzo and Matsuda, 2010; Matsuda and DeFronzo, 1999). ISI-Mat is a measure of glucose disposal during the LMMTT, representing a composite of both hepatic and muscular tissue insulin sensitivity (Matsuda and DeFronzo, 1999).

The insulinogenic index was used as a marker of early-phase insulin response and was calculated as follows: (Insulin30 – Insulin0) / (Glucose30 – Glucose0). The oral disposition index (DI), a measure of beta-cell function relative to insulin sensitivity during the LMMTT, was calculated as a product of ISI-Mat and insulin secretion index.

3.3.7 Statistical analyses

Data are presented as mean (SE) unless otherwise stated. ANOVA was used for comparisons of continuous variables, with *post hoc* Bonferroni corrections applied for group comparisons. The incremental changes in the post-meal plasma glucose and insulin concentrations between ethnic groups were assessed using repeated-measures ANOVA, with ethnic group set as between-subject factors and time as a within-subject factor. All analyses were carried out using the SPSS statistical analysis software version 19.0 (SPSS, Chicago, IL) and adjusted for age. A *P*-value of <0.05 was considered statistically significant.

3.4 Results

The mean (SE) for age and body mass index (BMI) was 25.9 (0.6) years and 21.3 (0.2) kg/m^2 respectively. Table 3-1 shows the baseline characteristics of study participants by ethnic groups. Chinese were significantly older than Malays and Asian-Indians (*P<0.05*), thus all subsequent analyses were age-adjusted. The BMI was not statistically different between the ethnic groups, but Asian-Indians had significantly higher waist circumference and percent body fat compared to Chinese and/or Malays. Fasting glucose concentrations were similar between the ethnic groups, but fasting insulin concentrations appeared higher among Asian-Indians compared to Chinese and Malays (*P>0.05*). Other metabolic parameters (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, AST and ALT) were not statistically different between ethnic groups.

Table 3-1 Baseline characteristics of study subjects by ethnicity

	Chinese (n=14)	**Malays (n= 21)**	**Asian-Indians (n= 24)**	***P-value (age adjusted)***
Age (years)	29.6 (1.7)[a,b]	25.2 (0.7)[a]	24.3 (0.7)[b]	-
BMI (kg/m^2)	21.5 (0.4)	20.9 (0.3)	21.6 (0.3)	*0.212*
Waist circumference (cm)	75.8 (1.1)[a]	73.9 (1.0)[b]	78.7 (0.9)[a,b]	***<0.001***
Percent body fat	19.4 (1.4)	17.5 (1.0)[a]	20.4 (1.0)[a]	***0.024***
Fasting glucose (mmol/L)	4.41 (0.09)	4.12 (0.10)	4.12 (0.07)	*0.382*
Fasting insulin (mU/L)	4.85 (0.65)	5.93 (0.90)	7.54 (0.92)	*0.103*
Total cholesterol (mmol/L)	5.10 (0.27)	4.60 (0.17)	4.43 (0.16)	*0.513*
LDL-cholesterol (mmol/L)	3.27 (0.24)	2.86 (0.15)	2.76 (0.12)	*0.650*
HDL-cholesterol (mmol/L)	1.34 (0.07)	1.34 (0.04)	1.33 (0.05)	*0.974*
Triglycerides (mmol/L)	1.08 (0.13)	0.86 (0.06)	0.76 (0.07)	*0.284*
Aspartate aminotransferase (U/L)	20.1 (1.5)	22.1 (1.3)	22.5 (1.0)	*0.158*
Alanine aminotransferase (U/L)	17.9 (2.1)	18.7 (1.4)	19.0 (1.1)	*0.190*

Data are presented as mean (SE). P-value for comparison among the ethnic groups adjusted for age. Variables with same superscript in each row are significantly different.

Table 3-2 Measures of insulin sensitivity and pancreatic beta-cell function

	Chinese (n=14)	**Malays (n= 21)**	**Asian-Indians (n= 24)**	***P-value (age adjusted)***
HOMA-IR	1.31 (0.08)[a]	1.40 (0.19)	2.00 (0.31)[a]	***0.017***
HOMA-B	133.0 (8.9)[a]	156.2 (21.0)[b]	284.6 (53.2)[a,b]	***0.008***
Euglycemic clamps derivative				
Insulin sensitivity index (ISI-clamp) per kg lean mass (mg/kg/min/mU/L)	14.2 (1.2)[a]	12.9 (1.2)[b]	8.8 (0.6)[a,b]	***0.001***
LMMTT derivatives				
Incremental area under curve – glucose (mmol•min/L)	157.0 (31.5)[a]	306.0 (29.5)[a]	224.6 (23.4)	***0.002***
Incremental area under curve – insulin (mU•min/L)	11138 (1272)	9963 (1316)	13061 (1282)	*0.188*
Insulinogenic index	83.3 (41.7)	34.6 (7.1)	51.9 (5.6)	*0.057*
Insulin secretion index (mU/mmol)	10.5 (1.2)	9.1 (1.2)	12.3 (1.1)	*0.136*
Matsuda insulin sensitivity index (ISI-Mat)	2.70 (0.49)[a,b]	1.69 (0.28)[a]	1.51 (0.16)[b]	***0.029***

Data are presented as mean (SE). Abbreviations: HOMA-IR, homeostatic model assessment – insulin resistance; HOMA-B homeostatic model assessment – beta-cell function; LMMTT, liquid mixed-meal tolerance test. P-value for comparison among the ethnic groups was adjusted for age or waist circumference. Variables with same superscripts in each row are significantly different

Figure 3-1 Incremental change in plasma glucose response following liquid mixed meal tolerance test between ethnic groups

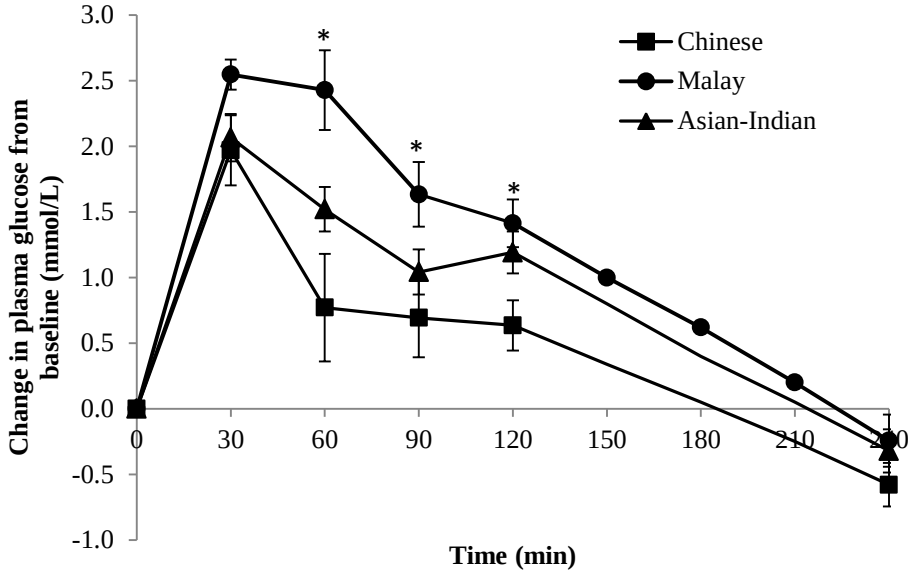

Figure 3-1. Post-meal plasma glucose response curves for Chinese (■), Malay (●) and Asian-Indians (▲) in response to liquid mixed meal. P-value for interaction between ethnic groups and post-meal glucose response = 0.011 (adjusted for age). There were significant differences in glucose response at 60 min between Chinese and Malay (*P<0.001*); 90 min between Chinese and Malay (*P=0.010*); 120 min between Chinese and Malay (*P=0.006*), Chinese and Asian-Indians (*P=0.039*).

Figure 3-2 Incremental change in plasma insulin response following liquid mixed meal tolerance test between ethnic groups

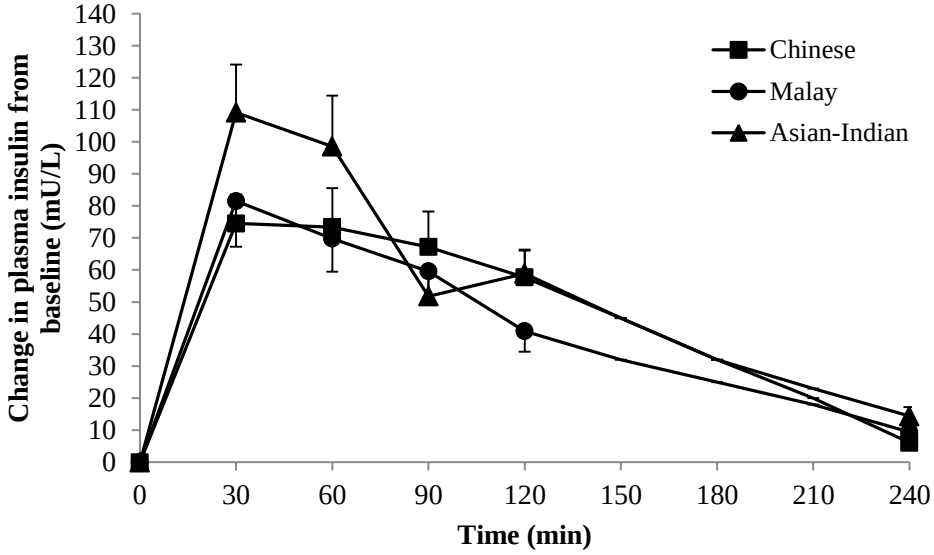

Figure 3-2. Post-meal plasma insulin response curves for Chinese (■), Malay (●) and Asian-Indians (▲) in response to liquid mixed meal. P-value for interaction between ethnic groups and post-meal glucose response = 0.164 (adjusted for age).

3.4.1 Glycemic and insulin response

The overall total and incremental AUC for glucose were greatest in Malays, followed by Asian-Indians and Chinese ($P<0.05$) (Table 3-2). Figure 3-1 shows the incremental change in plasma glucose concentration following the LMMTT. The post-meal glycemic excursion displayed a time by ethnic group interaction ($P_{interaction}=0.011$, adjusted for age).The plasma glucose concentrations were significantly higher in Malays compared with Chinese at 60 min and 90 min, and higher in Malays and Asian-Indians compared with Chinese at 120 min.

There were no statistical differences in total or incremental AUC for insulin between the ethnic groups (Table 3-2) although Malays appeared to have lower insulin response compared with Chinese or Asian-Indians. Figure 3-2 shows the incremental changes in the plasma insulin concentrations following the LMMTT. There was no significant difference in the post-meal plasma insulin responses between ethnic groups ($P_{interaction}= 0.164$).

3.4.2 Measures of insulin sensitivity

Asian-Indians were the least insulin sensitive compared with Chinese or Malays, based on ISI-clamp and HOMA-IR (Table 3-2). The trend for ISI-Mat was similar to ISI-clamp, being highest in Chinese and lowest in Asian-Indians. Similarly, adjusted ISI-clamp or ISI-Mat for waist circumference was lower in Asian-Indians compared with Chinese. Insulin sensitivity was no different between Chinese and Malays based on the euglycemic clamp, but was significantly lower in Malays compared with Chinese based on the LMMTT derivative.

3.4.3 Measures of pancreatic beta-cell function

In parallel with HOMA-IR, Asian-Indians showed a significantly higher HOMA-B compared with Chinese and Malays (Table 3-2), indicating a compensatory hyperinsulinemia in the presence of greater insulin resistance to maintain fasting normoglycemia. Chinese had higher insulinogenic index (marker of early-phase insulin response) than Malays or Asian-Indians, but Asian-Indians had higher insulin secretion index (total insulin response following the LMMTT) than Chinese or Malays. The ethnic differences in insulinogenic index and insulin secretion index however, did not reach statistical significance.

Figure 3-3 Mean (SE) oral disposition index derived from the liquid mixed meal tolerance test by ethnic groups.

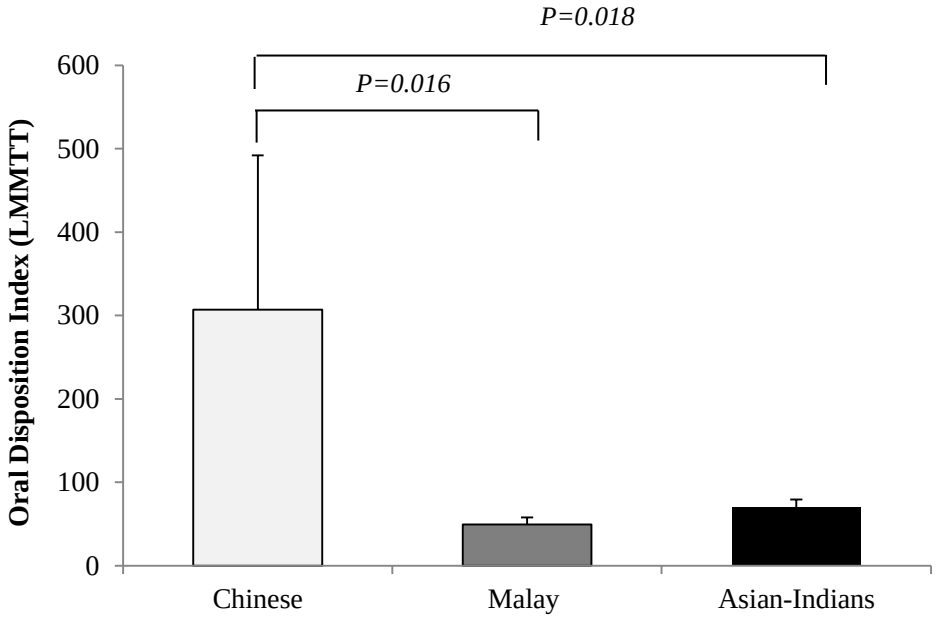

Figure 3-4 Relationship between insulin secretion index and Matsuda insulin sensitivity index

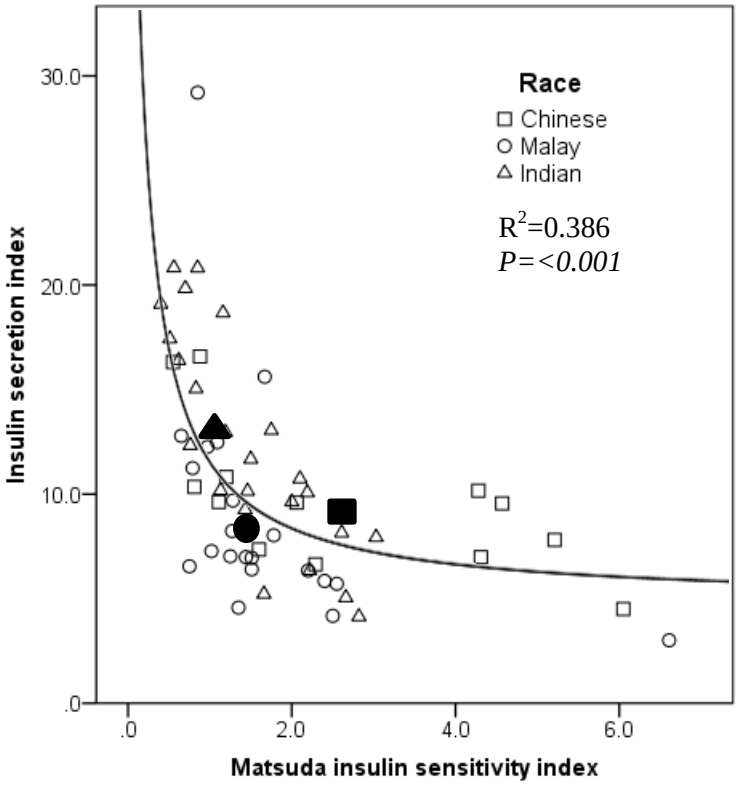

Figure 3-4. Scatterplots of insulin sensitivity (measured by euglycemic hyperinsulinemic clamp) vs insulin secretion index among Chinese (□), Malay (○) and Asian-Indians (△). Filled symbols represent the mean values for each ethnic group. The non-linear regression line is shown for the hyperbolic fit of the data.

3.4.4 Relationship between insulin sensitivity and pancreatic beta-cell function

The oral DI was significantly higher in Chinese compared with Malays or Asian-Indians (Figure 3-3). Figure 3-4 shows the hyperbolic relationship between the pancreatic beta-cell function (insulin secretion index) and insulin sensitivity (ISI-clamp). When we plotted the means of insulin secretion index and ISI-clamp by ethnicity, they were located at different points along the hyperbolic curve; Chinese and Asian-Indians along the hyperbolic DI curve indicating appropriate beta-cell

compensation for the prevailing insulin sensitivity, but Malays were found “falling off the DI curve”, indicating a lower beta-cell response relative to insulin sensitivity.

3.5 Discussion

In this study, we examined insulin sensitivity and beta-cell function between lean individuals of Chinese, Malay and Asian-Indians ethnicity. The results from this study add to the body of evidence that in addition to differences in insulin sensitivity, postprandial glucose response and beta-cell function differ between the Asian ethnic groups.

Our study showed that, among lean Asians, Malays exhibited higher postprandial glucose response but lower postprandial insulin response when compared with Asian-Indians or Chinese. The insulinogenic index, a marker for early phase insulin secretion, trended highest amongst the Chinese, followed by Asian-Indians and was the lowest amongst Malays. It has been suggested that a robust insulin response at the early phase after a meal is crucial in promoting hepatic glycogen storage and for the suppression of hepatic glucose production (Del Prato, 2003). A less robust insulin secretory capacity among the Malays might explain why this ethnic group displayed higher postprandial glucose excursion compared with Chinese or Asian-Indians.

The euglycemic-hyperinsulinemic clamp directly measures whole-body glucose disposal and is regarded the gold standard against which all other measures of insulin sensitivity must be compared with. Surrogate markers of insulin sensitivity such as HOMA-IR reflects more of hepatic insulin sensitivity since fasting glucose is determined by hepatic glucose production, which itself is primarily regulated by insulin. The ISI-Mat is an insulin sensitivity index derived from a dynamic physiologic meal challenge in order to understand glucose-insulin responses

(Matsuda and DeFronzo, 1999). Our findings from ISI-clamp, HOMA-IR and ISI-Mat supported previous results by showing that Asian-Indians were the least insulin sensitive, while Chinese were the most insulin sensitive (Khoo et al., 2014; Liew et al., 2003). In addition, Asian-Indians had higher total adiposity (in particular abdominal adiposity) compared with the other two Asian ethnic groups. An earlier study reported that among Japanese-American men who developed T2DM, insulin insensitivity, increased insulin secretion and increased intra-abdominal fat were already present before the onset of glucose intolerance (Bergstrom et al., 1990). This may help to explain why Asian-Indians were more prone to developing T2DM and other cardio-metabolic diseases at a lower body mass index and at younger age (Ramachandran et al., 2004).

Several studies reported that decreased insulin sensitivity and defective insulin secretion precedes the onset of dysglycemia (Bi et al., 2012; Chiasson and Rabasa-Lhoret, 2004). As long as the insulin sensitivity is matched by insulin secretion, normoglycemia is preserved, and a mismatch between these two parameters will therefore results in dysglycemia. This close relationship between insulin sensitivity and insulin secretion follows a hyperbolic curve, and the multiplication product of these two parameters is known as the disposition index (DI) (Kahn et al., 1993). The disposition index (DI) therefore reflects beta-cell function relative to the prevailing insulin sensitivity (Cobelli et al., 2007) and predicts incident diabetes beyond fasting and 2-hour glucose levels (Brouns et al., 2005; FAO/WHO, 1998). In prospective epidemiology studies, individuals who progressed from normoglycemia to T2DM exhibit the "falling off the DI curve" phenomenon, indicating a failure of insulin secretion to compensate for the degree of insulin resistance (Kahn, 2003).

In this study, we showed that the Chinese has the highest oral disposition index (DI) compared with Malays or Asian-Indians. Malays exhibited lower beta-cell

function (“falling off the curve”) compared with Chinese despite having similar insulin sensitivity. This suggests that Malays might have inadequate beta-cell secretory ability to compensate for their prevailing insulin sensitivity. We have further ascertained whether family history of diabetes might explain the lower beta-cell function amongst the Malays. The prevalence of first-degree relatives with diabetes mellitus in our study participants was 28.6% among Chinese, 19.0% among Malays and 16.7% among Asian-Indians (data not shown), indicating that family history of diabetes does not explain why Malays have lower beta-cell function compared with Chinese. Several candidate gene regions for T2DM have been discovered by genome-wide association studies and confirmed in various populations worldwide (Saxena et al., 2012; Sun et al., 2012). These candidate gene regions are likely to influence beta-cell function. One of them is KCNQ1 gene polymorphism, in particular a risk allele r2283228 has been associated with a 1.7 times higher odds of developing diabetes mellitus among Malaysia Malays (Saif-Ali et al., 2011). Whether genetic polymorphism in the KCNQ1 gene or other diabetes candidate genes may account for the ethnic differences in beta-cell function warrants further investigations.

To our knowledge, there was no prior publication that compared beta-cell function in relation to insulin sensitivity between Malay ethnicity and other ethnic groups. The reason why Malays have lower beta-cell function compared with Chinese despite similar insulin sensitivity is not clear. The finding of low beta-cell function among Malays has important implications. Based on the National Health Survey in Singapore, the age-standardized prevalence rate of obesity is the highest among Malays and has increased from 19.1% in 2004 to 24.0% in 2010 (Ministry of Health Singapore, 2010a). Countries like Indonesia and Malaysia, where the majority population consists of Malay origin, have seen a rapid increase in the prevalence of obesity (Khambalia and Seen, 2010; Roemling and Qaim, 2012). It is well established that insulin insensitivity is higher with greater obesity. Thus, with a background of

compromised beta-cell function, it is probable that this select [Malay] population will see a greater rise in the prevalence of impaired glucose tolerance and diabetes mellitus with increasing obesity. A recent population forecast using an individual-level simulation model, based on Markov chain Monte Carlo methods demonstrated that the rising prevalence of obesity will double the prevalence of diabetes mellitus among Singaporeans by 2050, with Malays and Asian-Indians being the most affected (Phan et al., 2014).

Our study has several strengths. We studied three major ethnic groups (Chinese, Malays and Asian-Indians) that represented the majority of ethnic groups living in Asia. This is a region where the prevalence of T2DM and cardiovascular disease are projected to increase over the next several decades (Yusuf et al., 2001). Our subjects were young, healthy and lean males, which allow us to determine the metabolic responses following a liquid mixed meal challenge prior to the onset of chronic diseases. We have used the gold standard for measurement of insulin sensitivity using the euglycemic-hyperinsulinemic clamp technique (DeFronzo et al., 1979; Goodarzi et al., 2011; Wallace and Matthews, 2002). We also carried out the liquid mixed meal challenge to investigate insulin secretory capacity instead of using an oral glucose tolerance test. As a mixed-nutrient load, the liquid mixed meal provides a more physiologic stimulus for assessing glucose and insulin homeostasis (Brodovicz et al., 2011; Caumo et al., 2000; Maki et al., 2010; Steil et al., 2004). Furthermore, the liquid mixed meal does not require chewing, thus it will not be a potential confounding factor in the post-meal glucose or insulin response (Ranawana et al., 2010a).

There are also limitations in this study. The number of subjects in this study was small and the difference in numbers among the different ethnic groups could have affected the resulted obtained. Thus, an additional validation using a larger sample size is needed to confirm these results. By using a liquid mixed-meal, we are

cognizant that differences in the nutrient absorption may contribute to the ethnic differences in the post-meal glucose response. Nonetheless, all of our subjects did not have any history of malabsorption nor was there evidence of lactose intolerance, thus we believe that differences in nutrient absorption play a minimal role in our study findings. Future studies should look into differences in nutrient absorption and the role of stool metagenomics in mediating the ethnic differences in postprandial glucose and insulin response. We did not measure incretin responses, which might contribute to the differences in the post-meal insulin response. However, one study showed that the higher early insulin response in African Americans compared with European Americans was not due to differences in circulating incretin concentrations (Higgins et al., 2008). Interpretation of insulin secretion and insulin sensitivity must also take into account hepatic insulin extraction and hepatic insulin sensitivity, both of which were not examined in this study. For future study, it would be useful to include females, taking into account the variation insulin resistance that occurs in different phases of the menstrual cycle.

In summary, we have shown that, in addition to ethnic differences in insulin sensitivity, lean Chinese, Malay and Asian-Indians also exhibit differences in beta-cell function. While lean Chinese and Asian-Indians show appropriate beta-cell function in relation to insulin sensitivity, lean Malays exhibit lower beta-cell function relative to their prevailing insulin sensitivity. With this background, Malays may face a rapid increase in the incidence of diabetes with rising prevalence of obesity, and that measures to maintain healthy body weight would be a key strategy to mitigate the development of T2DM in this population.

3.6 Acknowledgement

The candidate was responsible for data cleaning, statistical data analyses, data interpretation and wrote the manuscript. The manuscript is provisionally accepted with revision by Nutrition and Diabetes journal (Jun 2015).

CHAPTER 4a Physiological Digestive Factors and Their Influence on Glycemic and Insulin Response – A Literature Review

4a.1 Introduction

There is extensive evidence that biological digestive factors vary between individuals (Lassauzay et al., 2000; Mandel et al., 2010; Ranawana et al., 2011; Schwartz et al., 1995) and influence postprandial glycemic response (GR) and insulin response (IR) (Ranawana et al., 2011). Higher glycemic excursions and insulin response play a direct pathogenic role in the development of Type 2 Diabetes Mellitus (T2DM) (Bhupathiraju et al., 2014; Ludwig, 2002; Pawlak et al., 2004; Riccardi et al., 2008) and cardiovascular disease (Brand-Miller, 2003). However, no study to date has measured and compared physiological parameters such as mastication, salivary α-amylase activity (*AMY1* gene) and gastric emptying rate in a multi-ethnic Asian population, with varying prevalence of obesity and T2DM (Ministry of Health Singapore, 2010a).

This study was designed to investigate inter-individual differences in GR and IR and examine how physiological digestive factors contribute to inter-individual variability in GR and IR. Specifically, we also aimed to evaluate the influence of ethnicity contributing to the inter-individual variations. Through the understanding of the impact and role of ethnicity and inter-individual differences in physiological digestive factors on GR and IR, the study findings can potentially explain, in part, the varying susceptibility to obesity and T2DM between Chinese, Malays and Asian-Indians.

This chapter is divided into 5 sub-chapters. A review of the literature is described below, followed by a chapter on Methods (Chapter 4b) employed in this study. Subsequent chapters describe applicability of GI values of rice among Asian ethnic groups (Chapter 4c), a comparative study of *AMY1* gene copy numbers derived from blood, buccal swabs and saliva samples (Chapter 4d), and digestive determinants of glycemic response (Chapter 4e).

4a.2 Glycemic and insulin response

The postprandial glycemic response (GR) and the corresponding insulin response (IR) to carbohydrate foods are usually evaluated as the increase in the blood glucose or insulin response during a period of 2 h after the ingestion of a specific amount of carbohydrate food (Dickinson et al., 2002; Monro and Shaw, 2008).The postprandial blood glucose concentration is determined by the amount as well as the physical properties of the carbohydrate food such as the amylose to amylopectin ratio, degree of processing, presence of soluble dietary fibre (Foster-Powell et al., 2002), presence of minor constituents such as organic acids, phytates and polyphenols, cooking method used, as well as the co-ingestion of fat (Gentilcore et al., 2006) and protein (Gannon et al., 1993). These have all been extensively shown to influence the postprandial GR and insulin demand to carbohydrates.

Besides the digestibility of carbohydrates affecting blood glucose response, the postprandial GR has shown to vary considerably between individuals. A consistent observation in many studies is the wide within- and between-individual variations in GR and IR (Ranawana et al., 2011; Vega-Lopez et al., 2007; Wolever et al., 2008), with inter-individual differences being the greatest contributing factor to the variability. To our knowledge, there are no studies to date that have investigated biological factors contributing to inter-individual variability in GR and IR. We

therefore aim to focus on ethnicity, mastication, particle size distribution, salivary amylase activity (*AMY1* gene) and gastric emptying that may contribute to the variations in GR and IR among individuals. We hypothesize that differences in ethnicity, mastication, salivary amylase activity and gastric emptying modulates the variability of postprandial GR and IR.

4a.3 Ethnicity

The influence of ethnicity in GR has been reported extensively showing that Asians display a larger GR compared with Caucasians (Dickinson et al., 2002; Henry et al., 2008; Kataoka et al., 2013; Venn et al., 2010). In Singapore, the differing prevalence of obesity and T2DM among the three main ethnic groups indicates possibility of ethnic differences in susceptibility. Nevertheless, no studies to date have compared GR and examine the various physiological factors amongst the ethnic groups.

4a.4 Mastication and particle size of food

Mastication or chewing is the first step in the digestion process. The major physiological function of mastication is the mechanical disruption of food into small particles suitable for gastrointestinal absorption of nutrients (Pedersen et al., 2002). The extent of mastication may be influenced not only by innate habits, but also by the consistency, texture and taste of the food. Numerous studies have described a large variation between individuals in terms of the number of chews per mouthful and chewing time per mouthful (Lassauzay et al., 2000; Woda et al., 2006). Recent evidence has shown that mastication plays a crucial role in determining the postprandial GR (Ranawana et al., 2010; Suzuki et al., 2005). Healthy subjects who chewed more elicited a greater GR compared with those who chewed less, indicating

that the resultant particle size is an important factor contributing to the inter-individual variation in GR observed (Ranawana et al., 2010a). However, this observation holds true only for rice, which consist of intact grains, but not for spaghetti (highly milled starchy foods) in the study. In another study, thorough mastication elicited a *lower* postprandial plasma glucose concentration because of the potentiation of early-phase insulin secretion (Suzuki et al., 2005). These opposing findings underscore the need for further studies to elucidate physiological explanations by which mastication may be influencing the GR.

The particle size of food influences starch digestibility. Numerous studies demonstrated that food particle size was inversely associated with the rate of starch digestion rate (Bjorck et al., 1994; Ranawana et al., 2010a; Ranawana et al., 2010b). The smaller the particle size, the greater the surface area exposed to digestive enzymes, leading to an increased rate of digestion and consequently a higher glycemic response. In grains that are consumed whole, such as rice, physical breakdown of the grain is solely dependent on mastication (Ranawana et al., 2010b). A bolus of chewed grain typically contains a range of particle sizes, from intact grains to masticated grains that have been chewed, crushed and sheared between molar surfaces (Hoebler et al., 1998; Ranawana et al., 2010b). As dentition, chewing rate and eating behavior differ between individuals, it is likely that there are inter-individual differences in the degree of particle size reduction of whole grains consumed (Woda et al., 2006).

If the degree of habitual mastication have been reported to vary significantly between individuals and particle size reduction during normal mastication differs from person to person, such differences in oral processing parameters may in part, contribute to individual variability in glycemic response to a single food. Although previous studies have reported a possible association of mastication and particle size on GR, the effect of ethnicity has not been investigated. We hypothesized that those

who broke down rice to relatively smaller particles during mastication would demonstrate a greater rate of digestion and this in turn would influence the magnitude and pattern of the GR. This study provides useful information on a previously unexplored aspect that may potentially contribute to the inter-individual variation in GR to a single food, while attempting to demonstrate the practical importance of mastication in glycemia.

4a.5 Salivary α-amylase activity and *AMY1* gene

Saliva is an important lubricant secreted by the parotid, submandibular and sublingual glands in the mouth and play a vital role in mastication and swallowing (Lingstrom and Moynihan, 2003). Until recently, it was not recognized that salivary amylase added much to the digestion of carbohydrate foods as food is passed through the oral cavity quickly and the majority of carbohydrate digestion occurs in the small intestine via pancreatic amylase (Mandel and Breslin, 2012). However, earlier studies have alluded that salivary amylase may be of clinical importance. In the study by Read et al. (1986), postprandial blood glucose concentrations following ingestion of carbohydrate-rich foods (rice and potatoes), were higher if foods were chewed first, mixed with saliva and then swallowed rather than swallowed whole (Read et al., 1986). This finding highlighted the importance of salivary amylase in the initial starch hydrolysis process in the oral cavity (Hoebler et al., 1998). More recently, the presence of high concentrations of the enzyme α-amylase demonstrate that saliva could be important for the digestion of carbohydrates as the chewing of food and the production of saliva is the first step in the digestion process (Butterworth et al., 2011; Mandel et al., 2010).

In terms of inter-individual variability, the study by Mandel et al. (2010) demonstrated that the quantity and enzymatic activity of salivary amylase vary

considerably among individuals. Genetically, salivary amylase levels are influenced by individual copy number variation (CNV) of the *AMY1* gene on chromosome 1p21, which codes for salivary amylase (Bank et al., 1992). An individual can carry between 2 to 15 diploid copies of the *AMY1* gene in their genome (Mandel et al., 2010). In contrast, pancreatic amylase, produced by the gene *AMY2*, has not shown the copy number variability as *AMY1* does (Groot et al., 1989), suggesting that *AMY1* may be of greater significance to starch metabolism. Recent evidence suggests that populations who consume a traditional high-starch diet have higher copy numbers of the *AMY1* gene, with correspondingly higher concentrations of salivary amylase, than populations who consumed a low-starch (high protein) diet (Perry et al., 2007). This is suggestive of a nutritional evolution in response to the dramatic increase in the availability of carbohydrate foods in many populations since the agricultural revolution. The presence of highly refined processed carbohydrates have increased the number of *AMY1* copies (higher concentrations of salivary amylase) in selected populations to facilitate the digestion and metabolism of carbohydrates and influences the postprandial GR and IR (Mandel and Breslin, 2012; Perry et al., 2007). To our understanding, no studies have examined salivary α-amylase activity as well as *AMY1* gene copy numbers between ethnic groups in an Asian population with different prevalence of T2DM. From anthropological and ecological data (Noor, 2002; Norimah, 2008), it is known that the Chinese and Indian immigrants who came to Singapore 3 to 5 generations ago from populations in China and India have agriculturally-based diets and would generally consume a high carbohydrate, lower protein diet. On the other hand, the indigenous inhabitants of the Malay Archipelago would have relied primarily on fishing and hunting, and would have a diet containing a higher proportion of protein than carbohydrate. From this, we postulate that the Chinese and Asian Indians have higher numbers of *AMY1* gene and thus higher concentrations of salivary amylase compared to the Malays. How this relates to postprandial GR and IR still remains unclear. We thus aim to examine both *AMY1*

gene copy numbers and salivary amylase concentration among the three ethnicities and investigate their relationships with postprandial GR and IR.

4a.6 Gastric emptying

Gastric emptying is a physiological response to the presence of food and is a major determinant of postprandial glycemia (Berry et al., 2003; Horowitz et al., 1993; Rayner et al., 2001; Schwartz et al., 1995) and insulinemia (Phillips, 2006). This positive association between gastric emptying rates and postprandial GR and IR has implications for disease risk. Excessive glycemia and/or insulin demand on a chronic basis may eventually affect insulin secretory capacity leading to the development of T2DM (Dickinson et al., 2002).

Few gastric emptying studies have been performed in ethnic populations that are at high risk of developing diabetes. In healthy populations, existing evidence suggest that the gastric emptying of nutrients varies between ethnic groups and positively correlates with plasma glucose and serum insulin levels (Schwartz et al., 1995). To strengthen the hypothesis that gastric emptying rates vary between individuals, a recent study has shown significant between-individual variations in gastric emptying when all food-associated factors including ingested particle size were standardized (Ranawana et al., 2011). A faster emptying of food particles from the stomach would deliver a greater carbohydrate load into the duodenum and produce an augmented GR and IR. Data from this study suggest that the magnitude and pattern of the inter-individual variability in GR and IR may be mediated by differences in gastric emptying. To our knowledge, there are no studies that have examined the gastric emptying rates in a multi-ethnic Asian population. The positive correlations between the rate of gastric emptying with postprandial GR clearly demonstrates the role of gastric emptying in postprandial glucose homeostasis. We

aimed to investigate the gastric emptying rates between individuals of different ethnicities and to examine the relationship with postprandial GR and IR.

4a.7 Conclusion

It is evident from existing literature that even within the Asian population, GR and IR vary among ethnic groups. We therefore aim to conduct a detailed study to evaluate differences in postprandial GR and IR as well as variation in GI values among the three main ethnic groups in Singapore. Collectively, we aim to determine and strengthen current evidence on the inter-individual variability in digestive function as well as to investigate ethnic differences in our local population. From this study, we hope to determine whether physiological differences in digestive function can explain for the inter-individual variability in postprandial GR and IR and ultimately play a crucial role in the differing prevalence of obesity and diabetes in Singapore. One of the strengths of this study is that to date, no studies have compared individuals of non-Caucasian ethnicities, residing in and sharing a common environment as well as the detailed profiling and phenotyping of biological factors contributing to postprandial GR and IR in a single study.

Our study was uniquely designed to investigate the relationship between ethnicity and physiological factors (mastication, particle size distribution, salivary amylase activity, *AMY1* gene copy numbers and gastric emptying) contributing to inter-individual variability in GR and IR. Findings from this study will provide deeper insights into how biological variations between individuals can explain for the varying prevalence of T2DM and obesity among the three main ethnic groups in Singapore, which can be translated into better health outcomes through preventative health measures, improve quality of life and reduce the chronic disease burden for the country.

CHAPTER 4b Physiological Digestive Factors and Their Influence on Glycemic and Insulin response – Methods

4b.1 METHODS

4b.1.1 Subjects

Seventy-five healthy male participants, consisting of 25 Chinese, 25 Malays and 25 Asian-Indians were recruited for the study. Those who fulfilled all acceptable criteria (BMI 18.5 to <25 kg/m^2; age 18-45 years; both parents and grandparents were of the same ethnicity, blood pressure 110-120 / 70-90 mmHg; fasting blood glucose 4-6 mmol/L; not on prescription medication; non-smoking; no genetic or metabolic diseases) were included in the study and written informed consent was obtained.

During the screening visit, anthropometric measurements were taken and a health questionnaire (relating to medical history, smoking habits, history of any illness and use of any medications) was administered. All anthropometric measurements were made least 2 hours after food. Height and weight were measured without shoes by using a digital scale cum stadiometer (SECA 763, Germany). A digital blood pressure monitor (Omron HEM-907, Japan) was used to obtain blood pressure readings. Waist circumference was measured using a standard measuring tape (SECA tape measure, Germany) at the midpoint between the coastal margins of the ribs and the upper margin of iliac crest and the hip circumference at the widest level of the greater trochanters on both sides. Skin-fold measurements (biceps, triceps, sub-scapular and suprailiac) were taken, measured to the nearest 0.2mm in triplicates using a Holtain caliper (Holtain Ltd, Crymch, UK). Three measurements

were taken at each body site and the average measurement at each site was used for the computation of body fat percentage. Percentage body fat was also measured using air displacement plethysmography method (Bod Pod, Cosmed, USA).

On the day before the test visit, participants were reminded to avoid intake of alcohol, restrict caffeine-containing drinks, refrain from intense strenuous physical activity and to consume their usual meals. Participants were requested to fast overnight for 10-12 hours before each test visit.

This study was approved by the National Healthcare Group Domain Specific Review Board, Singapore. The study was registered at clinicaltrials.gov as NCT01804738 and all participants provided written informed consent prior to their participation in the study.

4b.1.2 Study procedures

A randomized, crossover, within-subjects, repeated-measure non-blind design was adopted. Participants arrived at the research centre between 8:30-9 am after 10-12 hours overnight fast. Following a 10 min rest, two fasting blood samples were obtained 5 min apart for baseline blood glucose and insulin levels. After which they were given either the reference food or test food with water to consume at a comfortable pace within 15 min. The reference food consisted of 50 g of glucose anhydrous powder dissolved in 250 ml water while the test foods were either Jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand) or Basmati rice (Dreamrice™, Singapore). These two rice varieties were chosen as they were commonly consumed by the local population. Jasmine rice was tested locally to be classified as high GI (Sun et al., 2014) while Basmati rice was tested in a local laboratory and certified low GI on its product packaging. Both rice samples were served in portions containing 50 g of available carbohydrates (difference between

total carbohydrates and dietary fibre). This amounted to 63.6 g (with 110 ml water) and 66.5 g (with 170 ml water) of uncooked Jasmine rice and Basmati rice respectively, cooked in individual portions in a rice cooker (Iona GL12 rice cooker, Singapore) and served with 250 ml of water. In total, the reference food were tested thrice and the test food tested once by each participant in a randomised order. This is in accordance with FAO/WHO recommendations (FAO/WHO, 1998) for the determination of GI value that each subject will test each test food once and the reference food three times in random order on separate days, with at least 2 days gap between measurements to minimise carry-over effects. During the entire study visit, the participants remain rested and in the research centre.

4b.1.3 Measurement of mastication rate

During rice consumption, mastication rate was measured using surface electrode electromyography, a method that was adopted from previous studies (Ranawana et al., 2010; Woda et al., 2006). Electromyograms were obtained using bipolar surface electrodes that were attached lengthwise along the left and right masseter muscles (Brown, 1994; Mioche and Martin, 1998; Ranawana et al., 2010a). The muscles were identified by palpating the area while the participants clenched their jaws. An additional earth electrode was placed on the right wrist. The electrodes were attached to a programmable data acquisition unit (DataLOG model P3X8; Biometrics Ltd, UK). Participants were asked to consume mouthfuls of the test food as they normally would. The number of mouthfuls to consume the test food, the number of chews per mouthful, and the time taken for each mouthful were quantified for each test food.

4b.1.4 Blood sampling

After the commencement of eating, further blood samples were taken at 15, 30, 45, 60, 90 and 120 min. The protocol used to measure blood glucose response was adopted from that described by Brouns et al (Brouns et al., 2005) and is in line with the FAO/WHO recommendations (FAO/WHO, 1998). Blood was obtained by finger-prick using sterile, single-use lancing device (Accu-check Safe-T-Pro Plus, Roche Diagnostics). Before a finger-prick, participants were encouraged to warm their hand to increase blood flow. To minimize plasma dilution, fingertips were not squeezed to extract blood bit were instead gently massaged starting from the base of the hand moving towards the tips. The first 2 drops of expressed blood were discarded, and the next drop was used for testing. Blood glucose was measured using a glucose dehydrogenase method (HemoCue 201 RT).

For the measurement of blood insulin levels at each time point, after blood glucose measurement, 300 μL of capillary blood (obtained from finger pricks) was collected into Microvette® capillary blood collection tubes treated with di Potassium EDTA (CB 300 K2E, Sarstedt) and stored in crushed ice immediately until the end of the study visit. The Microvette® tubes were centrifuged at 1000 x *g* for 12 min (Hettich Rotina 420R, UK), and the supernatant plasma transferred into individually labeled 1.5 ml microtubes (Axygen Scientific, Inc.), which were immediately transferred to a freezer held at −80°C until analysis of insulin. Plasma insulin concentrations were determined by electrochemiluminescence immunoassay using an automated analyzer (Cobas E411; Roche Diagnostics, Burgess Hill, UK). The Cobas system is a reliable method of blood insulin determination (Ranawana et al., 2011). The mean inter-assay CV was 1.13% and the mean intra-assay CV was 1.08%.

4b.1.5 Collection of buccal cells

For the collection of buccal cells, participants were asked to thoroughly rinse out their mouth twice with water in the fasted state. Cheek cells were collected using sterile foam-tip buccal cell collection swabs (Catch-All™ Sample Collection Swab, Epicentre, Madison, WI, USA), by rolling the collection swab firmly on the inside of the cheek, approximately 20 times on each side, making certain to move the swab over the entire cheek. The swab samples were air dried for 30 min at room temperature and then stored in original packaging at -80°C before analysis of *AMY1* gene copy numbers.

4b.1.6 Collection of saliva samples

Baseline saliva was collected in the fasted state and immediately after each test food has been consumed for the measurement of salivary amylase activity. In preparation for passive saliva collection, participants were asked to empty their mouths by swallowing all saliva and to rinse their mouth with water. They were then instructed to place a pre-weighed cotton swab (Salivette®, Sarstedt, Germany) in their mouth for 2 min to absorb passive saliva. The saturated cotton swab was re-weighed and then stored at -80°C until further analysis of salivary amylase activity. Upon thawing, the Salivette® tube was centrifuged for 10 min at 1500 g to yield clear saliva sample.

For a sub-set of 13 participants, additional saliva samples were collected for the comparative study of *AMY1* gene copy numbers between blood, buccal swab and saliva. In preparation for saliva collection, participants were instructed to empty their mouths by swallowing all saliva and to rinse their mouth with water. Whole saliva was collected using Oragene® DNA self-collection kit (DNA Genotek, Ottawa, ON, Canada). Participants were asked to deposit approximately 2 ml saliva through the

funnel into the collection tube until the amount of liquid saliva (excluding bubbles) reaches the fill line. When an adequate sample was collected, the funnel lid was then closed firmly. The collection tube was designed so that stabilizing liquid that is attached to the lid, mixes with the saliva when the funnel cap is securely fastened. This starts the initial phase of DNA isolation and stabilizes the saliva sample for long-term storage (DNA Genotek, 2011). Saliva samples were stored in a 4^{o}C refrigerator.

4b.1.7 Gastric emptying rate

Gastric emptying rate was estimated by using an ultrasound method described previously (Darwiche et al., 1999; Darwiche et al., 2003). Ultrasonographic measurements (LOGIQ P5, GE Healthcare, Korea) was performed at 0, 15, 30, 45, 60 and 90 min. Figures 4b-1 and 4b-2 show the ultrasound photos of the gastric antrum of the same participant at fasting and 15 min after rice consumption. The abdominal aorta and the left lobe of the liver were used as internal landmarks in each measurement of the gastric antrum to obtain the same standardized scanning level consistency. The participants were examined in the supine position, but were allowed to sit up between examinations. The degree of gastric emptying was expressed as the percentage change in the antral cross-sectional area between measurements taken at 15 and 90 min. At each examination, the longitudinal and antero-posterior diameters (D1 and D2 respectively) were measured thrice and the mean values were used to calculate the cross-sectional area of the gastric antrum (A_{Antrum}) as follows:

$$A_{Antrum} = \pi \times D_{1mean} \times D_{2mean} / 4$$

Gastric emptying rate (GER) was expressed as the percentage reduction in antral cross-sectional area from 15 to 90 min, calculated as follows:

$$GER = [(A_{Antrum90min} / A_{Antrum15min}) - 1] \times 100$$

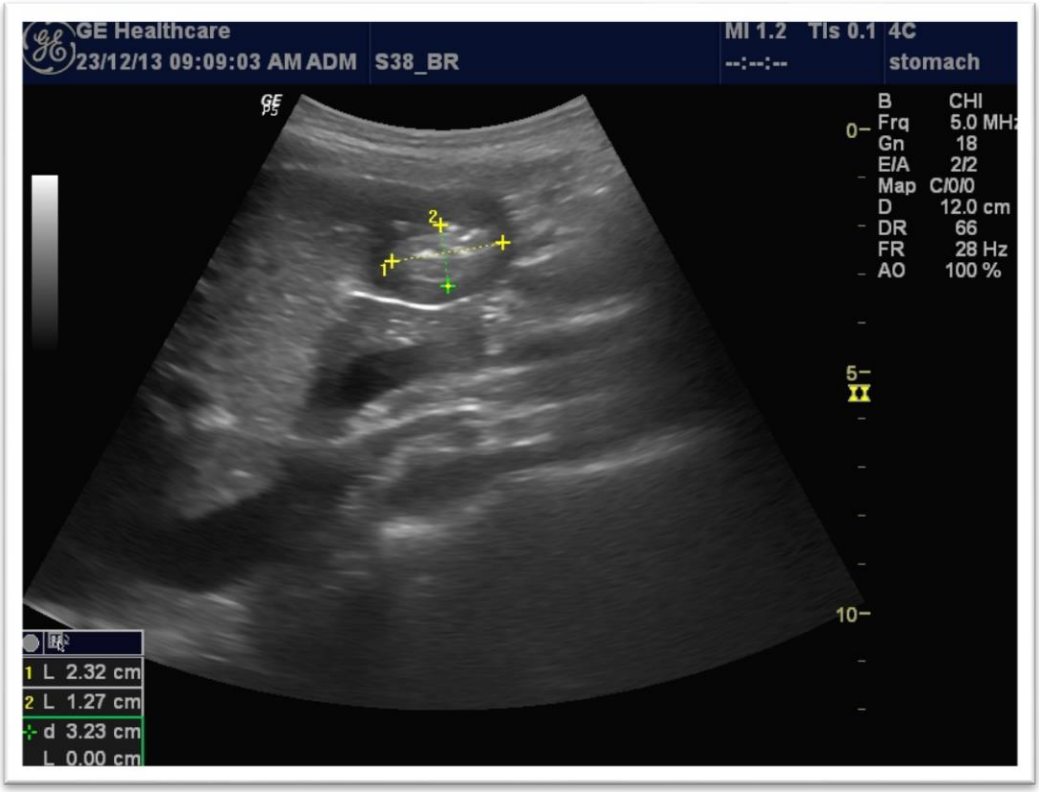

Figure 4b-1 Ultrasound photo of gastric antrum at fasting

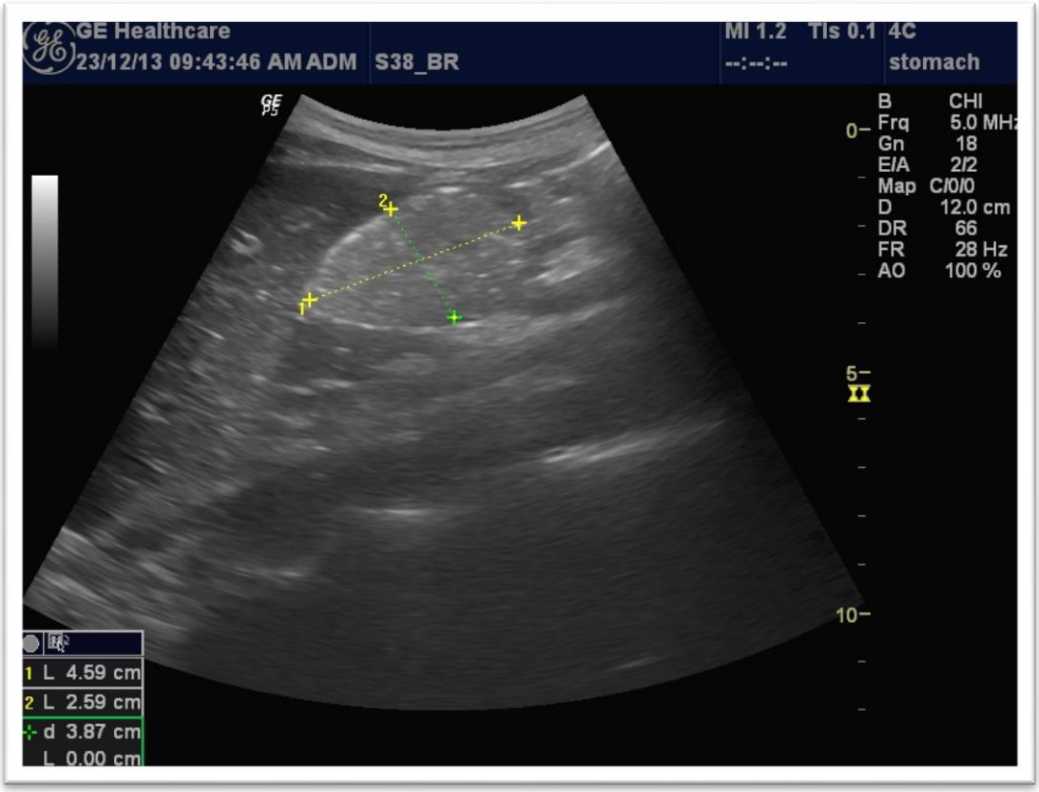

Figure 4b-2 Ultrasound photo of the gastric antrum 15 min after rice consumption

4b.1.8 Particle size distribution of masticated rice

At the end of the study visit where test food was provided, the participants were given a 100 g portion of the same cooked rice to determine the particle size distribution of masticated rice samples. The participants had to chew each mouthful to the point at which they felt the need to swallow. However, instead of swallowing, they would expectorate the contents into the preweighed plastic container provided. To maximize recovery of all chewed rice, they were provided with 150 ml of water to wash out their mouth between mouthfuls into the plastic container as well. All participants were advised not to swallow any of the rice as far as possible.

Particle size analysis was carried out within 10-15 min of obtaining the samples from the subjects. The samples were washed through a set of 3 sieves of different aperture sizes (mesh sizes: 2000 μm, 1000 μm, and 500 μm) (Endecotts Ltd, London, UK) under running water until the eluting liquid was free of rice particles. The retained particles on each sieve were transferred onto labelled pre-weighed aluminum foils and dried in an oven at 105°C overnight, which permitted the total evaporation of water to achieve a constant weight. The dried rice particles collected on each sieve was calculated as a percentage of the total sample weight (derived from dry weight of non-masticated rice sample). The dry weights of non-masticated rice samples was determined using 100g portions of cooked rice, dried in an oven at 105^{o}C overnight until a constant weight for each sample was achieved. Each rice variety was measured in triplicate. The mean (SD) dry weight of Jasmine rice was 39.31 (0.4) g with a moisture loss of 60.69% and the mean (SD) dry weight of Basmati rice was 34.89 (0.1) g with a moisture loss of 65.11%. Particle sizes calculated were greater than 2000 μm, less than 2000 μm to greater than 1000 μm, less than 1000 μm to 500 μm, and less than 500 μm (calculated by difference of weights).

4b.1.9 Salivary α-amylase activity

The α-amylase activity was measured using the Phadebas® Amylase test (Magle AB, Lund, Sweden) following the manufacturer's instruction. The saliva samples were first thawed to room temperature and then centrifuged (Hettich Rotina 420R, UK) for 10 min. Each saliva sample was diluted serially to a final solution 1:1000 using a buffer: 0.9 % NaCl (Merck), 0.2 % Bovine Serum Albumin (Sigma-Aldrich), 20 mM $CaCl_2$ (Merck). Briefly, 200 µl of diluted saliva and 4 ml deionized water were mixed and made in duplicate. 4.2 ml deionized water acted as reagent blank. All samples were pre-incubated in 10-12 ml centrifuge tubes at 37^{o}C for 5 min in a shaking water bath (WiseBath WSB, Korea). A Phadebas® Amylase test tablet was added to each tube and immediately vortexed for 10 seconds. All the tubes were replaced in the water bath for 15 min exactly in 37^{o}C +/- 0.5^{o}C. The reactions were stopped by adding 1 ml of 0.5 M NaOH and immediately vortexed. The test tubes were centrifuged at 1500 g for 5 min (Hettich Rotina 420R, UK). The supernatants were pipetted into cuvettes with 1 cm light path and the absorbance was measured at 620 nm with a Shimadzu UV-2600 spectrophotometer. The measured values were compared with a standard curve with the same batch number as the tablet container, which directly gave the α-amylase activity. The activity value was multiplied with the dilution factor for correct result. The differences in activity in the measured samples were obvious for a naked eye. The samples with highest amount of salivary α-amylase release more dye and are visualised as darker blue colour compared to the samples with lower amount of salivary α-amylase (Figure 4b.3).

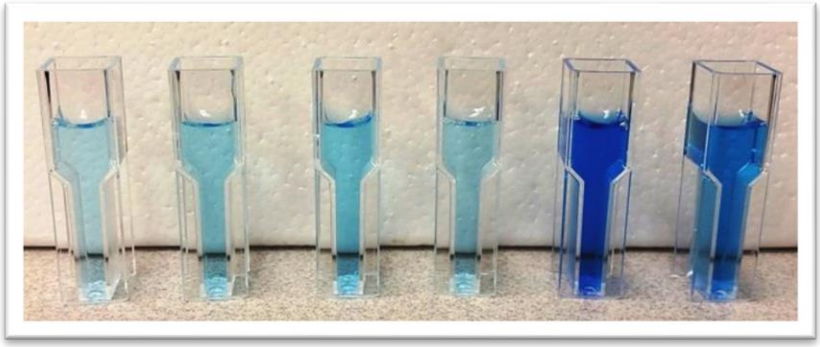

Figure 4b-3 Comparison of degree of colour between samples of varying salivary amylase activity levels

4b.1.10 Quantification of AMY1 gene copy numbers

DNA extraction

In order to determine diploid *AMY1* gene copy number, DNA was extracted from whole blood using QIAamp® DNA Mini Kit (Qiagen, Netherlands) as described by the manufacturer (Appendix 1). Briefly, approximately 200 µl whole blood sample was transferred to a 1.5 ml microcentrifuge tube containing 20 µl proteinase K. 200 µl Buffer AL was added and the sample was mixed by vortexing. The sample was incubated at 56^{o}C for 10 min. Then, 200 µl 100% ethanol was added and the sample was mixed. The resultant solution was added to a spin column, centrifuged, washed, and eluted as described by the manufacturer. The DNA was quantified and stored at -20^{o}C until qPCR analysis.

For buccal cells, DNA was also extracted using QIAamp® DNA Mini Kit (Qiagen, Netherlands), modified for the extraction of DNA from buccal swabs. Briefly, 600 µl PBS was added to a 2 ml microcentrifuge tube. The swab was placed in the tube and rotated repeatedly to dissolve the DNA on the buccal swab. 20 µl of proteinase K and 600 µl Buffer AL were then added. The sample was mixed by vortexing and incubated at 56^{o}C for 10 min. Thereafter, 600 µl of 100% ethanol was

added and the sample mixed. The swab was removed from the tube using a forceps. The resultant solution was added to a spin column, centrifuged, washed, and eluted as described by the manufacturer. The DNA was quantified and stored at -20^{o}C until qPCR analysis.

DNA was extracted from the saliva samples using the Oragene kit (DNA Genotek, Ottawa, ON, Canada) as described by the manufacturer. Briefly, the Oragene saliva sample was incubated at 50^{o}C for 1 hour. 500 µl sample was transferred to a 1.5 ml microcentrifuge tube, 20 µl of Oragene purifier added, and the sample was mixed by inversion and incubated on ice for 10 min. The sample was then centrifuged for 5 min at 15,000 x *g* and the supernatant was transferred to a new microcentrifuge tube. 600 µl of 95% ethanol was added; the sample was mixed by inversion 10 times and incubated at 10 min at room temperature. The sample was then centrifuged for 2 min at 15,000 x *g*, the supernatant was discarded, 250 µl of 70% ethanol was added and then incubated at room temperature for 1 min. The ethanol was removed and the DNA was dissolved in 100 µl TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). The sample was then vortexed. To ensure complete rehydration of the DNA, the sample was incubated at 50^{o}C for 1 hour with occasional vortexing. The DNA samples were quantified and stored at -20^{o}C until qPCR analysis.

Quantification of DNA

The concentration of 1 µl DNA sample was determined using the absorbance method (NanoDrop 1000 v3.7.1, Thermo Scientific). Absorbance of ultraviolet light at wavelengths of 260 nm and 280 nm were used to calculate the optical density (OD) 260/280 ratio. This is to compare the ratio of nucleic acid concentration in the sample (OD 260 nm) to that of protein and organics (OD 280 nm) contaminants. A ratio of 1.8 was considered ideal for the OD 260/280 ratio, indicating limited protein and

organic contamination. The average DNA concentration was taken from 2 OD readings.

To validate the accuracy of the absorbance method (NanoDrop) in quantifying DNA concentration, we took a random subset of 4 subjects' samples (blood, buccal swab and saliva), diluted at 10 ng/µl and measured their DNA concentrations simultaneously using the absorbance method (NanoDrop) as well as an ultra-sensitive fluorescent nucleic acid stain (Quant-iT™ PicoGreen®, Invitrogen), according to their assay protocols.

Quantitative Polymerase Chain Reaction (qPCR) for the AMY1 gene

The method used to determine diploid *AMY1* gene copy numbers was adapted from previously published work (Mandel et al., 2010). Extracted DNA from blood, cheek cells and saliva samples were standardized to 5 ng/ul. A TaqMan Copy Number Assay for *AMY1* (Assay ID Hs07226362_cn) and RNAse P reference assay (44003328, Applied Biosystems, Foster City, CA) were used with TaqMan Genotyping Master Mix (Applied Biosystems), according to product literature. 20 µl reactions (final DNA concentration of 1 ng/ul) were run in quadruplicates on an ABI Prism 7500 Real-Time PCR System (Applied Biosystems). Each individual's blood, cheek cells and saliva DNA were present in the same plate. For blood DNA, we run the qPCR experiments using duplicate plates, assessed the concordance between duplicate plates and the predicted copy number calculated by averaging the predictions between duplicate plates.

Values extracted following analysis with the SDS software were analysed using Copy Caller Software v2.0 (Applied Biosystems). *AMY1* diploid copy number was estimated using a standard curve constructed from a reference DNA sample

NA18972 (Coriell Cell Repositories, Camden, NJ) previously determined to have 14 *AMY1* diploid copies by qPCR and Fiber FISH (Perry et al., 2007).

Quantitative Polymerase Chain Reaction (qPCR) for the human actin beta 2 gene

A subset of 7 subjects' blood, buccal cells and saliva samples were used to validate and compare gene copy numbers for a housekeeping human actin beta 2 gene. The qPCR method used was described above. Human actin beta 2 gene copy numbers were analysed using median C_T with calibrator sample set at 2 copies.

4b.1.11 Sample size calculation

One study on ethnic differences in postprandial glycemia (Henry et al., 2008) between UK Caucasians and Asian Indians, observed at least a 59 $mmol.min^{-1}.L^{-1}$ difference in glycemic response between the 2 groups with standard deviations of 79 $mmol.min^{-1}.L^{-1}$ and 47 $mmol.min^{-1}.L^{-1}$ respectively. At the 5% level of significance with power of 80%, a minimum sample size of 21 subjects for each group would be required. Studies on *in vivo* assessment of GI have been based on 10 subjects, to take into account inter-individual variations (Brouns et al., 2005; FAO/WHO, 1998).

A minimum of 10 subjects for each group would be required to detect a difference of 112kU/L in salivary amylase concentration, with standard deviations of 31.7kU/L and 95.2kU/L respectively at the 5% level of significance with power of 90% (Mandel and Breslin, 2012).

In the study by Schwartz et al (1995), there was a significant difference in mean gastric half-emptying rate (9.7 min) between 18 male non-Hispanic Whites and 18 male Mexican American with standard deviations of 19.5 min and 19.9 min respectively.

One study showed significant differences in inter-individual mastication rate (chews per mouthful) and particle size distribution between rice and spaghetti (Ranawana et al., 2010). With a calculated effect size of 0.42, a sample size of 11, the experimental design had a power of 81%.

Therefore, we have decided to recruit a total of 75 subjects with 25 Chinese, 25 Malays and 25 Asian-Indians for the study.

CHAPTER 4c Glycemic and Insulin Responses, Glycemic Index and Insulinemic Index Values of Rice Between Three Asian Ethnic Groups[1]

4c.1 Abstract

Introduction – Asians exhibit larger glycemic response (GR) and insulin response (IR) than Caucasians, predisposing to increased risk of Type 2 diabetes mellitus (T2DM). We aimed to determine the GR and IR as well as the Glycemic Index (GI) and Insulinemic Index (II) of two rice varieties amongst the three ethnic groups in Singapore.

Methods – A total of seventy-five healthy males (25 Chinese, 25 Malays, and 25 Asian-Indians) were served equivalent available carbohydrate amounts (50 g) of test foods (Jasmine rice and Basmati rice) and a reference food (glucose) on separate occasions. Postprandial blood glucose and plasma insulin concentrations were measured at fasting (-5 and 0 min) and at 15, 30, 45, 60, 90 and 120 min after food consumption. Using the trapezoidal rule, GR, IR, GI and II values were determined.

Results – The GR did not differ between ethnic groups for Jasmine and Basmati rice. The IR were consistently higher among Asian-Indians for Jasmine rice (*P=0.002*) and Basmati rice (*P=0.002*) compared to Chinese and Malays, likely due to compensatory

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hyperinsulinemia to maintain normoglycemia. The GI and II of both rice varieties did not differ significantly between ethnicities. The overall mean GI (SD) for Jasmine and Basmati rice were 91 (21) and 59 (15) respectively. The overall mean II (SD) for Jasmine rice was 76 (26) and Basmati rice was 57 (24).

Conclusion – We conclude that the GI values presented for Jasmine and Basmati rice were applicable to all three ethnic groups in Singapore. Future studies should include deriving the II for greater clinical utility in the prevention and management of T2DM.

4c.2 Introduction

Numerous studies have showed that Asians, Chinese and non-Caucasians exhibit a 2-3 fold larger postprandial glycemic and insulin responses than Caucasians (Chan et al., 2009; Dickinson et al., 2002; Henry et al., 2008; Venn et al., 2010). In a recent study, the glycemic response (GR) following rice consumption was over 60% greater amongst Chinese compared with Europeans (Kataoka et al., 2013). Higher blood glucose concentrations stimulate increased production of insulin, resulting in a state of hyperinsulinemia. The chronically increased insulin demand may eventually result in pancreatic beta-cell failure, and, as a consequence, impaired glucose tolerance leading to type 2 diabetes mellitus (T2DM) (Pawlak et al., 2004).

Rice is a staple carbohydrate of many Asian populations, providing up to two-thirds of daily energy intake in Southeast Asian countries (Kataoka et al., 2013), making it a major contributor to the overall glycemic load of most Asian diets (Hu et al., 2012; Ministry of Health Singapore, 2010b; Murakami et al., 2006; Villegas et al., 2007). The extent to which different varieties of rice influence postprandial glycemia has potential relevance in the prevention and treatment of obesity and T2DM in Asia, where the recent increase in prevalence outweighs other parts of the world (Yoon et al., 2006). In many studies, rice consumption, especially white rice has been implicated in the deterioration of glucose metabolism, which leads to an increased risk of T2DM (Hu et al., 2012; Nanri et al., 2010; Villegas et al., 2007). This is likely mediated by the glycemic potency of rice, as measured by their dietary glycemic index (GI), a classification of carbohydrates according to their effect on postprandial glycemia (Jenkins et al., 1981; Wolever et al., 1991).

The question whether the GI varies between ethnicities is equivocal. Some studies showed no ethnic differences in GI values (Chan et al., 2001; Henry et al., 2008) but other studies demonstrated significant effects of ethnicity on the GI value of white bread (Wolever et al., 2009) and rice (Kataoka et al., 2013). One of the

major impediments in the use of GI tables worldwide has been the uncertainty of the applicability to different ethnic groups. If the standard classifications of low, medium and high GI differ between Asians of different ethnicities, this has important implications for dietary recommendations for individuals living in a multi-ethnic Asian population, especially those with T2DM. The insulinemic index (II), derived similarly to GI, measures the extent to which a food raises plasma insulin levels (Holt et al., 1997; Miller et al., 1995; Wolever et al., 1991). The insulinemic effects of foods may be relevant to the prevention of T2DM as prolonged postprandial insulinemia may play a role in the pathogenesis of T2DM (Reaven, 2005) and associated chronic diseases (Nilsson et al., 2003; Takahashi et al., 2006).

The population in Singapore comprises three major ethnic groups, Chinese, Malays and Asian-Indians. Yet there are significant differences in prevalence of obesity and T2DM between our three ethnic groups; the Malay population has the highest prevalence of obesity (24.0%) while our Asian-Indian population has the highest prevalence of T2DM (17.2%) (Ministry of Health Singapore, 2010a). This offers us an unusual opportunity to study the effect of different Asian ethnicities on postprandial glycemic and insulin responses as well as GI and II values to the same food.

The aims of this study were to examine glycemic and insulin responses as well as to determine the GI and II of two varieties of commonly consumed rice among the ethnic groups in Singapore. This is the first study known to the authors to examine the role of three ethnic groups residing in a shared and common multi-ethnic Asian environment. The results obtained will provide insights into the metabolic effects of consuming the same food among different ethnic groups.

4c.3 Methods

The methods for this study were described in Chapter 4b.

4c.3.1 Statistical analyses

One study on ethnic differences in postprandial glycemia (Henry et al., 2008) between UK Caucasians and Asian Indians, observed at least a 59 mmol.min-1.L-1 difference in glycemic response between the 2 groups with standard deviations of 79 mmol.min-1.L-1 and 47 mmol.min-1.L-1 respectively. At the 5% level of significance with power of 80%, a minimum sample size of 21 subjects for each group would be required. Studies on *in vivo* assessment of GI have been based on 10 subjects, to take into account inter-individual variations (Brouns et al., 2005; FAO/WHO, 1998). As we were investigating ethnic differences, a sample size of 25 from each ethnic group (total sample size of 75) was considered adequate for the current study.

The glycemic response data was analyzed using "change in GR", which was calculated by taking the difference between the blood glucose reading at each time point and mean baseline blood glucose value (based on 2 baseline values taken at 5 min apart). This represented the relative increment in glycemic response at any time point compared with the baseline value. The total glycemic response over 120 min was expressed as the IAUC ignoring the area under the baseline using the trapezoidal rule ((Brouns et al., 2005; FAO/WHO, 1998)). The insulin response data was analysed in the same manner as the glycemic response data, using "change in IR". These data were then used to construct insulin response curves and calculate the IAUC (using the trapezoidal rule).

The intention-to-treat analysis included data from all participants. All physiologically valid data were included in the computation of GI and II. In the

calculation of GI, the absolute IAUC glucose values for each rice was expressed as a percentage of the mean IAUC glucose values of the reference food, and the resulting values were averaged to obtain the GI value for each rice. The II, which measures the insulin response of food, was calculated similarly to GI, where the absolute IAUC insulin values for each rice was expressed as a percentage of the mean IAUC glucose values of the reference food.

Results were presented as means (standard deviation) where appropriate. Differences in glycemic and insulin response and GI values were analysed using one way ANOVA with *post-hoc* Bonferroni correction for multiple comparisons. The temporal glycemic and insulin response over 120 min were analysed using repeated measures ANOVA with *post-hoc* Bonferroni correction. Paired t-tests were used to compare the IAUC and rates of decline from 45 min to 60 min, 60 min to 90 min and from 90 min to 120 min for Jasmine rice and Basmati rice. Correlation analyses were conducted to determine relationships between GI, II with body composition (waist circumference and percent body fat) and metabolic indices (HOMA-IR, a marker of insulin resistance and HOMA-B, a measure of steady-state beta-cell function). Statistical analyses were conducted using the SPSS version 19 (SPSS Inc, Chicago, Ill). A *P*-value of <0.05 was considered statistically significant.

4c.4 Results

Table 4c-1 compared the characteristics of the participants by ethnicity. There were no differences in age, BMI, blood pressure, fasting glucose and fasting insulin between the 3 groups. However, waist circumference (*P=0.010*) and percent body fat (*P=0.035*) were significantly higher among the Asian-Indians compared to Chinese and Malays. The HOMA-IR was highest for Asian-Indians (*P<0.001*) compared to Chinese and Malays. In parallel with HOMA-IR, Asian-Indians showed

a significantly higher HOMA-B (*P=0.020*) compared to Chinese and Malays. This indicated a compensatory hyperinsulinemia in the presence of greater insulin resistance amongst Asian-Indians to maintain fasting normoglycemia.

Table 4c-1 Anthropometric characteristics by ethnicity

	Chinese (n=25)		**Malays (n= 25)**		**Asian-Indians (n= 25)**		***P-value****
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	23.0	1.3	23.0	2.3	24.1	2.0	*0.072*
Weight (kg)	63.8	6.9	65.8	7.1	68.7	8.5	*0.073*
Height (cm)	172.6	6.0	170.8[a]	6.0	176.2[b]	7.0	*0.012*
BMI (kg/m^2)	21.4	1.8	22.5	1.7	22.1	1.9	*0.085*
Systolic blood pressure (mmHg)	125	11	127	8	124	7	*0.528*
Diastolic blood pressure (mmHg)	76	9	73	8	72	10	*0.247*
Mean fasting glucose (mmol/l)	4.55	0.31	4.46	0.25	4.54	0.32	*0.484*
Mean fasting insulin (mU/l†)	7.43	2.85	9.10	3.38	8.10	3.93	*0.225*
Waist circumference (cm)	75.5	5.4	72.7[a]	4.4	77.7[b]	6.9	*0.010*
Body fat (%)	16.0[a]	5.9	17.8	5.2	20.5[b]	6.8	*0.035*
Mean HOMA-IR	1.45[a]	0.53	1.43[a]	0.51	2.16[b]	0.89	*<0.001*
Mean HOMA-B	165.7[a]	86.1	172.6	76.8	229.2[b]	93.3	*0.020*

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment – insulin resistance; HOMA-B, homeostatic model assessment – beta cell function. Data presented as mean (SD).
[a,b] Mean values within each row with unlike superscript letters were significant different (*P<0.05*).
* P-values represent comparison between the ethnic groups.
†To convert insulin from mU/l to pmol/l, multiply by 6.945

The glycemic response (GR) incremental area under the curve (IAUC) did not differ between ethnic groups for glucose reference, Jasmine and Basmati rice (Table 4c-2). Mean intra-individual variations in GR to the three glucose reference were 17.3% CV for Chinese, 17.1% CV for Malays and 16.0% CV for Asian-Indians (*P=0.841*). These values were consistent with previously reported variability for repeat tests of the reference foods in normal subjects (Brand-Miller et al., 2009; Henry et al., 2008; Wolever et al., 2008a). The inter-individual variations in GR to the reference food were 28.8% CV for Chinese, 35.8% CV for Malays and 37.6% CV for Asian-Indians. There were also no significant differences in temporal GR patterns for the three groups for glucose reference (Figure 4c-1) ($P_{interaction}=0.685$), Jasmine rice (Figure 4c-3) ($P_{interaction}=0.757$) and Basmati rice (Figure 4c-5) ($P_{interaction}=0.837$).

Basmati rice showed an initial faster rate of decline from 45 min to 60 min (mean difference 2.2 mmol/L/min, *P=0.002*) than Jasmine rice. However, the later postprandial rate of decline was significantly more rapid for Jasmine rice from 90 min to 120 min (mean difference 6.7 mmol/L/min, *P=<0.001*) compared to Basmati rice.

In contrast, insulin responses (IR) between ethnic groups showed marked differences. The insulin IAUC were consistently higher in Asian-Indians compared with Chinese and Malays for glucose reference (*P<0.001*), Jasmine rice (*P=0.002*) and Basmati rice (*P<0.001*). The temporal IR patterns for glucose reference, Jasmine and Basmati rice differed considerably between ethnic groups (Figures 4c-2, 4c-4 and 4c-6). Time point analyses showed that the IR for glucose reference and Jasmine rice at 45, 60, 90 and 120 min (Figure 4c-2 and 4c-4) were significantly higher for Asian-Indians than Chinese and Malays (*P<0.05*). For basmati rice, the IR at 15, 30, 45, 60 and 90 min (Figure 4c-4) were also significantly higher for Asian-Indians compared with Chinese and Malays (*P<0.05*).

As shown in Table 4c-2, the GI of Jasmine rice (*P=0.957*) and Basmati rice (*P=0.277*) did not differ between ethnic groups. The overall mean GI (SD) for Jasmine rice was 91.1 (20.7) (high GI) and Basmati rice was 59.3 (15.1) (medium GI). The GI values for both rice varieties were not significantly different between ethnic groups after adjusting for body fat or waist circumference. Similarly, the II for Jasmine rice (*P=0.681*) and Basmati rice (*P=0.197*) were not different between ethnic groups. The overall mean II (SD) for Jasmine rice was 75.8 (25.9) and Basmati rice was 56.7 (23.8). Adjustment for body composition (body fat or waist circumference) made no difference to the II values between ethnic groups.

Correlation analyses indicated that GI and II for Jasmine rice and Basmati rice were not related to any of the body composition measurements (waist circumference and percent body fat) or metabolic indices (HOMA-IR and HOMA-B)

(*P>0.05*). These results demonstrate that GI and II were independent of individuals' body composition and metabolic status, making them valid properties of food.

Between rice varieties, we observed that Jasmine rice showed consistently higher glycemic and insulin response as well as significantly higher GI (*P=0.001*) and II (*P<0.001*) values (Table 4c-2) compared to Basmati rice for each ethnic group (Figures 4c-7 and 4c-8).

Table 4c-2 Glycemic and insulin responses to glucose, Jasmine rice and Basmati rice, as incremental area under the curve (IAUC)

Ethnicity	Glucose reference				Jasmine rice								Basmati rice							
	IAUC glucose (mmol•min/L)		IAUC insulin (mU•min/L)		IAUC glucose (mmol•min/L)		IAUC insulin (mU•min/L)		GI		II		IAUC glucose (mmol•min/L)		IAUC insulin (mU•min/L)		GI		II	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chinese	237.1	68.2	4350[a]	1763	213.8	70.3	3137[a]	1377	91.2	19.2	75.0	28.1	131.6	48.6	2043[a]	866	55.7	13.3	49.9	21.7
Malays	224.2	80.3	4500[a]	1468	201.0	62.1	3515[a]	1275	92.0	20.0	79.4	20.7	140.1	52.6	2724[a]	1334	62.6	13.1	61.6	21.7
Asian-Indians	240.1	90.2	7436[b]	4159	206.6	66.3	5035[b]	2714	90.2	23.4	73.0	28.9	139.3	62.9	4067[b]	2395	59.5	18.1	58.5	27.1

Abbreviations: GI, glycemic index; IAUC, incremental area under the curve; II, insulinogenic index. Data are presented as mean (SD). Mean values within a column with different superscript letters are significantly different ($P<0.05$).

Figure 4c-1 Temporal blood glucose response curves for glucose reference beverage among Chinese, Malays and Asian-Indians

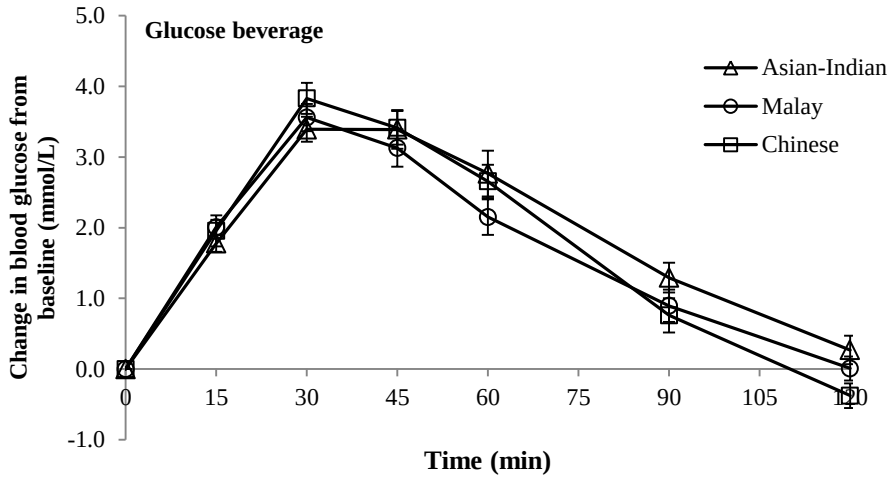

Figure 4c-1. Temporal blood glucose response curves for glucose reference beverage among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in glucose response from baseline over 120 min following the consumption of glucose beverage. Values are means, with their standard errors represented by vertical bars. Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P=0.685*).

Figure 4c-2 Temporal plasma insulin response curves for glucose reference beverage among Chinese, Malays and Asian-Indians

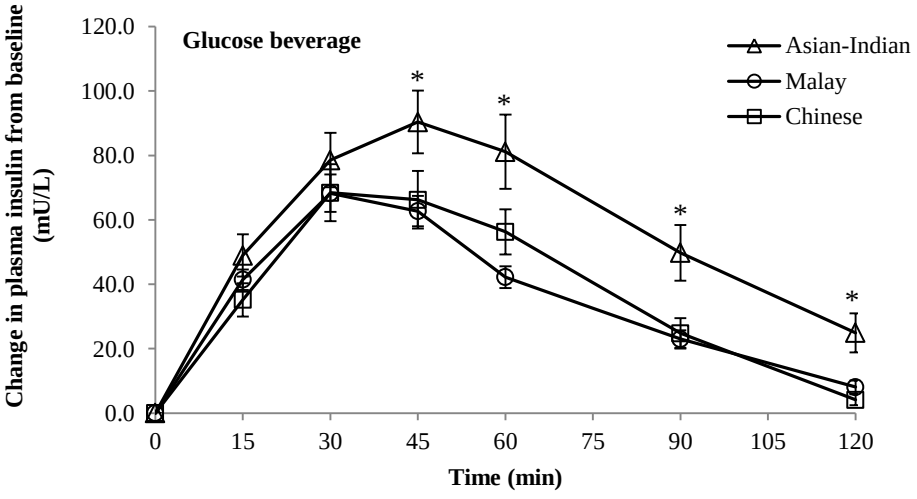

Figure 4c-2. Temporal plasma insulin response curves for glucose reference beverage among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in insulin response from baseline over 120 min following the consumption of glucose beverage. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different at a specific time point (*P<0.05*). Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P=0.003*). To convert insulin from mU/l to pmol/l, multiply by 6.945.

Figure 4c-3 Temporal blood glucose response curves for Jasmine rice among Chinese, Malays and Asian-Indians

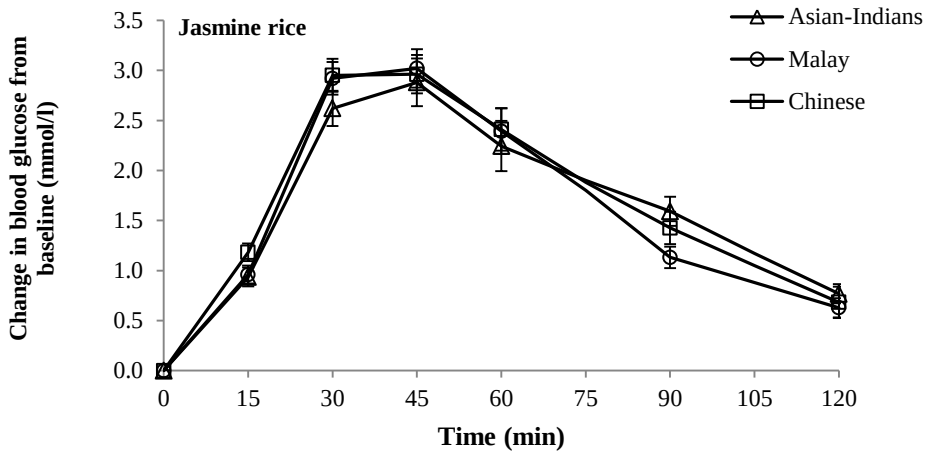

Figure 4c-3. Temporal blood glucose response curves for Jasmine rice among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in glucose response from baseline over 120 min following the consumption of Jasmine rice. Values are means, with their standard errors represented by vertical bars. Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P=0.757*).

Figure 4c-4 Temporal plasma insulin response curves for Jasmine rice among Chinese, Malays and Asian-Indians

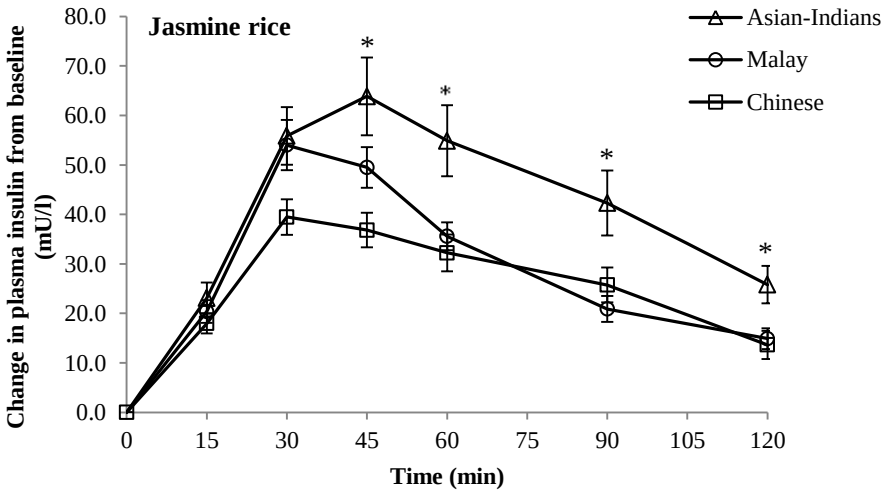

Figure 4c-4. Temporal plasma insulin response curves for Jasmine rice among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in insulin response from baseline over 120 min following the consumption of Jasmine rice. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different at a specific time point (*P<0.05*). Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P=0.002*). To convert insulin from mU/l to pmol/l, multiply by 6.945.

Figure 4c-5 Temporal blood glucose response curves for Basmati rice among Chinese, Malays and Asian-Indians

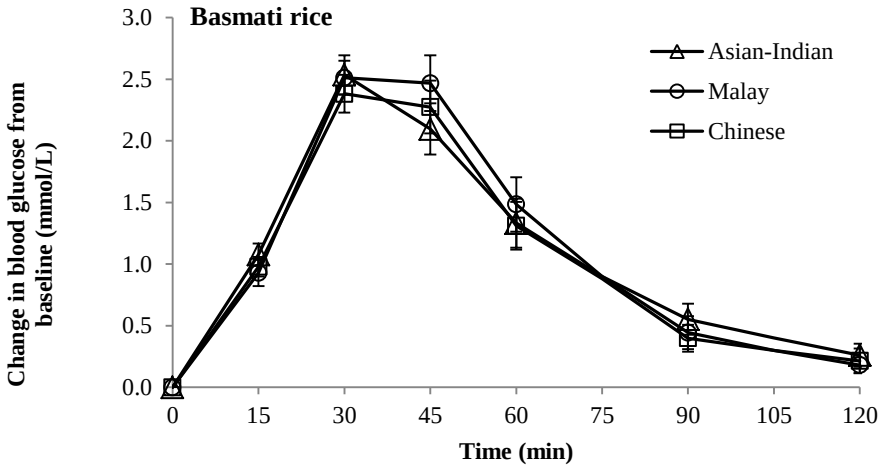

Figure 4c-5. Temporal blood glucose response curves for Basmati rice among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in glucose response from baseline over 120 min following the consumption of Basmati rice. Values are means, with their standard errors represented by vertical bars. Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P=0.837*).

Figure 4c-6 Temporal plasma insulin response curves for Basmati rice among Chinese, Malays and Asian-Indians

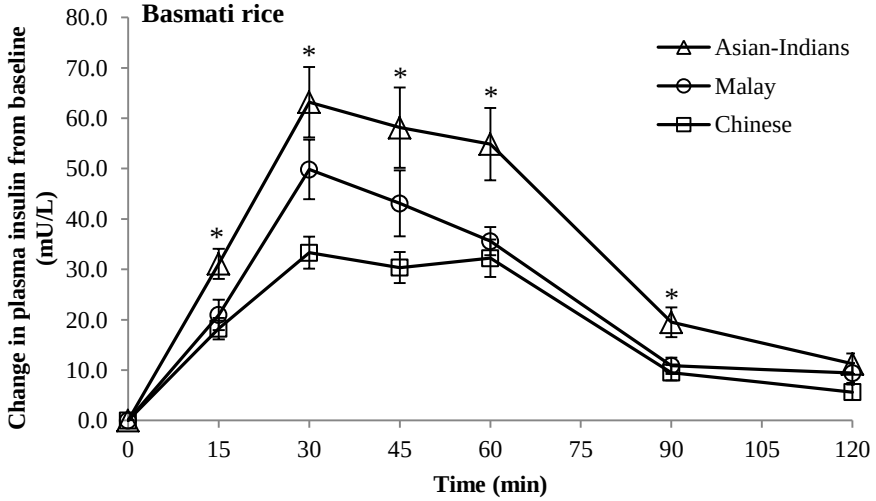

Figure 4c-6. Temporal plasma insulin response curves for Basmati rice among Chinese (□), Malays (○) and Asian-Indians (△). Data are represented as change in insulin response from baseline over 120 min following the consumption of Basmati rice. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different at a specific time point (*P<0.05*). Repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests revealed an overall difference between ethnic groups (*P<0.001)*. To convert insulin from mU/l to pmol/l, multiply by 6.945.

Figure 4c-7 Comparison of glycemic response between ethnic groups following consumption of Jasmine rice and Basmati rice

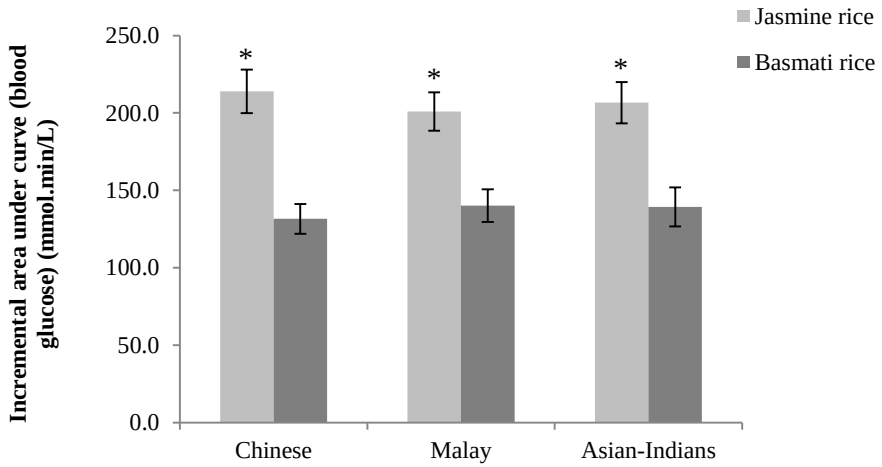

Figure 4c-7. Comparision of incremental area under curve (IAUC) for glycemic response between ethnic groups following consumption of Jasmine rice ( ) and Basmati rice ( ). Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different in IAUC glycemic response between rice varieties for each ethnic group (*P<0.05*).

Figure 4c-8 Comparison of plasma insulin response between ethnic groups following consumption of Jasmine rice and Basmati rice

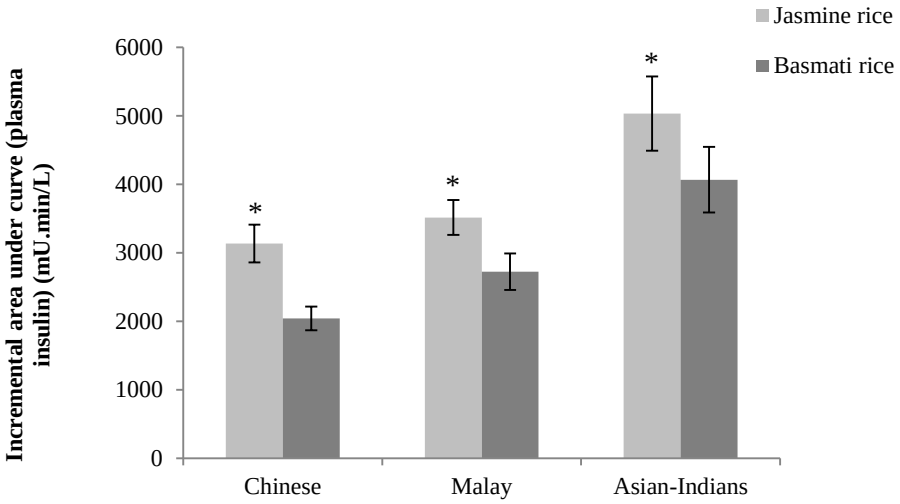

Figure 4c-8. Comparision of incremental area under curve (IAUC) for plasma insulin response between ethnic groups following consumption of Jasmine rice ( )and Basmati rice ( ). Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different in IAUC insulin response between rice varieties for each ethnic group (*P<0.05*).

4c.5 Discussion

This was the first study that examined the role of three ethnicities in a multi-ethnic Asian environment on postprandial glycemic and insulin responses as well as GI and II values to two commonly eaten rice varieties. We observed no ethnic variations in glycemic response (GR) to Jasmine and Basmati rice yet there were considerable differences in insulin response (IR).

Even though there were no differences in fasting glucose and fasting insulin levels, Asian-Indians mounted significantly higher insulin excursions for both Jasmine and Basmati rice compared to Chinese and Malays, even after adjustment for percent body fat or waist circumference. These differences in IR were likely due to a compensatory hyperinsulinemia in the presence of greater insulin resistance amongst the Asian-Indians to maintain fasting normoglycemia. Genetically, Asian-Indians have a high prevalence of insulin resistance (Liew et al., 2003) that may underlie their greater than normal tendency to develop diabetes and cardiovascular disease compared to Chinese and Malays (Mak et al., 2003; Ministry of Health Singapore, 2010a; Misra and Vikram, 2004). In the pathogenesis of T2DM, both beta-cell dysfunction and decreased insulin sensitivity play key roles (Kahn, 2003). If insulin sensitivity decreases, insulin secretory response of pancreatic beta-cells must increase to preserve normal glucose tolerance. This was evidenced by significantly higher HOMA-IR (a steady-state measure of insulin sensitivity) and HOMA-B (a measure of pancreatic beta-cell function) amongst the Asian-Indians compared to Chinese and Malays. Chronic elevated insulin levels leads to impaired action and an exhaustion of pancreatic beta -cells (Hayashi et al., 2013). Further deterioration of beta -cell function and insulin sensitivity will result in worsening of glucose tolerance and risk for T2DM (Hayashi et al., 2013; Lorenzo et al., 2010; Weyer et al., 1999). Although the GI values for each rice variety were similar between ethnic groups, the same rice presented different stress to Asian-Indians and their pancreatic beta islet cells,

resulting in higher compensatory insulin response for both Jasmine and Basmati rice, when compared with the Malays and Chinese. This may thus be an important contributing factor to the higher risk of T2DM in this ethnic group.

We did not find any significant differences in GI and II values to Jasmine rice and Basmati rice between Chinese, Malays and Asian-Indians. This study lends support to the findings of previous research concerning the influence of ethnicity in GI values of foods. In the UK, there were no difference in GI values of biscuits and breakfast cereals between Asian-Indians and Caucasians (Henry et al., 2008). Using the standard classification for low (≤55), medium (56-69) and high GI (≥70) (Foster-Powell et al., 2002; International Standards Organisation, 2010; Standards Australia, 2007), the overall GI for Jasmine rice was classified as high GI, while Basmati rice was considered medium GI in this study. While it is known that Jasmine rice, with its low amylose content (11% - 18%) (Suwansri and Meullenet, 2004) elicits a greater glycemic and corresponding greater insulin response, it was unexpected that Basmati rice, an intermediate amylose rice (20% - 25%) (Bhattacharjee et al., 2002) and claimed low GI on its packaging, was classified as medium GI in our study (overall group mean as well as ethnicity mean). In Singapore, there is a lack of GI database for locally tested foods and thus there is a pressing need to uncover rice varieties which are truly low GI since rice in the major carbohydrate staple in this country(Ministry of Health Singapore, 2010b).

In this study, we observed no differences in II values of both rice varieties despite ethnic differences in IR. The IR is a biological response to fluctuations in blood glucose levels. The II, however is an index of the insulinemic effects of food. The computation of II normalizes an individual's insulin response to a test food against a reference food, similar to the computation of the GI. Therefore, while insulin response may differ, II on the other hand remained consistent between individuals. Our study showed that both GI and II are properties of food, not

influenced by the anthropometric and metabolic status of the participants. We have shown that both GI and II were similar between ethnic groups regardless of their degree of insulin sensitivity and adiposity. Recent evidence has also showed that II values of rice were not different between healthy, hyperinsulinemic and T2DM individuals although the II was correlated with metabolic status (Lan-Pidhainy and Wolever, 2011).

Our findings and existing evidence have shown that the consumption of a high GI food like Jasmine rice leads to higher postprandial glycemic response, more rapid decline in glucose levels from 60 min onwards and a correspondingly augmented insulin response compared to a lower GI food (Jenkins et al., 2002). A recent study had demonstrated that the consumption of a high GI meal led to a rapid decline in blood glucose levels (Ludwig, 2002; Ludwig et al., 1999). In addition, brain activity was increased in regions related to food intake, reward and craving in the late postprandial phase (Lennerz et al., 2013). This triggered excessive hunger and a preference for foods, such as high GI foods that rapidly restored blood glucose to normal. (Campfield et al., 1996; Strachan et al., 2004). This could lead to overeating and contribute to problems like overweight and obesity. These findings have considerable potential clinical significance given the global epidemic of obesity and T2DM and the especially high rates in Asian countries (Chan et al., 2009), where white rice is the major contributor to the overall glycemic load of the diet (Hu et al., 2012; Ministry of Health Singapore, 2010b; Murakami et al., 2006; Villegas et al., 2007). There was growing evidence that high intakes of cooked white rice (≥420 g per day or ≥3 bowls per day) were associated with a significantly increased risk of T2DM, especially in Japanese women (Nanri et al., 2010). Amongst middle-aged Chinese women, an increased risk of T2DM was not apparent until the women consumed more than 625g of rice (Villegas et al., 2007). Despite the positive findings of rice consumption and increased risk of T2DM, rice intake was not associated with

diabetes in a Chinese population (Yu et al., 2011) and amongst Japanese men (Nanri et al., 2010) , and added no additional risk of CVD in Japanese men and women (Eshak et al., 2014). Therefore, evidence is still equivocal on rice intake and risk of T2DM in Asian populations.

Nonetheless, rice is a major dietary staple in Asian populations and it appeared that Asian populations were more susceptible to the adverse effects of high intakes of white rice compared to the Western populations (Hu et al., 2012). In Singapore, the mean (SD) rice intake among the population was 361.8 (5.7) g/day, with Chinese, Malays and Asian-Indians consuming 364.9 (6.5) g, 348.1 (15.5) g and 352.7 (19.2) g respectively (Ministry of Health Singapore, 2010b). This puts the population as being high consumers of white rice. It is therefore prudent to advice on consumption of rice with a lower GI that may reduce the glycemic load of the diet. Additionally, a lower insulin response can help to improve long term metabolic control in individuals who are insulin resistant and thereby reducing the future risk of T2DM.

The International Tables of the Glycemic Index (Atkinson et al., 2008) reported a wide range of GI values for different rice varieties, due to differences in ratio of amylose to amylopectin, physical and chemical characteristics, other botanical structures and processing methods (Goddard et al., 1984; Larsen et al., 1996; Larsen et al., 2000; Miller et al., 1992; Panlasigui et al., 1991). GI values vary even when considering the same variety of rice. For example, the GI values for Jasmine rice range from 48 to 109 and Basmati rice range from 43 to 69 (Atkinson et al., 2008; S.U.G.I.R.S., 2011). As the composition of rice and other carbohydrate-containing foods may vary according to where they are grown, hence the recommendation that the GI of such foods are best tested locally (Foster-Powell et al., 2002). In this study, we have used two locally available and commonly consumed rice varieties among the ethnic groups. The cooking method used was as per the

manufacturers' instructions and represented how the population would cook these two rice varieties. There is currently no cut-offs nor classification for II values to food. Future work can thus focus on establishing the corresponding II values when determining the GI of foods for greater clinical utility.

The strengths of this study are that it measured the glycemic response of three ethnic groups fed identical foods within a common environmental milieu. We standardized the cooking method by using the exact amount of water to cook each rice variety in the same rice cooker to ensure consistency in rice texture. Using a standardized cooking method also allows a valid and unbiased comparison to be made between ethnic groups by removing the confounding effects of cooking methods. Moreover, we have simultaneously measured the corresponding insulin response to gain a better understanding of the interplay between postprandial glucose and insulin responses as well as to determine both the GI and II values. However, we recognised that the limitation of this study was that we have only tested two rice varieties and it may not be feasible to extrapolate these findings to all carbohydrate foods. However, these rice varieties were the most commonly consumed amongst the ethnic groups in Asia and are the major contributors to the overall glycemic load of the Asian diet.

In summary, the present study has shown that there were no significant differences in the GI and II values to two commonly eaten rice varieties between the three ethnic groups. This suggests that GI values of Jasmine rice and Basmati rice determined in this study are applicable to all three ethnic groups in Singapore. Although there are currently no cut-offs for II values, our results showed that II is a property of food, independent on the metabolic status of the individuals. Future studies testing the GI of foods may include testing the concurrent insulin response to derive the II for greater clinical utility in the prevention and management of T2DM. Despite similar GI values between ethnic groups, the same rice presented greater pancreatic beta -cell stress to Asian-Indians, resulting in higher compensatory insulin

response, and possibly conferring higher risk of T2DM in this ethnic group. Preserving beta-cell function in at-risk populations is a critical factor in the prevention of T2DM onset (Defronzo, 2009). Therefore, therapeutic prevention strategies should focus on encouraging the consumption of rice varieties with lower GI especially amongst the high-risk groups to maintain glucose homeostasis.

4c.6 Acknowledgements

The candidate was responsible for the conception and design of the study, carried out the study, was responsible for the analysis of the blood samples, carried out the statistical analysis and drafted the manuscript.

The data in this chapter is published in the British Journal of Nutrition (paper is attached at the end of the thesis).

CHAPTER 4d Within-individual Differences in *AMY1* Gene Copy Numbers Derived from Blood, Buccal cells and Saliva

4d.1 Abstract

Introduction – The *AMY1* gene, encoding salivary α-amylase, has variable copy number variants in the human genome. We aimed to determine *AMY1* gene copy numbers derived from blood, cheek cells and saliva from the same individual.

Methods – Thirteen participants (3 Chinese, 5 Malays and 5 Asian-Indians) were recruited. All participants provided blood, buccal cells and saliva samples. DNA was extracted from the samples and the quantity and purity of DNA examined by absorbance method (Nanodrop). Quantitative polymerase chain reaction experiments were conducted to quantify *AMY1* gene copy numbers. We used binomial test to determine agreement of *AMY1* copy numbers of buccal cells and saliva, using blood copy numbers as the reference.

Results – The mean (SD) for age and BMI was 23.7 (2.1) years and 21.6 (1.9) kg/m^2 respectively. The mean values of the optical density 260/280 ratios were 1.87, 1.89 and 1.89 for genomic DNA purified from blood, buccal swab and saliva respectively, were close to the ideal value of 1.8. There was no difference in blood *AMY1* copy numbers between runs for the same individual (*P=0.196*) with an inter-assay CV of 4.5% and median difference of 0.24 copy. Using blood as the reference sample, buccal cells (*P=0.022*) and saliva (*P=0.022*) *AMY1* copy numbers differed

significantly from blood. The difference in copy numbers ranged from -4 to +3 between blood and buccal cells, with a median difference of -1 copy (*P=0.003*). For blood and saliva, the copy numbers ranged from -8 to +3, with a median difference of -1 copy (*P=0.003*).

Discussion – Despite buccal cells and saliva samples being good alternatives to blood samples to obtain high quality genomic DNA, it is pertinent that a single biological sample be used for determining *AMY1* gene copy numbers due to the large within-individual variability between different biological samples.

4d.2 Introduction

There has been recent interest in copy number variants (CNVs) of the *AMY1* gene and its association with obesity via carbohydrate metabolism (Falchi et al., 2014). *AMY1* gene, encoding salivary α-amylase is located in a gene cluster on 1p21. Evolutionary analysis revealed that the *AMY1* gene was derived from duplication of an ancestral pancreatic amylase gene, with the insertion of a retrovirus sequence that is responsible for tissue-specific expression (Meisler and Ting, 1993). The *AMY1* gene has a very high sequence identity showing extensive CNV ranging from 2 diploid copies (1 of paternal and 1 of maternal origin) to as many copies as 16 copies (Perry et al., 2007). The CNV of *AMY1* was strongly related with the amount of α-amylase in saliva (Mandel and Breslin, 2012; Mandel et al., 2010; Perry et al., 2007). Low levels of *AMY1* CNVs are associated with decreased levels of α-amylase in the saliva and an increased risk of obesity (Falchi et al., 2014).

Until recently, blood samples are the preferred choice of genetic material because they yield sufficient amounts of genomic DNA for genetic studies (Sun and Reichenberger, 2014). The quality of genomic DNA from blood is high without contamination with foreign DNA (Philibert et al., 2008). However, the collection of blood samples may not always be possible. Study subjects may be reluctant to provide blood samples, thereby reducing participating rates. Therefore, less invasive procedures for collecting DNA are needed.

Buccal sampling as an alternative to venous blood sampling has been investigated previously. Several studies have found that exfoliated buccal epithelial cells are promising alternative sources of DNA (Cozier et al., 2004; Garcia-Closas et al., 2001; King et al., 2002; Mulot et al., 2005). Another alternative source of DNA is whole saliva. The collection of saliva provides DNA with better quality than from buccal swabs (Rogers et al., 2007). Recently, a study showed that saliva collected using the Oragene kit yielded high quantity and high quality DNA for genetic studies

(Nunes et al., 2012). However, there is still reluctance to using DNA extracted from saliva samples or buccal cyto-brushes (Hansen et al., 2007) for genotyping. The main reasons are concerns over reduced yield and quality of DNA extracted as well as the abundance of bacterial DNA (Marenne et al., 2011). Furthermore, data is lacking on whether these biological samples can be used interchangeably for the genotyping of a specific gene of interest, in this case *AMY1* gene.

Whether the performance of DNA from buccal cells and saliva is comparable to that from blood for the determination of *AMY1* gene copy numbers is currently unknown. To our knowledge, no study to date has evaluated whether there are within-individual differences in *AMY1* gene copy numbers from biological samples derived from blood, buccal cells or saliva. Most have assumed concordance in gene copy numbers as they are derived from the same person. However, DNA quality and quantity vary using different methods of DNA collection (Hansen et al., 2007). Furthermore, copy number variants differ in different tissues of the same individual (Piotrowski et al., 2008). We hypothesize that there are within-individual differences in *AMY1* gene copy number derived from blood, cheek cells and saliva that can be attributed to differences in DNA quality. In this study, we aim to compare DNA yield and quality of three DNA sampling methods (blood, buccal swab and saliva) and determine *AMY1* gene copy numbers derived from blood, cheek cells and saliva from the same individual. Findings from this study will provide confidence in the use of different biological samples for the determination of *AMY1* gene copy numbers.

4d.3 Methods

4d.3.1 Subjects

A random sub-set of thirteen subjects (3 Chinese, 5 Malays and 5 Asian-Indians) out of a total 75 subjects from the main study were recruited for this study.

4d.3.2 Methods

The methods for this sub-study were described in Chapter 4b.1.10.

4d.3.3 Statistical analyses

Data are presented as means (standard deviation), unless otherwise stated. We conducted the binomial test to determine agreement of *AMY1* copy numbers of buccal cells and saliva, using blood copy numbers of the same individual as the reference, weighted by frequency. Paired t-test was used to compare difference in blood *AMY1* copy numbers between duplicate plates. A *P*-value of <0.05 was considered statistically significant. Statistical analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

4d.4 Results

The descriptive characteristics of the participants were presented in Table 4d-1. The participants were young and healthy with no presence of glucose intolerance.

Table 4d-1 Baseline characteristics of participants

Parameter (n=13)	**Mean (SD)**
Age (year)	23.7 (2.1)
Height (cm)	171.8 (6.2)
Weight (kg)	63.8 (6.1)
BMI[a] (kg/m^2)	21.6 (1.9)
Waist circumference (cm)	72.5 (5.2)
Body fat[b] (%)	17.8 (5.9)
Systolic blood pressure (mmHg)	128 (8)
Diastolic blood pressure (mmHg)	74 (7)
Fasting glucose[c] (mmol/L)	4.5 (0.3)

Data are presented as mean (SD).

Table 4d-2 Comparison of DNA yield and quality according to DNA collection method

Method of DNA collection	Blood		Buccal cells (swabs)		Saliva	
Amount of sample used	100-200 µl	*Range*	1 swab	*Range*	500 µl	*Range*
DNA concentration (ng/ µl)	71.0 (34.5)	30.1-150.3	40.5 (19.6)	9.5-83.6	235.1 (224.7)	34.7-815.3
Actual DNA concentration used (ng)	4.83 (0.50)	3.9-5.4	4.08 (0.47)	3.3-4.7	4.32 (0.50)	3.4-5.0
Optical density 260/280 nm ratio	1.87 (0.05)	1.80-1.96	1.89 (0.07)	1.80-2.00	1.89 (0.04)	1.80-1.94
Diploid *AMY1* gene copy numbers	6.0 (4.0-8.0)	2.0–10.0	7.0 (5.0-8.5)	3.0-10.0	7.0 (4.5-9.5)	3.0-16.0

Data are presented as means (SD), except *AMY1* gene copy numbers where data are presented as median (inter-quartile range).

Figure 4d-1 Diploid *AMY1* gene copy numbers of 13 participants.

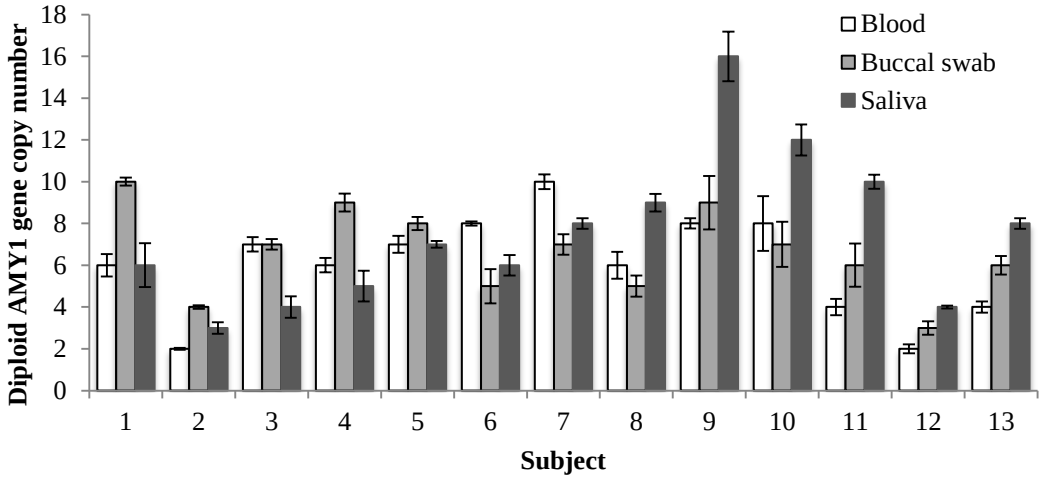

Figure 4d-1. A comparison of within-individual AMY1 gene copy numbers derived from blood, buccal cells and saliva. Error bars on bar charts are standard deviation.

The quantity and purity of DNA examined by absorbance method (Nanodrop) were shown in Table 4d-2. The estimated amount of DNA extracted from 100-200 µl blood samples varied between 30.1 to 150.3 µg with a mean of 71.0 µg, from buccal swabs between 9.5 to 83.6 µg with a mean of 40.5 µg, from 500 µl Oragene saliva samples between 34.7 to 815.3 µg with a mean of 235.1 µg. The quality of DNA obtained was assessed by the ratio of absorbance at 260 nm and 280 nm. The mean

values of the optical density 260/280 ratios were 1.87, 1.89 and 1.89 for genomic DNA purified from blood, buccal swab and saliva respectively, were close to the ideal value of 1.8 (Abraham et al., 2012), indicating acceptably low protein and organic contamination. A subset validation of DNA concentration using absorbance method (NanoDrop) and an ultra-sensitive fluorescent nucleic acid stain (Quant-iT™ PicoGreen®, Invitrogen) protocol showed no difference in the mean DNA concentration between these two methods ($P<0.05$) (data not shown).

The median diploid *AMY1* gene copy numbers for blood, buccal cells and saliva were 6, 7 and 7 respectively (Table 4d-2). Figure 4d-1 illustrated the *AMY1* copy numbers for blood, buccal cells and saliva for each participant. Within each participant, *AMY1* copy numbers differ depending on which biological sample was used for genotyping. Using blood as the reference sample, buccal cells ($P=0.022$) and saliva ($P=0.022$) copy numbers differed significantly from blood. The difference in copy numbers ranged from -4 to +3 between blood and buccal cells, with a median of 2 copies ($P<0.001$). For blood and saliva, the copy numbers ranged from -8 to +3, with a median of 3 copies ($P=0.001$).

When we compared the copy numbers from blood between separate runs, there was no difference in blood *AMY1* copy numbers between runs for the same individual ($P=0.196$) with an inter-assay CV of 4.5% and median difference of 0.24 copy.

In our validation sub-study using a housekeeping human actin beta 2 gene, we found no difference in copy numbers of this gene between blood, buccal cells and saliva (data not shown). Notably these 7 subjects showed variation in their *AMY1* gene copy numbers (Table 4d-4).

Table 4d-3 Comparison of *AMY1* gene copy numbers derived from blood between qPCR runs

Subject	Run 1	Run 2	Standard deviation
1	5.83	6.06	0.16
2	2.12	2.44	0.23
3	6.70	6.92	0.16
4	6.00	6.32	0.23
5	7.13	7.02	0.08
6	8.02	7.9	0.08
7	10.30	10.18	0.08
8	6.08	6.04	0.03
9	8.11	7.52	0.42
10	7.61	8.02	0.29
11	4.08	4.3	0.16
12	1.77	2.48	0.50
13	4.29	4.26	0.02

Each run was conducted in quadruplicates. The standard deviation (SD) was derived from the mean of the quadruplicates.

Table 4d-4 Comparison of *AMY1* gene copy numbers in a subset of 7 subjects

Subject	Blood	Buccal cells	Saliva
2	2	4	3
3	7	7	4
4	6	9	5
9	8	9	16
10	8	7	12
11	4	6	10
12	3	3	4

4d.5 Discussion

In this study, we demonstrated that we were able to obtain high quality DNA, free of contaminants and of adequate yield for genotyping from all biological samples collected – blood, buccal cells and saliva.

We tested whether *AMY1* gene copy numbers differed between blood, buccal cells and saliva from the same individual. Our results showed that unexpectedly, *AMY1* copy numbers varied between blood and buccal cells as well as between blood and saliva despite sufficient amount of high quality DNA. We showed within-individual variability when different biological samples from the same individual were used to determine *AMY1* gene copy numbers. We postulate that this could be because *AMY1* gene is a highly polymorphic gene showing extensive copy number variation ranging from 2 diploid copies to as many as 16 copies (Mandel et al., 2010; Perry et al., 2007).This has implications for studies using different biological samples for genotyping gene copy numbers that are highly polymorphic.

From our findings, *AMY1* copy numbers ranged from 2 to 16 copies, consistent with published literature. It is known that the relative difference between copy numbers becomes more difficult to discriminate as copy number increases (Hollox, 2012). Nonetheless, analyses of copy number should produce results that are identical within individuals unless there is evidence for tissue mosaicism in which different cell types contain different copy numbers (Notini et al., 2008).

Genetic studies often require the recruitment of large numbers of participants and ascertainment of DNA samples. In the past, most blood samples were obtained by venipuncture requiring trained staff. Using collection methods that do not unduly burden study participants, yet increasing DNA yield and quality, is therefore integral to improving participating rates for genetic studies. In this study, we found the mean DNA yield from saliva was highest, followed by blood then buccal cells. This might

be because we have used only 100-200 µl of blood sample and a single buccal swab, resulting in lower DNA yield compared to saliva. Nonetheless, our results showed that blood, buccal cell and saliva samples yielded good DNA quality for down-stream qPCR application for the determination of *AMY1* copy numbers.

The primary strengths of the current study include using the same 13 individuals in a randomized crossover comparison. One concern was that saliva may contain significant proportions of DNA from oral bacteria and/or food. If this had been the case, the true concentration of human DNA added to each assay would be lower than the calculated concentration and the amplified signals would have been reduced. To validate our findings, we ran a copy number study of control gene (human beta actin A) and the copy numbers were not different between different sources of sample, suggesting that our method of quantifying gene copy numbers is valid. Moreover, bacteria, fungi and potentially food remnants in the oral cavity can be reduced by rinsing the mouth thoroughly prior to saliva donation (Sun and Reichenberger, 2014), which we adhered to in this study.

The primers and probes of the TaqMan qPCR assay employed in our analyses specifically target a region within exon 1 of the *AMY1* gene, which is absent in the *AMYP1* pseudogene, therefore ensuring specificity of the qPCR assay for *AMY1*. The sample NA18972 was specially selected as a reference sample due to the fact that its copy number has been independently measured and confirmed in several published studies using methods including Fiber FISH, qPCR and next-generation sequence analysis (Perry et al., 2007; Sudmant et al., 2010)

We recognize several limitations for this study. We did not examine the amount of bacterial DNA present in our samples. However, it is known that buccal and saliva samples are contaminated with bacterial DNA. Bacterial contamination primarily depends on the way the samples are kept after collection. To minimize the

effect of contaminating DNA, we had participants refrain from eating and rinse their mouths out with water prior to sample collections. We immediately refrigerate and froze the saliva and buccal swab samples respectively to avoid and minimize bacterial growth. Moreover, the Oragene sample kit contains an antibacterial agent, which prevents the growth of bacteria between time of collection and the time of DNA purification (Hansen et al., 2007). Compared to swabs, which contain only 11% human DNA (Garcia-Closas et al., 2001), saliva samples contained an average human DNA yield of 68% (Rylander-Rudqvist et al., 2006). The quality of genomic DNA was examined by measurements of the 260/280 ratio by NanoDrop analysis. The 260/280 nm ratios from DNA from blood were comparable to buccal cells and saliva. This suggests that these samples were unlikely to be contaminated with proteins, which can overestimate the amount of DNA in these samples. These results suggest that DNA purified from buccal swab and saliva in this study were suitable for downstream application like qPCR for the determination of *AMY1* gene copy numbers. As a result of our small sample size, we were unable to examine ethnic and age differences, which have been suggested to affect the yield of DNA collection methods (Garcia-Closas et al., 2001; Le Marchand et al., 2001).

In summary, both buccal swabs and saliva provided high quality DNA with acceptably low protein and organic contamination, and successful qPCR amplification. Our findings of excellent yields and quality for both buccal swabs and whole-saliva make them suitable for genetic studies. However, despite buccal cells and saliva samples being good alternatives to blood samples to obtain high quality genomic DNA, it is pertinent that a single biological sample be used for determining *AMY1* gene copy numbers due to the large within-individual variability between different biological samples. This is important and applicable especially for the determination of other genes with wide-ranging copy numbers.

4d.6 Acknowledgement

The candidate was responsible for the conception and design of the study, carried out the study, responsible for the analysis of the samples, carried out the statistical analyses. This manuscript is currently under preparation.

CHAPTER 4e The Role of Digestive Factors in Determining Glycemic Response in a Multi-ethnic Asian Population

4e.1 Abstract

Background and objective – There is wide inter-individual differences in glycemic response (GR). We aimed to examine key digestive parameters that influence inter-individual and ethnic differences in GR in healthy Asian individuals.

Methods – Seventy-five healthy male subjects (25 Chinese, 25 Malay, and 25 Asian-Indians) were served equivalent available carbohydrate amounts (50 g) of Jasmine rice (JR) and Basmati rice (BR) on separate occasions. Postprandial blood glucose concentrations were measured at fasting (-5 and 0 min) and at 15-30 min interval over 180 min. Mastication parameters (number of chews per mouth and chewing time per mouthful), saliva α-amylase activity, *AMY1* gene copy numbers and gastric emptying rate were measured to investigate their relationships with GR.

Results – The GR for Jasmine rice was significantly higher than for Basmati rice ($P<0.001$). The median number of *AMY1* gene copies was 6, with a range of 2 to 15. There was a significant positive relationship between *AMY1* copy number and α-amylase activity (*P=0.002*). There were no significant ethnic differences in GR. For both rice varieties, the number of chews per mouthful was positively associated with the GR (JR, *P=0.011*; BR, *P=0.005*) while chewing time per mouthful showed a

negative association (JR, *P=0.039*; BR, *P=0.016*). Ethnicity, salivary α-amylase activity, particle size distribution, gastric emptying rate and *AMY1* gene copy numbers were not significant contributors to GR (*P>0.05*).

Conclusion – Mastication parameters contribute significantly to GR. Eating slowly and having larger food boluses before swallowing (less chewing), both potentially modifiable, may be beneficial in glycemic control.

4e.2 Introduction

Postprandial glycemia has been linked to several chronic diseases such as obesity, type 2 diabetes mellitus (T2DM) and heart disease (Augustin et al., 2002; Jenkins et al., 2002). A consistent observation in many studies is the wide inter-individual variations in glycemic response (GR) (Ranawana et al., 2011; Vega-Lopez et al., 2007; Wolever et al., 2008a). This suggests that variations in physiological digestive factors may contribute to these differences in GR. The influence of ethnicity on GR has been reported extensively showing that Asians display a larger GR compared with Caucasians (Dickinson et al., 2002; Henry et al., 2008; Kataoka et al., 2013; Venn et al., 2010). In Singapore, the differing prevalence of obesity and T2DM among the three main ethnic groups suggest the possibility of ethnic differences in susceptibility; Malays have the highest prevalence of obesity while the Asian-Indians have the highest prevalence of T2DM (Ministry of Health Singapore, 2010a) compared to the Chinese. No studies to date have examined how physiological factors amongst the ethnic groups impact the GR. In this study, we wish to investigate key digestive parameters that influence and impact inter-individual and ethnic differences in GR.

Recent evidence has shown that rice elicited the greatest inter-individual variations in GR compared to spaghetti and carbohydrate drinks (Ranawana and Henry, 2011). Rice is an intrinsic staple for many Asian diets, providing up to two-thirds of daily energy intake in Southeast Asian countries (Kataoka et al., 2013). This makes rice a major contributor to the overall glycemic load of most Asian diets (Hu et al., 2012; Murakami et al., 2006; Villegas et al., 2007). We have recently demonstrated that two commonly consumed rice varieties, Jasmine rice and Basmati rice have high and medium GI respectively (Chapter 4c). As diabetes rates are increasing at alarming proportions in Asian countries (Chan et al., 2009), it is

important to examine and understand key digestive parameters affecting the digestion of this carbohydrate staple.

The first step in the digestion process involves mastication. Recent evidence has shown that mastication plays a crucial role in determining the postprandial GR (Ranawana et al., 2010a; Suzuki et al., 2005). Furthermore, studies have described large variations in chewing parameters between individuals, such as the number of chews per mouthful and chewing time per mouthful (Lassauzay et al., 2000; Woda et al., 2006). This potentially contributes to the variability in GR.

Saliva is an important lubricant secreted by the parotid, submandibular and sublingual glands in the mouth and play a vital role in mastication and swallowing (Lingstrom and Moynihan, 2003). An earlier study showed that postprandial blood glucose concentrations following ingestion of carbohydrate foods, were higher if foods were chewed first, mixed with saliva and then swallowed rather than swallowed whole (Read et al., 1986). This highlighted the importance of salivary α-amylase in the initial starch hydrolysis process in the oral cavity (Hoebler et al., 1998). There has been recent interest in copy number variation (CNV) of the *AMY1* gene and its association with obesity via carbohydrate metabolism (Falchi et al., 2014). An individual can carry between 2 to 16 diploid copies of the *AMY1* gene on chromosome 1p21, which codes for salivary α-amylase (Mandel et al., 2010; Perry et al., 2007). The CNV of *AMY1* is strongly related to the amount of α-amylase in saliva (Mandel and Breslin, 2012; Mandel et al., 2010; Perry et al., 2007). Low levels of *AMY1* CNVs are associated with decreased levels of α-amylase in the saliva and an increased risk of obesity (Falchi et al., 2014). With large inter-individual variations *AMY1* gene copy numbers, we wish to explore whether salivary factors are important determinants of the GR.

Gastric emptying is an important determinant of blood glucose concentrations by controlling the delivery of carbohydrate to the small intestine (Berry et al., 2003; Horowitz et al., 1993; Marathe et al., 2013). Gastric emptying accounts for about 35%

of the variance in the GR to oral glucose and/or carbohydrate-containing meals in health (Horowitz et al., 1993) and T2DM (Jones et al., 1996) and is therefore one of the key digestive processes that we wish to examine in this study.

To our knowledge, there are no studies to date that have investigated numerous digestive factors contributing to inter-individual variability in GR and possible inter-ethnic differences in GR. We therefore aim to focus on mastication, salivary α-amylase activity, *AMY1* gene copy numbers and gastric emptying rate that contribute to the variations in GR.

4e.3 Methods

The methods for this study were described in Chapter 4b.1.1 – 4b.1.9.

4e.3.1 Statistical Analyses

The total glycemic response (GR) over 180 min for each rice variety was expressed as the incremental area under curve (IAUC) ignoring the area under the baseline using the trapezoidal rule (Brouns et al., 2005; FAO/WHO, 1998). Differences in IAUC between Jasmine rice and Basmati rice were analysed using paired t-tests. Correlation analyses and trend analyses were performed to determine the relationships between particle size distribution and mastication parameters as well as saliva α-amylase and *AMY1* copy numbers. The linear regression procedure was used to determine the relationship between the digestive factors and the IAUC for each rice variety. Statistical analyses were conducted using the SPSS version 19 (SPSS Inc, Chicago, Ill). Data were presented as means (standard deviation) where appropriate.

One study on ethnic differences in postprandial glycemia (Henry et al., 2008) between 34 UK Caucasians and 13 Asian Indians, observed at least a 59 $mmol.min^{-1}.L^{-1}$ difference in glycemic response between the 2 groups with standard deviations

of 79 $mmol.min^{-1}.L^{-1}$ and 47 $mmol.min^{-1}.L^{-1}$ respectively. At the 5% level of significance with power of 80%, a minimum sample size of 21 subjects for each group would be required. As we were also investigating ethnic differences, a sample size of 25 from each ethnic group was considered adequate for the current study, with a total sample size of 75.

4e.4 Results

The anthropometric measurements of the participants were within the acceptable normal limits for BMI, fasting blood glucose, and blood pressure (Table 4e-1).

Table 4e-1 Baseline characteristics of participants

Characteristics	**Mean (%)**
Ethnicity	
Chinese	25 (33.3)
Malays	25 (33.3)
Asian-Indians	25 (33.3)
	Mean (SD)
Age (years)	23.4 (2.0)
Weight (kg)	66.1 (7.7)
Height (cm)	173.2 (6.7)
BMI (kg/m^2)	22.0 (1.8)
Systolic blood pressure (mmHg)	125 (9)
Diastolic blood pressure (mmHg)	74 (9)
Mean fasting glucose (mmol/L)	4.52 (0.29)
Waist circumference (cm)	75.3 (5.9)
Body fat (%)	18.1 (6.2)

Figure 4e-1 illustrates the change in GR following the consumption of Jasmine and Basmati rice. The IAUC for Jasmine rice was significantly higher than for Basmati rice (*P<0.001*). For Jasmine rice, peak glucose was observed at 45 min while Basmati rice peak GR was observed earlier at 30 min. The GR for Jasmine rice and Basmati rice were not associated with other baseline characteristics except body fat percentage.

Figure 4e-1 Comparison of incremental change in the blood glucose concentration

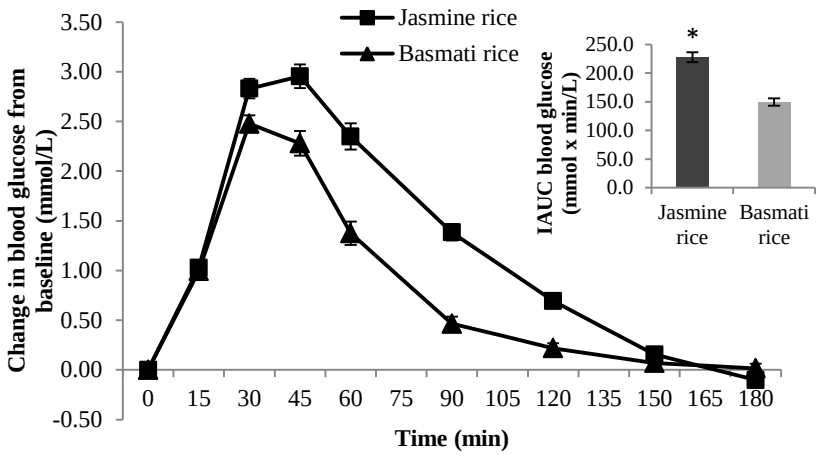

Figure 4e-1 Comparison of incremental change in the blood glucose concentration following consumption of 50g available carbohydrate of Jasmine rice and Basmati rice (n=75). Error bars are standard errors. There is a significant difference in incremental area under the curve (IAUC) for blood glucose response between Jasmine rice and Basmati rice (*P<0.001*).

The median number of *AMY1* gene copies was 6, with a range of 2 to 15 (Appendix 2). In Figure 4e-2, we observed that salivary α-amylase activity increased as *AMY1* gene copy number increased (*P=0.002*). However, fasting salivary α-amylase activity and *AMY1* gene copy numbers were not correlated with postprandial GR for Jasmine rice (salivary α-amylase activity: R=-0.032, *P=0.791*; *AMY1:* R=0.072, *P=0.542*) and Basmati rice (salivary α-amylase activity: R=0.192, *P=0.117*; *AMY1:* R=0.091, *P=0.4*37).

Figure 4e-2 Correlation of salivary α-amylase activity and *AMY1* gene copy numbers

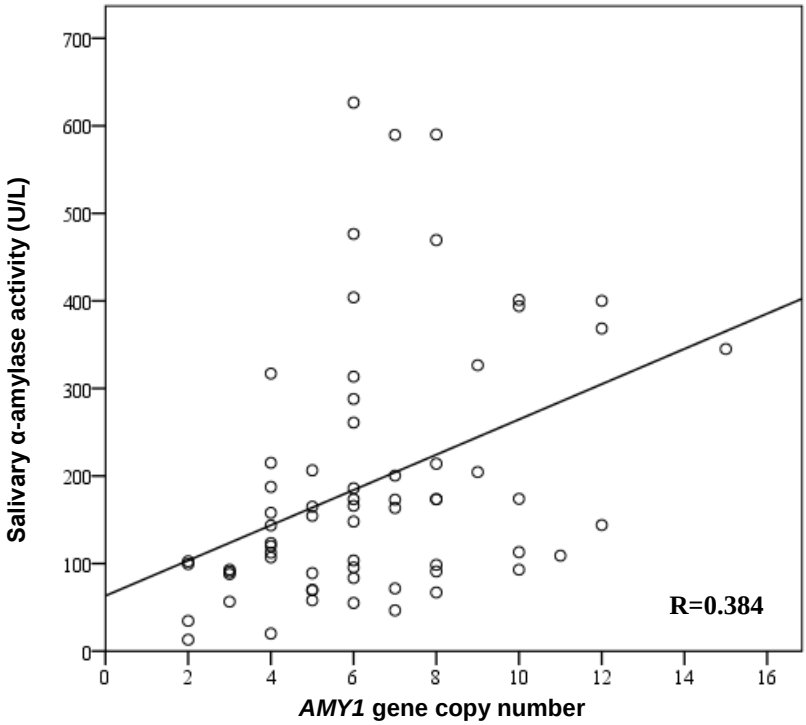

Figure 4e-2 Correlation of salivary α-amylase activity and *AMY1* gene copy numbers. There was a significant positive relationship between *AMY1* copy number and amylase activity (*P=0.002*).

We observed significant correlations between the mastication parameters and particle size distribution of the masticated rice (Table 4e-2). For both rice varieties, particle sizes distribution were generally related to both chews per mouthful and chewing time, but more so for chews per mouthful. We found that the greater the number of chews per mouthful, the fewer the particles size of more than 2000 μm but a greater percentage of smaller particle sizes of less than 500 μm for Jasmine rice (*P for trend <0.001*) and Basmati rice (*P for trend = 0.008*). The relationships between chewing time and particle sizes were more pronounced for larger particle sizes of more than 2000 μm but less so for smaller particle sizes of less than 500 μm.

Table 4e-2 Correlation between mastication parameters and particle size distribution in masticated rice

Particle size distribution (%)	Jasmine rice				Basmati rice			
	No. of chews per mouthful		Chewing time per mouthful		No. of chews per mouthful		Chewing time per mouthful	
	R	*P*	R	*P*	R	*P*	R	*P*
>2000 µm	-0.405	*<0.001*	-0.292	*0.011*	-0.364	*0.001*	-0.236	*0.042*
>1000-<2000 µm	0.423	*<0.001*	0.338	*0.003*	0.307	*0.007*	0.228	*0.049*
>500 - <1000 µm	0.514	*<0.001*	0.387	*0.001*	0.397	*0.001*	0.221	*0.057*
<500 µm	0.318	*0.005*	0.215	*0.064*	0.340	*0.003*	0.214	*0.065*
P for trend	*<0.001*		*0.008*		*0.008*		*0.208*	

Table 4e-3 details the regression analyses of various determinants of GR to Jasmine and Basmati rice. For both rice varieties, the number of chews per mouthful was positively associated with the GR while chewing time per mouthful were negatively associated with GR. Adjustment for total adiposity did not alter the relationships between mastication parameters and GR. Ethnicity, fasting salivary α-amylase activity, particle size distribution, gastric emptying rate and *AMY1* gene copy numbers were not significant contributors to GR (*P>0.05*).

Table 4e-3 Regression analysis of determinants of glycemic response to Jasmine and Basmati rice

Parameters	Jasmine rice			Basmati rice		
	β	*P-value*	95% CI	β	*P-value*	95% CI
Race (Malay)	0.035	*0.838*	-49.014, 60.221	0.185	*0.243*	-15.606, 60.259
Race (Asian-Indian)	0.067	*0.659*	-37.149, 58.304	0.172	*0.266*	-16.230, 57.761
No. of chews per mouthful	0.732	*0.011*	0.639, 4.841	0.944	*0.005*	0.730, 3.966
Chewing time taken per mouthful (s)	-0.531	*0.039*	-5.640, -0.150	-0.774	*0.016*	-4.877, -0.533
Saliva α-amylase activity (fasted) (U/L)	0.055	*0.686*	-0.088, 0.132	0.241	*0.088*	-0.014, 0.203
Particle size <500 µm	0.205	*0.657*	-4.363, 6.862	0.395	*0.505*	-3.832, 7.699
Particle size >2000 µm	0.345	*0.481*	-2.935, 6.158	0.656	*0.281*	-2.102, 7.095
Gastric emptying rate (%)	-0.276	*0.057*	-3.660, 0.57	-0.013	*0.922*	-1.213, 1.099
Diploid AMY1 gene copy numbers	0.144	*0.273*	-3.148, 10.936	0.042	*0.757*	-4.664, 6.380

Race (Chinese) was used as the reference group.

4e.5 Discussion

In this study, we examined the physiological processes involved in the postprandial regulation of carbohydrate metabolism following the consumption of two rice varieties.

Jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand), an aromatic low-medium amylose white rice (11-18%) (Suwansri and Meullenet, 2004) is commonly consumed in South East Asia and was recently found in a local study to have a high GI value of 75.8 (Tan et al., 2015). Basmati rice (Dreamrice^{TM}, Singapore) has a medium amylose content (20-25%) (Bhattacharjee et al., 2002) and have a medium GI value of 56.7 (Tan et al., 2015). We showed that the glycemic response IAUC was significantly higher for Jasmine compared to Basmati rice. Our findings reinforced existing evidence that the consumption of a high-GI food such as Jasmine rice led to higher postprandial GR (Ranawana et al., 2010a; Sun et al., 2014; Tan et al., 2015) as well as a rapid decline in blood glucose levels (Ludwig, 2002; Ludwig et al., 1999). This can lead to excessive hunger (Campfield et al., 1996; Strachan et al., 2004) and consequently overeating, in order to restore blood glucose to normal. This can contribute to problems such as overweight, obesity and increased risk of T2DM, especially in Asian populations where white rice is the major contributor to the overall glycemic load of the diet (Hu et al., 2012; Murakami et al., 2006; Villegas et al., 2007). It is therefore prudent to advice on consumption of rice with a lower GI that may reduce the glycemic load of the diet to reduce future risk of T2DM.

Our findings showed that increased *AMY1* copy numbers was positively associated with salivary α-amylase activity. This is consistent with existing evidence illustrating that individuals with high α-salivary amylase activity had more *AMY1* gene copies within their genomes than did the low salivary α-amylase activity individuals (Mandel and Breslin, 2012; Mandel et al., 2010). We did not observe any

associations between salivary α-amylase activity or *AMY1* copy numbers with postprandial GR. Research has shown that individuals with high endogenous salivary α-amylase activity had improved glycemic homeostasis than those with low salivary α-amylase activity (Mandel and Breslin, 2012), following the ingestion of a starch solution, which did not take into account individual differences in starch digestion. Findings from our study, using two commonly consumed rice varieties and measuring key digestive parameters, did not demonstrate this relationship with glucose homeostasis. This suggests that the genetic link between *AMY1* and carbohydrate metabolism may be modulated by other mechanisms other than through influencing GR. This warrants future investigation into possible biological explanations underpinning the link between *AMY1*, salivary α-amylase amounts and activity with carbohydrate metabolism.

The median *AMY1* copy number in this study (6 copies) was higher than that reported by Mandel et al. (2010) (Mandel et al., 2010) (4 copies). Perry et al. (2007) (Perry et al., 2007) noted that individuals from populations with traditionally high-starch diets (7 copies) have on average more *AMY1* copies than those with low-starch diets (5 copies). We further investigated whether there were ethnic differences in *AMY1* gene copy numbers among the participants in this study. We would have expected ethnic differences in *AMY1* gene copies due to inherent differences in traditional cultural diets in a multi-ethnic Asian population. Unexpectedly, we found no ethnic differences in *AMY1* gene copy numbers between Chinese, Malays and Asian-Indians. The reason behind this is currently unclear. Further investigation in future studies is integral in view of recent evidence showing reduced *AMY1* copy number was associated with increased BMI and obesity risk (Falchi et al., 2014). Nonetheless, the results provided in this study provided an overview of the *AMY1* gene copy number variation in a multi-ethnic Asian population. This can serve as a

reference for further genetic population studies, which may correlate these polymorphisms with other phenotypic features.

We found significant correlations between mastication parameters and the particle distribution of the mastication food. In this study, the number of chews per mouthful were consistently correlated with the particle size distribution, in which the greater the number of chews, the larger the proportion of smaller particle sizes (500 to <2000 μm). As an intact grain, the starch in rice needs to be released for digestion via mechanical breakdown (Kaur et al., 2015). Thorough chewing increases the bioaccessibility of nutrients to enzymatic activity due to increased breakdown of the food matrix (Al-Rabadi et al., 2009) and increases digestion rate (O'Dea et al., 1981).

In this study, we observed that mastication parameters but not particle size, predicted GR despite the close correlations between these two factors. For both rice varieties, our results showed a positive relationship between the number of chews at each mouthful and the GR. These findings were corroborated by a local recent study, which showed that the GR was attenuated by lesser chews per mouthful as a result of increased particle sizes (Sun et al., 2015). Our results suggest that mastication rate may be a significant predictor of GR and that chewing less will result in a lower GR. We postulate that more mastication will result in finer particle size, which aids in the breakdown of the complex carbohydrate structure, resulting in higher GR, though we are unable to show the significant correlation with particle sizes as measured by our method, which may have its limitation.

The time taken for chewing each mouthful, or eating rate was inversely associated with GR. It has been reported that a longer oral processing time (eating slowly) leads to a more pronounced anorexigenic gut hormone (PYY and GLP-1) response (Kokkinos et al., 2010). GLP-1 potentiates insulin secretion and regulates glucose homeostasis (Ronveaux et al., 2015). This then leads to a lower overall GR, which we postulate is a possible mechanism for the observed results in this study.

Eating at a physiologically moderate pace produce a more pronounced anorexigenic gut peptide response (PYY and GLP-1) than eating very fast (Kokkinos et al., 2010), which elicited a weaker anorexigenic gut hormone response. Collectively, evidence has shown that a shorter oral processing time or a fast eating rate, can promote overeating (Andrade et al., 2008; de Wijk et al., 2008; Zijlstra et al., 2009). As this study provided a fixed amount of rice for each participant, we hypothesized that eating fast could lead to greater consumption of food within a shorter period of time, thus resulting in a higher GR. Further studies are pertinent to determine the mechanisms involved in the satiety response between fast and slow eaters. Taken together, these results suggest that eating slowly and having larger food boluses before swallowing (less chewing) may be beneficial in glycemic control.

Gastric emptying rate was not associated with GR for Basmati rice although there was a marginal relationship for Jasmine rice. There is still not a consistent picture regarding the role of gastric emptying on the GR with studies suggesting that higher gastric emptying rate results in a lower GR (Mourot et al., 1988; Zhu et al., 2013), or a higher GR (Berry et al., 2003; Ranawana et al., 2011). These discrepant results may be due to differences in the methods used in measuring gastric emptying or characteristics of the test meal such macronutrient composition and food viscosity (Zhu et al., 2013). We are not aware of any studies that have examined the impact of rice consumption on gastric emptying between Asian ethnicities. These findings have thus provided evidence for similar gastric emptying capacity between Asian ethnicities, at least for two commonly consumed rice varieties.

The strengths of this study were that it measured GR together with key physiological digestive factors of three ethnic groups fed identical foods within a common environmental milieu. To our understanding this was the first study that examined numerous digestive parameters simultaneously (salivary α-amylase, mastication rate, particle size distribution and gastric emptying) and its association

with glycemic response within the same individual and between ethnic groups. We have also provided an overview of the *AMY1* gene copy number variation in a multi-ethnic Asian population, which can serve as a reference for further genetic population studies among Asian ethnicities. Through our findings, we have understood the relationships between mastication parameters (chews per mouthful and chewing rate) and glycemic response, which are potentially modifiable eating behaviour. Although the difference in GI between Jasmine rice and Basmati rice, the association between *AMY1* copy numbers and salivary *α*-amylase activity as well as the correlation between mastication parameters and particle distribution have been previously shown, they were reported in Caucasian populations. It is known that Asians and Caucasians have different glycemic response profile but data in Asian population is lacking. Thus, we wish to demonstrate these findings in our multi-ethnic Asian population.

However, we recognised that the limitation of this study was that we did not include intestinal transit time, which is another factor known to influence GR (Clegg and Shafat, 2010). Mastication force was also not measured as chewing force is an important measurement when making inferences to the oral stage of digestion. We also did not measure appetite-related hormones, which was a limitation as these exert incretin effect and influences glycemia. However, we did not wish to hamper recruitment rate due to increased blood collection as well as budget constraint. Salivary α-amylase concentration was not determined in this study, but it has been found that higher *AMY1* gene copy numbers and higher α-salivary activity highly correlates with higher concentrations of salivary α-amylase (Mandel and Breslin, 2012; Mandel et al., 2010). However, it might be possible to have two individuals with the same concentration of salivary α-amylase, with rather different levels of salivary enzymatic activity, and vice versa. We also consider low power as a potential explanation for the absence of relationship between postprandial GR with salivary α-

amylase activity, *AMY1* copy numbers, gastric emptying rate as well as ethnic differences in *AMY1* copy numbers.

In summary, we have determined the contribution of key digestive parameters on GR. The mastication parameters showed a significant relationship with GR – the greater the number of chews at each mouthful and a shorter chewing time (faster eating rate), the higher the GR. We recommend that future work can focus on measuring the gut hormones profile, where further understanding of the satiety mechanisms will provide greater insight into the physiological factors influencing inter-individual and ethnic variability in GR.

4e.6 Acknowledgement

The candidate was responsible for the conception and design of the study, carried out the study, responsible for the analysis of the samples, carried out the statistical analysis and drafted the manuscript.

The manuscript is provisionally accepted with revision by the European Journal of Nutrition (Jun 2015).

CHAPTER 5 Summary and Integration

This thesis presents the findings of studies relating to diet in individuals with Type 2 Diabetes Mellitus (T2DM), comparing the postprandial responses following liquid meal and rice in lean healthy subjects from the three ethnic groups, and examined the physiological digestive factors that influence glycemic and insulin responses.

5.1 Ethnic dietary differences in individuals with T2DM

The focus of the first study was on the ethnic differences in dietary habits, especially the Glycemic Index (GI) of the diet among individuals with T2DM in Singapore. We found ethnic differences in metabolic profiles, where Malays were more obese and had higher total and LDL-cholesterol than Chinese and Asian-Indians. In terms of dietary intake, Malays and Asian-Indians consumed more carbohydrates while Chinese consumed more protein. Although the total fat intake was similar across ethnic groups, Malays consumed the greatest proportion of energy in the form of saturated fat and Chinese the least. This seemed to explain, at least to some extent, the higher LDL-cholesterol observed amongst the Malays. Our findings provided some evidence that dietary factors contribute to the observed differences in metabolic measures amongst the three ethnic groups.

Carbohydrate intake, in particular its quantity and quality was a focal point in this study. Advice regarding reducing intake of beverages and sweetened drinks was paramount for the Malays who obtained about twice as much carbohydrate from these sources as the other two groups. Food-based interventions would need to focus on the

quantity of these carbohydrate sources, especially amongst the Malays where high intakes of sugar was common.

In investigating the quality of carbohydrate consumed, we found that all three ethnic groups had dietary GI values classified within the medium category of between GI 56 to 69, comparable to numerous studies reporting that people with T2DM already consume a moderate GI diet. Clinical trials have shown that low GI diets improve glycemic control in diabetes (Brand-Miller et al., 2003), increase insulin sensitivity (Frost et al., 1998; Rizkalla et al., 2004), and beta-cell function (Wolever and Mehling, 2002), reduce food intake (Warren et al., 2003) and body weight (Ebbeling et al., 2003; McMillan-Price et al., 2006), influence memory (Kaplan et al., 2000) and may reduce serum cholesterol (Kelly et al., 2004). Therefore we suggest that population and individual level interventions targeting changes in carbohydrate quality and quantity in the dietary management of people with T2DM need to keep cultural/ethnic food preferences in mind. In particular, more education needs to be focused on the Malay population, as it appeared that this ethnic group had both less healthy dietary and metabolic profiles. Dietary advice to lower GI foods is clearly of critical relevance to all three ethnic groups for optimal glycemic control. Further prospective interventional studies are required to determine optimal dietary habits for individuals in the management of individuals with T2DM in a multi-ethnic Asian population.

5.2 Ethnic differences in insulin sensitivity and beta-cell function among Asian men

Several studies have reported the importance of insulin secretory defect in relation to decreased insulin sensitivity in the development of glucose intolerance among Asians. (Fukushima et al., 2004; Kodama et al., 2013). In view of the varying

susceptibility to T2DM in the three major ethnic groups in Singapore, we examined the beta-cell function in relation to insulin sensitivity among individuals of Chinese, Malay and Asian-Indian ethnicity.

In a cohort of young, lean and healthy Singaporean, we demonstrated that the Asian-Indians were the least insulin sensitive and had greatest total adiposity (in particular abdominal adiposity) among the three major Asian ethnic groups. In addition to the differences in insulin sensitivity, these lean and young Chinese, Malays and Asian-Indians also exhibit differences in beta-cell function. Following a mixed-meal challenge, the Malay subjects showed higher glucose excursion compared with Asian-Indians and Chinese. However, there were no significant ethnic differences in the incremental insulin response. The oral disposition index, which reflects beta-cell function relative to the prevailing insulin sensitivity, was the lowest in Malays, followed by Asian-Indians and Chinese. This suggests that Malays might have inadequate beta-cell secretory ability to compensate for their prevailing insulin sensitivity

To our knowledge, there was no prior publication that compared the beta-cell function in relation to insulin sensitivity between Malay ethnicity and other ethnic groups. Compared to lean Chinese, lean Asian-Indian males were less insulin sensitive but they were able to compensate by increasing insulin secretion to maintain normoglycemia. Lean Malays on the other hand, showed less compensatory insulin secretion to prevailing insulin sensitivity. In essence, the paths leading to T2DM might differ between Malay and Asian-Indian ethnic groups. With this background, we postulate that the Malays may be facing a rapid increase in the incidence of diabetes with rising prevalence of obesity, and that measures to maintain healthy body weight would be key to mitigate this epidemic.

5.3 Investigating inter-individual differences in glycemic and insulin responses

There is extensive evidence that biological digestive factors vary between individuals, which influence the postprandial glycemic response (GR) and insulin response (IR). This study was designed to investigate inter-individual differences in GR and IR and examine how physiological digestive factors contribute to inter-individual variability in GR and IR. Specifically, we also aimed to evaluate the influence of ethnicity contributing to the inter-individual variations.

5.3.1 The glycemic and insulin response

Using two commonly consumed rice varieties, we have shown that the GR did not differ between ethnic groups for Jasmine rice and Basmati rice. The IR, on the other hand, was consistently higher among Asian-Indians for both rice varieties. This could be attributed to compensatory hyperinsulinemia in the presence of insulin resistance (evidenced by higher HOMA-IR amongst the Asian-Indians) to maintain normoglycemia amongst the Asian-Indians compared to the Chinese and Malays.

5.3.3 The Glycemic index (GI) and Insulinemic Index (II)

The glycemic index (GI) and insulinemic index (II) of both rice varieties did not differ significantly between ethnicities, with Jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand) being classified as high GI (GI 91) and Basmati rice (DreamriceTM, Singapore) as medium GI (GI 59) (Tan et al., 2015). While it is known that Jasmine rice, with its low amylose content (11% - 18%) (Suwansri and Meullenet, 2004) has a high GI (Atkinson et al., 2008), it was unexpected that Basmati rice, an intermediate amylose rice (20% - 25%)

(Bhattacharjee et al., 2002) and claimed low GI on its packaging, was classified as medium GI in our study. This was reflected in the overall group mean as well as ethnicity mean. In Singapore, there is a paucity of a local GI database and thus there is an urgent need to uncover carbohydrate alternatives that are truly low GI and tested in our local population.

The overall II for Jasmine rice was 76 and Basmati rice was 57, which reflect that Jasmine rice elicits a greater insulin response than Basmati rice, independent of the participants' metabolic status. There is currently no cut-offs nor classification for II values to food. Future work can thus focus on establishing the corresponding II values when determining the GI of foods for greater clinical utility in the prevention and management of T2DM.

5.3.2 Different glycemic and insulin responses between studies

The earlier study alluded to ethnic differences in GR following a liquid mixed-meal challenge with Malays having the highest glucose excursion compared with Asian-Indians and Chinese. There were no significant differences in the post-meal plasma insulin responses between ethnic groups. On the other hand, this current study did not observe any ethnic differences in GR following rice consumption but insulin responses between ethnic groups were markedly different.

We postulate that the disparate results between the two studies were due to the differing test meals with respect to the overall caloric content and nutrient composition (Table 5-1). The regulation of postprandial glucose homeostasis involves both the direct stimulation of insulin release by the digested food as well as the secretion of incretin hormones, namely gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Drucker and Nauck, 2006; Meier and Nauck, 2005; Vollmer et al., 2008). It is well established that fat and protein ingestion stimulate

GLP-1 and GIP secretion (Deacon, 2005; Elliott et al., 1993; Herrmann et al., 1995). GLP-1 stimulates insulin secretion in a glucose-dependent manner following ingestion of carbohydrates (Suzuki et al., 2011) and suppresses glucagon secretion (Cummings and Overduin, 2007). Similarly, GIP stimulates insulin release from pancreatic beta-cells in response to a meal (Kieffer, 2003). An earlier study reported that the quantitative contribution of incretin effect to overall postprandial insulin secretion is about 50-70% (Shuster et al., 1988), depending on size of the meal and its composition.

Table 5-1 A comparison of the nutrient composition of test foods between studies

Study	Test food	Type	Amount of carbohydrate consumed (g)	Energy (kcal)	Protein (% energy)	Carbohydrate (% energy)	Fat (% energy)
SAMS	Ensure Plus	Liquid	81	600	16.7	53.9	29.4
Inter-individual differences in GR and IR	Jasmine rice	Solid	50	222	8.1	90.5	1.3
	Basmati rice	Solid	50	233	10.7	87.5	1.8

From Table 5-1, we observed that for the SAMS study, the liquid mixed-meal has a balanced-macronutrient composition. Jasmine and Basmati rice contain minimal fat and protein and were served in 50g available carbohydrate portion, whereas Ensure Plus with almost twice the amount of absolute carbohydrate and nearly three times the caloric content compared to rice was provided.

The much higher carbohydrate load and caloric content of the Ensure Plus liquid meal may also present significantly “greater” challenge and “stress” to the Malay subjects, whom with less beta-cell function, manifested greater glycemic response compared to the “lesser” challenge of the rice. A follow-up study using comparable carbohydrate load of liquid meal and rice will yield important results to support or refute our postulation.

The presence of protein and fat in Ensure Plus would have triggered significant incretin secretion action of GLP-1 and GIP and enhanced the effect of insulin to maintain glucose homeostasis. With increased insulin secretion in the presence of insulin resistance (Asian-Indians) or less robust pancreatic beta-cell function (Malays), the insulin response between ethnic groups might have been altered, resulting in similar response between the ethnic groups. The similar insulin response may have augmented the difference in glycemic response observed between the ethnic groups following consumption of the liquid mixed-meal.

We are cognizant that differences in the nutrient absorption of a liquid mixed-meal versus rice may underlie the differences in results observed following a liquid mixed-meal versus rice consumption. The provision of rice in the second study allowed a more thorough investigation of the various factors that are involved in digestion of solid foods. Mastication is a potential confounding factor in the post-meal glucose and insulin responses as it is established in the literature that postprandial responses differ depending on whether food is chewed and then swallowed or swallowed whole (Read et al., 1986). In addition, the difference in gastric emptying rates between liquid meal and solid food (Hellmig et al., 2006) is another important factor differentiating the results from the two studies.

Although the liquid meal study was conducted as a separate study, the profile of the subjects was similar between both studies. We were interested to examine the trend of early incremental rise in blood glucose levels between the two studies. For the liquid Ensure Plus meal, the rate of early incremental rise in blood glucose for was lower than that of Basmati rice and Jasmine rice, at least for Chinese and Asian-Indians (Table 5-2). This is to be expected as Ensure Plus is classified as low GI, which is known to result in a more gradual rise in blood glucose levels as compared to a higher GI food (Wolever et al., 1991). It is interesting that for the Malays, the rate of glucose increase is similar between low GI Ensure Plus and medium GI

Basmati rice. This provided additional evidence for a lower early phase insulin response because of less robust pancreatic beta-cell function amongst the Malays.

Table 5-2 Comparison of the rate of increase of glucose and insulin from 0 to 30 min by ethnicity

Type of food	Rate of increment from 0 to 30 min	Chinese	Malay	Asian-Indians	*P-value*
Ensure Plus liquid mixed meal (low GI)	Glucose increment (mmol•min/L)	0.0657 (0.0332)	0.0849 (0.0176)	0.0689 (0.0295)	*0.068*
	Insulin increment (mU•min/L)	2.48 (1.12)	2.72 (2.18)	3.64 (2.44)	*0.191*
Basmati rice (medium GI)	Glucose increment (mmol•min/L)	0.0792 (0.0255)	0.0837 (0.0230)	0.0845 (0.0265)	*0.720*
	Insulin increment (mU•min/L)	1.11 (0.54)	1.66 (0.98)	2.11 (1.17)	*0.001*
Jasmine rice (high GI)	Glucose increment (mmol•min/L)	0.0980 (0.0275)	0.0973 (0.0272)	0.0869 (0.0305)	*0.305*
	Insulin increment (mU•min/L)	1.32 (0.61)	1.80 (0.85)	1.86 (0.97)	*0.042*

Data are presented as mean (SD)

Integrating all the postprandial glucose and insulin responses observed, it appears that for the Asian-Indians, who are consistently found in two studies to be less insulin sensitive despite being young and lean, will benefit from consuming foods that are much lower in GI. This would reduce the insulin demand in order to combat the greater rise in blood glucose levels following the consumption of a more rapidly digested food (higher GI food). Chronically elevated insulin levels leads to eventual pancreatic beta-cell exhaustion (Hayashi et al., 2013) and predisposing to T2DM (Hayashi et al., 2013; Lorenzo et al., 2010; Weyer et al., 1999). Conversely, Malays were shown to exhibit lower beta-cell function in relation to insulin sensitivity. Measures to maintain healthy body weight could be a key strategy to mitigate the development of beta-cell dysfunction and eventual overt T2DM in this ethnic group.

5.3.4 AMY1 gene copy numbers derived from blood, buccal cells and saliva

We conducted a sub-study to investigate within-individual differences *AMY1* gene copy numbers derived from blood, cheek cells and saliva. We found that *AMY1* gene copy numbers from the same individual were different depending on which biological sample was used. We postulate that this could be because *AMY1* gene is a highly polymorphic gene showing extensive copy number variation ranging from 2 diploid copies to as many as 16 copies (Perry et al., 2007). It is therefore pertinent that a single biological sample is used for determining *AMY1* gene copy numbers due to the large within-individual variability between different biological samples. This is applicable especially for the determination of other genes of high copy numbers.

5.4 Limitations

We recognized some limitations to our collective findings. For the rice study, we did not measure incretin responses, which may provide mechanistic answers to the findings. This was because we did not wish to hamper recruitment rate due to increased blood collection. We also had to take into account budget limitation. Moreover, the measurement of incretin responses would have defeated the primary purpose of study, which examined digestive factors and their influence on glycemic and insulin responses. For future study, it would be useful to include females, although we need to take into account the variation insulin resistance that occurs in different phases of the menstrual cycle.

5.4 Conclusion

Through a collection of studies in this thesis, we have comprehensively profiled the dietary habits of Asian ethnicities with T2DM in Singapore, understood glucose homeostasis and beta-cell function relative to insulin sensitivity following a

liquid mixed meal challenge amongst lean and healthy individuals, and derived some physiological explanations for ethnic variability in glycemic and insulin responses through measuring factors that are involved in food digestion.

With Asian diets being markedly different from a western diet, the comprehensive profiling of dietary intake of Asians with T2DM will allow more targeted nutrition intervention in diabetes management. From our findings in Chapter 3, we postulated that the pathways to T2DM might differ between Malay and Asian-Indian ethnic groups. This understanding of the early pathogenesis of T2DM among the susceptible ethnic groups is thus critical to designing effective prevention health-care strategies. Public health strategies aimed at prevention of weight gain and obesity will probably be more cost effective than treatment of consequences such as diabetes (Yoon et al., 2006). Additionally, through measuring physiological processes that are involved in postprandial regulation of carbohydrate metabolism, we found that digestive function contributes significantly to glycemic response.

Findings from this study have provided deeper insights into how biological variations between individuals can explain for the varying prevalence of obesity and T2DM among the three main ethnic groups in Singapore. Dietary intervention and preventative health measures can thus reduce the chronic disease burden and improve the quality of life for the country.

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Appendix 1

Protocols for purification of DNA from blood, buccal swabs and saliva

a) DNA extraction from blood using QIAamp® DNA Mini Kit (Qiagen, Netherlands)

(Adapted from QIAamp DNA Mini and Blood Mini Handbook 06/2012)

Procedure

1. Pipette 20 µl QIAGEN Protease stock solution (or proteinase K) into a 1.5 ml microcentrifuge tube.
2. Add up to 200 µl blood sample to the microcentrifuge tube.
3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15s.
4. Incubate at 56°C for 15 min. Briefly centrifuge to remove drops from inside the lid.
5. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15s. Briefly centrifuge to remove drops from inside the lid.
6. Carefully apply the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim.
7. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.
8. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.
9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for

1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.

10. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim.

11. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

12. Place the QIAamp Mini spin column in a new 2 ml collection tube and centrifuge at full speed for 1 min.

13. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube.

14. Carefully open the QIAamp Mini spin column and add 50 µl Buffer AE or distilled water.

15. Incubate at room temperature (15–25°C) for 5 min, and then centrifuge at full speed for 1 min.

16. The eluted DNA was stored at –20°C until further analysis.

b) DNA extraction from buccal swabs using QIAamp® DNA Mini Kit (Qiagen, Netherlands)

(Adapted from QIAamp DNA Mini and Blood Mini Handbook 06/2012)

Procedure

1. Pipette 20 µl QIAGEN Protease stock solution (or proteinase K) and 600ul of Buffer AL into a 2ml microcentrifuge tube.

2. Place buccal swab in a 2 ml microcentrifuge tube. Add 600ul of PBS to the sample. Mix immediately by vortexing for 15 s. Incubate at room temperature for 5 min.

3. Incubate at 56°C for 15 min. Briefly centrifuge to remove drops from inside the lid.

4. Add 600ul of ethanol (96–100%) to the sample, and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.

5. Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim.

6. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

7. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.

8. Repeat step 5 by applying up to 700 µl of the remaining mixture from step 4 to the QIAamp Mini spin column. (Repeat step 5 until all the samples have been loaded into the spin column, the DNA in the sample will bind to the membrane on the spin column)

9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the filtrate.

10. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim.

11. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

12. Place the QIAamp Mini spin column in a new 2 ml collection tube and centrifuge at full speed for 1 min.

13. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube.

14. Carefully open the QIAamp Mini spin column and add 50 µl Buffer AE or distilled water.

15. Incubate at room temperature (15–25°C) for 5 min, and then centrifuge at full speed for 1 min.

16. The eluted DNA was stored at –20°C until further analysis.

c) DNA extraction from saliva using prepIT.L2P (DNA Genotek, Ottawa, ON, Canada)

Procedure

1. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.
2. Incubate the sample at 50°C in a water bath for minimum 1 hour.
3. Transfer 500 µl of the sample to a 1.5 ml microcentrifuge tube.
4. Add 20 µl of PT-L2P to the microcentrifuge tube and mix by vortexing for a few seconds.
5. Incubate on ice for 10 min. Centrifuge at 15,000 x *g* for 5 min.
6. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.
7. To 500 µl of supernatant, add 600 µl of room temperature ethanol (95-100%). Mix gently by inversion 10 times.

8. Allow the sample to stand at room temperature for 10 min to allow the DNA to fully precipitate.
9. Place the tube in the microcentrifuge in a known orientation. Centrifuge at 15,000 x *g* for 2 min.
10. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.
11. Ethanol wash: Carefully add 250 µl of 70% ethanol. Let the sample stand at room temperature for 1 min. Completely remove the ethanol without disturbing the pellet.
12. Add 100 µl of TE solution to dissolve the DNA pellet. Vortex for at least 5 s.
13. To ensure complete rehydration of the DNA (pellet and smear), incubate at 50°C for 1 hour with occasional vortexing.
14. The fully rehydrated DNA was stored at –20°C until further analysis.

<u>Quantification of DNA using NanoDrop 1000 version 3.7.1</u> (Thermo Scientific, USA)

1. Turn on the NanoDrop machine.
2. On the monitor, select and open the NanoDrop program.
3. Select 'Nucleic Acid'
4. Gently clean the machine with clean-wine and distilled water.
5. Place 1 µl of nuclease-free water on the reader to and click 'OK' to initiate the machine.
6. Wipe off the water with clean-wipe. Place 1 µl of nuclease-free water on the reader and click 'Blank'
7. Wipe off the water with clean-wipe. Load 1 µl of sample on the reader and click 'Measure'.

8. Wipe off the sample using clean-wipe, then clean with nuclease-free water, dry the reader. Then, load 1 µl of another sample
9. Repeat step 8 for other samples

The purity of the DNA sample is determined by the UV absorbance, A_{260}/A_{280} ratio, and should be between 1.7-2.0

Quantification of *AMY1* gene copy number using TaqMan® Copy Number Assays

Note: All DNA samples were thawed on ice

Dilution of the DNA samples

1. Dilute each sample to 5 ng/µL using nuclease-free water to make a 5X stock solution.
2. Use the NanoDrop machine to determine the concentration and A_{260}/A_{280} ratio should be greater than 1.7

Prepare the reactions for liquid DNA

Calculate the volumes of components needed, based on the reaction volume and the number of reactions. Include excess volume in your calculations to provide for the loss that occurs during reagent transfers.

(Note: 4 replicates were performed for each DNA sample)

For a 96 well plate, each well contained the following:

S/N	Reaction mixture component	Volume per well (µL)
1.	2X TaqMan® Genotyping Master Mix	10.0
2.	20X TaqMan® Copy Number Assay	1.0
3.	20X TaqMan® Copy Number Reference Assay	1.0
4.	Nuclease-free water	4.0
5.	DNA sample* (to be added last)	4.0
	Total	**20**

*For non-template control (NTC), substitute the DNA sample with nuclease-free water

1. Completely thaw the TaqMan Copy Number Assays and the TaqMan® Copy Number Reference Assays. Gently vortex the assays to mix them, then centrifuge the tubes briefly to bring contents to the bottom of the tube.
2. Swirl to thoroughly mix the TaqMan® Genotyping Master Mix.
3. Combine the required volumes of reaction components in microcentrifuge tubes.
4. Invert or flick the tubes to mix the contents thoroughly, then centrifuge the tubes briefly.
5. Pipette 16µL of reaction mixture into the wells of the reaction plate that you prepared.
6. Vortex the DNA samples that was prepared and diluted.
7. Add 4µL of DNA (5 ng/µL) into the wells containing the reaction mixture (Note: Substitute with 4µL of nuclease-free water for NTC wells).
8. Seal the reaction plate with optical adhesive film, then centrifuge the reaction plate briefly to remove any bubbles.

Running the plate with Applied Biosystems 7500 Fast Real-Time PCR System

1. Turn on the machine.
2. Open the programme, 7500 System Software Version 1.3.1.
3. Select ‘File’ then ‘New’.
4. To run the plate, under ‘Assay’ select ‘Absolute Quantification (Plate) then click ‘Next’.
5. Under ‘Find’, select ‘*AMY1* gene’ with FAM dye and ‘RNase gene’ with VIC dye
6. Select ALL the wells.
7. Under ‘Task’, change your reference dye to ‘ENDO’ and click ‘Finish’.
8. Select the wells and name them accordingly.
9. Select the empty wells and omit them accordingly.
10. Under ‘Instrument’, check that you have the different stages as stated in protocol,

    Stage 1 – 95°C for 10 min (1 rep)

    Stage 2 – 95°C for 15 s (40 reps)

    Last – 60°C for 1 min

11. Ensure that your sample volume is 20 µl.
12. Always ‘Save’ the settings every time you make changes to the settings.
13. Open the reader, ensure the reader is placed with the correct plate holder and load.
14. Click ‘Start’, wait for the time lapse to appear to ensure everything is running well before leaving the test to run.

Appendix 2

Diploid *AMY1* gene copy numbers of individual subjects.

Subject	Ethnicity	Diploid *AMY1* copies	Standard deviation	Copies (integer)
1	Chinese	6.58	1.10	7
2	Indian	2.10	0.11	2
3	Indian	3.31	0.07	3
4	Indian	4.50	0.74	5
5	Chinese	6.19	0.49	6
6	Chinese	2.27	0.10	2
7	Chinese	5.53	0.16	6
8	Chinese	8.14	0.57	8
9	Chinese	11.87	0.72	12
10	Chinese	5.83	0.13	6
11	Chinese	3.79	0.66	4
12	Chinese	4.76	0.37	5
13	Chinese	6.01	0.24	6
14	Chinese	5.74	0.59	6
15	Chinese	7.48	0.28	7
16	Chinese	4.35	0.64	4
17	Chinese	10.59	0.30	11
18	Malay	6.16	0.25	6
19	Chinese	2.12	0.08	2
20	Chinese	2.97	0.04	3
21	Chinese	6.00	0.25	6
22	Chinese	6.70	0.06	7
23	Chinese	4.13	0.16	4
24	Indian	5.64	0.17	6
25	Chinese	7.56	0.25	8
26	Chinese	8.91	0.10	9
27	Chinese	3.54	0.08	4
28	Chinese	5.04	0.06	5
29	Indian	11.13	0.51	11
30	Indian	5.04	0.32	5
31	Chinese	4.08	0.16	4
32	Indian	15.22	0.17	15
33	Indian	4.76	0.03	5
34	Indian	12.22	0.20	12
35	Indian	6.00	0.06	6
36	Indian	7.13	0.16	7
37	Indian	8.02	0.00	8
38	Indian	10.30	0.03	10
39	Indian	4.03	0.67	4
40	Indian	2.04	0.11	2
41	Indian	4.71	0.01	5
42	Indian	3.70	0.20	4

43	Malay	9.93	0.44	10
44	Indian	2.91	0.04	3
45	Chinese	3.45	0.16	3
46	Indian	7.21	0.04	7
47	Indian	4.51	0.07	5
48	Malay	10.15	0.49	10
49	Indian	7.62	0.17	8
50	Indian	4.95	0.07	5
51	Malay	10.46	0.14	10
52	Indian	6.03	0.30	6
53	Malay	5.99	0.01	6
54	Indian	10.06	0.06	10
55	Indian	6.08	0.14	6
56	Malay	11.61	0.24	12
57	Malay	4.48	0.17	4
58	Malay	3.93	0.55	4
59	Malay	6.20	0.20	6
60	Malay	8.44	0.03	8
61	Malay	4.21	0.01	4
62	Malay	10.11	0.21	10
63	Malay	9.47	0.61	9
64	Malay	10.07	0.47	10
65	Malay	8.11	0.44	8
66	Malay	7.61	0.16	8
67	Malay	8.12	0.34	8
68	Malay	6.42	0.45	6
69	Malay	6.01	0.21	6
70	Malay	5.56	0.11	6
71	Malay	4.08	0.28	4
72	Malay	7.76	0.88	8
73	Malay	7.01	0.18	7
74	Malay	1.77	0.49	2
75	Malay	4.29	0.10	4

Publications

1. **V.M.H. Tan**, T. Wu, C.J. Henry, Y.S. Lee. Glycaemic and insulin responses, glycaemic index and insulinaemic index values of rice between three Asian ethnic groups. British Journal of Nutrition *Apr 2015*; 113 (8): 1228-36.

2. **V.M.H. Tan**, S.Q. Ooi, J. Kapur, T. Wu, Y.H. Chan, J. Henry, Y.S. Lee. The role of digestive factors in determining glycemic response in a multi-ethnic Asian population (manuscript under review)

3. **V.M.H. Tan**, Y.S. Lee, K. Venkataraman, E.Y.H. Khoo, E.S. Tai, Y.S. Chong, P. Gluckman, M.K.S. Leow, C.M. Khoo. Ethnic differences in insulin sensitivity and beta-cell function among Asian men (manuscript under review).

4. **V.M.H. Tan**, S.Q. Ooi, Y.H. Chan, C.J. Henry, Y.S. Lee. Within individual differences in *AMY1* gene copy numbers derived from blood, buccal cells and saliva. (manuscript in preparation)

Oral and poster presentations

1. **V.M.H. Tan**, T. Wu, C.J. Henry, Y.S. Lee (2015). Glycemic index of rice do not differ between Asian ethnic groups. *(Oral presentation at the 9th Asian Pacific Congress of Clinical Nutrition, Kuala Lumpur, Malaysia, 26-29 Jan 2015)*

2. **V.M.H. Tan,** K. Venkataraman, E.S. Tai, Y.S. Lee, M.K.S. Leow, C.M. Khoo. (2013) Glycemic responses following a mixed-meal tolerance test among Chinese, Malay and Asian-Indians in Singapore. (*Poster presentation at the 20th International Congress of Nutrition, Granada, Spain, 15-20 Sept 2013*)

3. **V. Tan**, F.Y. Ong, Y.L. Tan, K. Venkataraman, K. Bhaskaran, J.I. Mann, K.O. Lee, E.S. Tai, Y.S. Lee, E.Y.H. Khoo. (2012). Ethnic differences in dietary glycemic measures of individuals with Type 2 Diabetes Mellitus in Singapore. (*Oral presentation at the International Congress of Dietetics 2012, Sydney, Australia, 5-8 Sept 2012*)

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British Journal of Nutrition, page 1 of 9
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Glycaemic and insulin responses, glycaemic index and insulinaemic index values of rice between three Asian ethnic groups

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Abstract

Asians exhibit larger glycaemic response (GR) and insulin response (IR) than Caucasians, predisposing to an increased risk of type 2 diabetes mellitus (T2DM). We aimed to determine the GR and IR as well as the glycaemic index (GI) and insulinaemic index (II) of two rice varieties among three ethnic groups in Singapore. A total of seventy-five healthy males (twenty-five Chinese, twenty-five Malay and twenty-five Asian-Indians) were served the available equivalent carbohydrate amounts (50 g) of test foods (Jasmine rice and Basmati rice) and a reference food (glucose) on separate occasions. Postprandial blood glucose and plasma insulin concentrations were measured at fasting (−5 and 0 min) and at 15, 30, 45, 60, 90 and 120 min after food consumption. Using the trapezoidal rule, GR, IR, GI and II values were determined. The GR did not differ between ethnic groups for Jasmine rice and Basmati rice. The IR was consistently higher for Jasmine rice (P=0·002) and Basmati rice (P=0·002) among Asian-Indians, probably due to compensatory hyperinsulinaemia to maintain normoglycaemia. The GI and II of both rice varieties did not differ significantly between ethnicities. The overall mean GI for Jasmine rice and Basmati rice were 91 (SD 21) and 59 (SD 15), respectively. The overall mean II for Jasmine rice was 76 (SD 26) and for Basmati rice was 57 (SD 24). We conclude that the GI values presented for Jasmine rice and Basmati rice were applicable to all three ethnic groups in Singapore. Future studies should include deriving the II for greater clinical utility in the prevention and management of T2DM.

Key words: Glycaemic response: Insulin response: Glycaemic index: Jasmine rice: Basmati rice: Ethnic groups: Asians

Numerous studies have shown that Asians, Chinese and non-Caucasians exhibit a 2–3-fold larger postprandial glycaemic response (GR) and insulin response (IR) than Caucasians[1–4]. In a recent study, GR following rice consumption is over 60 % greater among Chinese compared with Europeans[5]. Higher blood glucose concentrations stimulate increased production of insulin, resulting in a state of hyperinsulinaemia. The chronically increased insulin demand may eventually result in pancreatic β-cell failure and, as a consequence, impaired glucose tolerance, leading to type 2 diabetes mellitus (T2DM)[6].

Rice is a staple carbohydrate of many Asian populations, providing up to two-thirds of daily energy intake in Southeast Asian countries[5], making it a major contributor to the overall glycaemic load of most Asian diets[7–10]. The extent to which different varieties of rice influence postprandial glycaemia has potential relevance in the prevention and treatment of obesity and T2DM in Asia, where the recent increase in prevalence outweighs other parts of the world[11]. In many studies, rice consumption, especially white rice, has been implicated in the deterioration of glucose metabolism, which leads to an increased risk of T2DM[9,10,12]. This is probably mediated by the glycaemic potency of rice, as measured by their dietary glycaemic index (GI), a classification of carbohydrates according to their effect on postprandial glycaemia[13,14].

The question whether GI varies between ethnicities is equivocal. Some studies have shown no ethnic differences in GI values[3,15]; however, other studies have demonstrated significant effects of ethnicity on the GI value of white bread[16] and rice[5]. One of the major impediments in the use of GI tables worldwide has been the uncertainty of the applicability to different ethnic groups. If the standard

Abbreviations: GI, glycaemic index; GR, glycaemic response; HOMA-B, homeostatic model assessment-β cell function; HOMA-IR, homeostatic model assessment-insulin resistance; IAUC, incremental AUC; II, insulinaemic index; IR, insulin response; T2DM, type 2 diabetes mellitus.

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British Journal of Nutrition

British Journal of Nutrition

classifications of low, medium and high GI differ between Asians of different ethnicities, this has important implications for dietary recommendations for individuals living in a multi-ethnic Asian population, especially for those with T2DM. Insulinaemic index (II), derived similar to GI, measures the extent to which a food raises plasma insulin levels[13,17,18]. The insulinaemic effects of foods may be relevant to the prevention of T2DM as prolonged postprandial insulinaemia may play a role in the pathogenesis of T2DM[19] and associated chronic diseases[20,21].

The population in Singapore comprises three major ethnic groups: Chinese; Malays; Asian-Indians. Yet, there are significant differences in the prevalence of obesity and T2DM between our three ethnic groups. The Malay population has the highest prevalence of obesity (24·0 %), while our Asian-Indian population has the highest prevalence of T2DM (17·2 %)[22]. This offers us an unusual opportunity to study the effect of different Asian ethnicities on postprandial GR and IR as well as GI and II values to the same food.

The aims of the present study were to examine GR and IR as well as to determine the GI and II of two varieties of commonly consumed rice among the ethnic groups in Singapore. This is the first study known to the authors to examine the role of three ethnic groups residing in a shared and common multi-ethnic Asian environment. The results obtained will provide insights into the metabolic effects of consuming the same food among the different ethnic groups.

Methods

Subjects

A total of seventy-five healthy male participants, consisting of twenty-five Chinese, twenty-five Malays and twenty-five Asian-Indians, were recruited for the present study. The participants initially underwent a screening visit to assess eligibility, which comprised of a health assessment, where anthropometric measurements (Table 1) were taken and a health questionnaire (relating to medical history, smoking habits, history of any illness and use of any medications) was administered. All anthropometric measurements were made at least 2 h after food consumption. Height and weight were measured without shoes by using a digital scale cum stadiometer (SECA 763). A digital blood pressure monitor (Omron HEM-907) was used to obtain blood pressure readings. Waist circumference was measured using a standard measuring tape (SECA tape measure) at the midpoint between the coastal margins of the ribs and the upper margin of iliac crest and the hip circumference at the widest level of the greater trochanters on both sides. Percentage of body fat was measured by air displacement plethysmography method (Bod Pod; Cosmed).

Those who fulfilled all acceptable criteria (BMI 18·5 to $<25\,kg/m^2$, age 18–45 years, both parents and grandparents were of the same ethnicity, blood pressure 110–120/70–90 mmHg, fasting blood glucose 4–6 mmol/l, not on prescription medication, non-smoking, and no genetic or metabolic diseases) were included in the present study. On the day before the test visit, participants were asked to avoid intake of alcohol, restrict caffeine-containing drinks, refrain from intense strenuous physical activity and to consume their usual meals. Participants were also requested to fast overnight for 10–12 h before each test visit.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the National Healthcare Group Domain Specific Review Board, Singapore. All the participants provided written informed consent before their participation in the study. This was registered at clinicaltrials.gov as NCT01804738.

Study procedures

A randomised, cross-over, within-subject, repeated-measure non-blind design was adopted. Participants arrived at the research centre between 08.00 and 09.00 hours after 10–12 h

Table 1. Anthropometric characteristics by ethnicity
(Mean values and standard deviations)

	Chinese (*n* 25)		Malay (*n* 25)		Asian-Indians (*n* 25)		
	Mean	SD	Mean	SD	Mean	SD	*P**
Age (years)	23·0	1·3	23·0	2·3	24·1	2·0	0·072
Weight (kg)	63·8	6·9	65·8	7·1	68·7	8·5	0·073
Height (cm)	172·6	6·0	170·8[a]	6·0	176·2[b]	7·0	0·012
BMI (kg/m^2)	21·4	1·8	22·5	1·7	22·1	1·9	0·085
Systolic blood pressure (mmHg)	125	11	127	8	124	7	0·528
Diastolic blood pressure (mmHg)	76	9	73	8	72	10	0·247
Mean fasting glucose (mmol/l)	4·55	0·31	4·46	0·25	4·54	0·32	0·484
Mean fasting insulin (mU/l†)	7·43	2·85	9·10	3·38	8·10	3·93	0·225
Waist circumference (cm)	75·5	5·4	72·7[a]	4·4	77·7[b]	6·9	0·010
Body fat (%)	16·0[a]	5·9	17·8	5·2	20·5[b]	6·8	0·035
Mean HOMA-IR	1·45[a]	0·53	1·43[a]	0·51	2·16[b]	0·89	<0·001
Mean HOMA-B	165·7[a]	86·1	172·6	76·8	229·2[b]	93·3	0·020

HOMA-IR, homeostatic model assessment-insulin resistance; HOMA-B, homeostatic model assessment-β cell function.
[a,b] Mean values within each row with unlike superscript letters were significantly different ($P<0·05$).
* *P* values represent comparison between the ethnic groups.
† To convert insulin from mU/l to pmol/l, multiply by 6·945.

British Journal of Nutrition

of overnight fast. Following a 10 min rest, two fasting blood samples were obtained 5 min apart for determining baseline blood glucose and insulin levels. After that, participants were given either a reference food or a test food with water to consume at a comfortable pace within 15 min. The reference food consisted of 50 g of glucose anhydrous powder dissolved in 250 ml water, while the test food was Jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand) or Basmati rice (Dreamrice™, Singapore). These two rice varieties were chosen as they were commonly consumed by the local population. Jasmine rice was tested locally to be classified as high GI[23], while Basmati rice was tested in a local laboratory and certified as low GI on its product packaging. Both rice samples were served in portions containing 50 g of available carbohydrates (difference between total carbohydrates and dietary fibre). This amounted to 63·6 g (with 130 ml water) and 66·5 g (with 170 ml water) of uncooked Jasmine rice and Basmati rice, respectively, cooked in individual portions in a rice cooker (Iona GL12 rice cooker, Singapore) and served with 250 ml water. In total, the reference food was tested thrice, and the test food was tested once by each participant in a randomised order. This is in accordance with FAO/WHO recommendations[24] for the determination of GI value that each subject will test each test food once and the reference food thrice in a random order on separate days, with at least 2 d gap between measurements to minimise carry-over effects.

After the commencement of eating, further blood samples were taken at 15, 30, 45, 60, 90 and 120 min. The protocol used to measure blood glucose response was adopted from that described by Brouns *et al.*[25] and is in line with the FAO/WHO recommendations[24]. Blood sample was obtained by finger prick using sterile, single-use lancing device (Accu-check Safe-T-Pro Plus; Roche Diagnostics). Before a finger prick, participants were encouraged to warm their hand to increase blood flow. To minimise plasma dilution, fingertips were not squeezed to extract blood but were gently massaged starting from the base of the hand moving towards the tips. The first two drops of expressed blood were discarded, and the next drop was used for testing. Blood glucose was measured using the HemoCue 201+ Glucose analyzer (HemoCue 201 RT).

For the measurement of blood insulin levels at each time point, after blood glucose measurement, 300 μl of capillary blood (obtained from finger pricks) was collected into Microvette® capillary blood collection tubes treated with di-potassium EDTA (CB 300 K2E; Sarstedt) and stored in crushed ice immediately until the end of the study visit. The Microvette® tubes were centrifuged at 4500 rpm for 12 min (Rotina 420R; Hettich), and the supernatant plasma was transferred into individually labelled 1·5 ml microtubes (Axygen Scientific, Inc.), which were immediately transferred to a freezer held at −80°C until analysis of insulin. Plasma insulin concentrations were determined by electrochemiluminescence immunoassay using an automated analyzer (Cobas E411; Roche Diagnostics). The Cobas system is a reliable method of blood insulin determination[26].

Table 2. Glycaemic and insulin responses to glucose, Jasmine rice and Basmati rice, as incremental AUC (IAUC)
(Mean values and standard deviations)

	Glucose reference				Jasmine rice						Basmati rice					
	IAUC glucose (mmol × min/l)		IAUC insulin (mU × min/l)*		IAUC glucose (mmol × min/l)		IAUC insulin (mU × min/l)*		GI		II		IAUC glucose (mmol × min/l)		IAUC insulin (mU × min/l)*		GI		II	
Ethnicity	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chinese	237·1	68·2	4350[a]	1763	213·8	70·3	3137[a]	1377	91·2	19·2	75·0	28·1	131·6	48·6	2043[a]	866	55·7	13·3	49·9	21·7
Malay	224·2	80·3	4500[a]	1468	201·0	62·1	3515[a]	1275	92·0	20·0	79·4	20·7	140·1	52·6	2724[a]	1334	62·6	13·1	61·6	21·7
Asian-Indians	240·1	90·2	7436[b]	4159	206·6	66·3	5035[b]	2714	90·2	23·4	73·0	28·9	139·3	62·9	4067[b]	2395	59·5	18·1	58·5	27·1

GI, glycaemic index; II, insulinogenic index.
[a,b] Mean values within a column with unlike superscript letters are significantly different ($P<0·05$).
* To convert IAUC insulin from mU × min/l to pmol × min/l, multiply by 6·945.

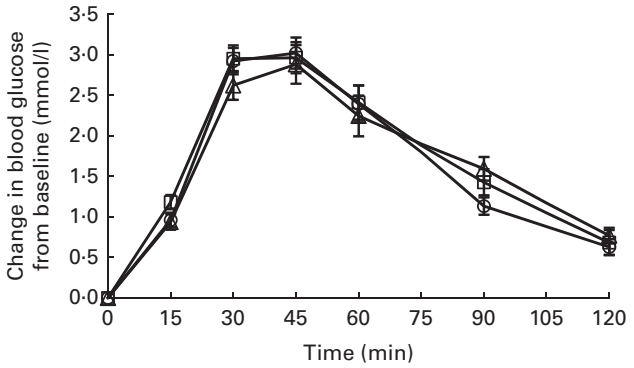

Fig. 1. Temporal blood glucose response curves for Jasmine rice among Chinese (-□-), Malays (-○-) and Asian-Indians (-△-). Data are represented as change in glucose response from baseline over 120 min following the consumption of Jasmine rice. Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA with *post hoc* Bonferroni multiple-comparison tests revealed an overall difference between ethnic groups (P=0·757).

We computed homeostatic model assessment-insulin resistance (HOMA-IR) as follows:

fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5.

Homeostatic model assessment-β cell function (HOMA-B) was calculated using the following formula:

(20 × fasting insulin (mU/l))/(fasting glucose (mmol/l) − 3.5).

(To convert insulin in mU/l to pmol/l, multiply by 6·945).

Statistical analyses

One study on ethnic differences in postprandial glycaemia[3] between UK Caucasians and Asian Indians has observed at least a 59 mmol/min per litre difference in GR between the two groups with standard deviations of 79 and 47 mmol/min per litre, respectively. At the 5% level of significance with power of 80%, a minimum sample size of twenty-one subjects for each group would be required. Studies on *in vivo* assessment of GI have been based on ten subjects, to take into account inter-individual variations[24,25]. As we were investigating ethnic differences, a sample size of twenty-five from each ethnic group (total sample size of seventy-five) was considered adequate for the present study.

The GR data was analysed using 'change in GR', which was calculated by taking the difference between the blood glucose reading at each time point and mean baseline blood glucose value (based on two baseline values taken at 5 min apart). This represented the relative increment in GR at any time point compared with the baseline value. The total GR over 120 min was expressed as the incremental AUC (IAUC) ignoring the area under the baseline using the trapezoidal rule[24,25]. The IR data was analysed in the same manner as the GR data, using 'change in IR'. These data were then used to construct IR curves and calculate the IAUC (using the trapezoidal rule).

The intention-to-treat analysis included data from all the participants. All physiologically valid data were included in the computation of GI and II. In the calculation of GI, the absolute IAUC glucose values for each rice variety was expressed as a percentage of the mean IAUC glucose values of the reference food, and the resulting values were averaged to obtain the GI value for each rice variety. The II, which measures the IR of food, was calculated similar to GI, where the absolute IAUC insulin values for each rice variety were expressed as a percentage of the mean IAUC glucose values of the reference food.

Results were expressed as means with standard deviations where appropriate. Differences in GR and IR and GI values were analysed using one-way ANOVA with *post hoc* Bonferroni correction for multiple comparisons. The temporal GR and IR over 120 min were analysed using repeated-measures ANOVA with *post hoc* Bonferroni correction. Paired t tests were used to compare the IAUC and rates of decline from 45 to 60 min, 60 to 90 min and 90 to 120 min for Jasmine rice and Basmati rice. Correlation analyses were conducted to determine the relationships between GI, II with body composition (waist circumference and percentage of body fat) and metabolic indices (HOMA-IR and HOMA-B). Statistical analyses were conducted using the SPSS version 19 (SPSS, Inc.). A P value $<0·05$ was considered statistically significant.

Results

Table 1 compares the characteristics of the study participants by ethnicity. There were no differences in age, BMI, blood pressure, fasting glucose level and fasting insulin level between the three groups. However, waist circumference (P=0·010) and percentage of body fat (P=0·035) were significantly higher among the Asian-Indians compared with Chinese and Malays. The HOMA-IR, a marker of insulin resistance, was highest for Asian-Indians ($P<0·001$) compared with Chinese and Malays. In parallel with HOMA-IR, Asian-Indians showed a significantly higher HOMA-B (P=0·020) compared with Chinese and Malays. This indicated a compensatory hyperinsulinaemia in the presence of greater insulin resistance among Asian-Indians to maintain fasting normoglycaemia.

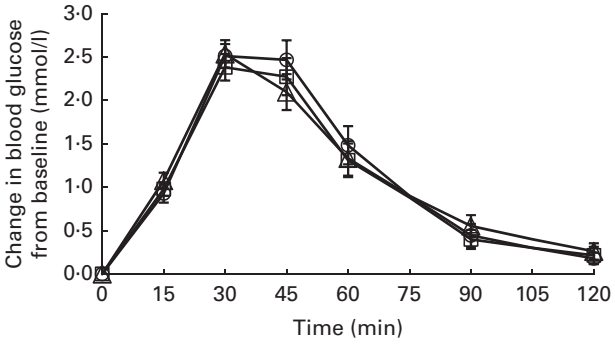

Fig. 2. Temporal blood glucose response curves for Basmati rice among Chinese (-□-), Malays (-○-) and Asian-Indians (-△-). Data are represented as change in glucose response from baseline over 120 min following the consumption of Basmati rice. Values are means, with their standard errors represented by vertical bars. Repeated-measures ANOVA with *post hoc* Bonferroni multiple-comparison tests revealed an overall difference between ethnic groups (P=0·837).

British Journal of Nutrition

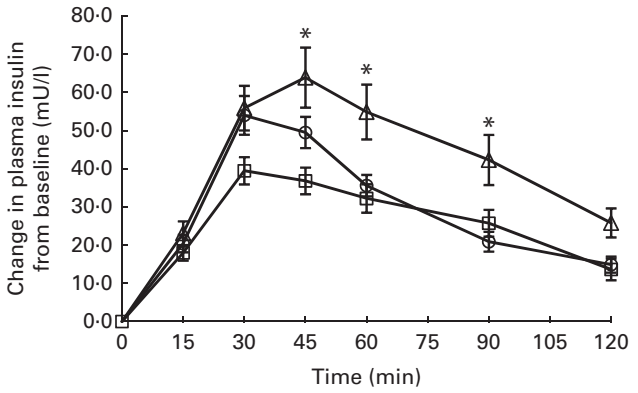

Fig. 3. Temporal plasma insulin response curves for Jasmine rice among Chinese (-□-), Malays (-○-) and Asian-Indians (-△-). Data are represented as change in insulin response from baseline over 120 min following the consumption of Jasmine rice. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different at a specific time point ($P < 0·05$). Repeated-measures ANOVA with *post hoc* Bonferroni multiple-comparison tests revealed an overall difference between ethnic groups ($P=0·002$). To convert insulin from mU/l to pmol/l, multiply by 6·945.

The GR IAUC did not differ between ethnic groups for glucose reference, Jasmine rice and Basmati rice (Table 2). The mean intra-individual variations in GR to the three glucose reference were 17·3 % CV for Chinese, 17·1 % CV for Malays and 16·0 % CV for Asian-Indians ($P=0·841$). These values were consistent with previously reported variability for repeat tests of the reference foods in normal subjects[3,27,28]. The inter-individual variations in GR to the reference food were 28·8 % CV for Chinese, 35·8 % CV for Malays and 37·6 % CV for Asian-Indians. There were also no significant differences in temporal GR patterns for the three groups for Jasmine rice ($P_{interaction} = 0·757$; Fig. 1) and Basmati rice ($P_{interaction} = 0·837$; Fig. 2). Basmati rice showed an initial faster rate of decline from 45 to 60 min (mean difference 2·2 mmol/l per min; $P=0·002$) than Jasmine rice. However, the later postprandial rate of decline was significantly more rapid for Jasmine rice from 90 to 120 min (mean difference 6·7 mmol/l per min; $P<0·001$) compared with Basmati rice.

In contrast, IR between the ethnic groups showed significant differences. The insulin IAUC was consistently higher in Asian-Indians compared with Chinese and Malays for glucose reference ($P<0·001$), Jasmine rice ($P=0·002$) and Basmati rice ($P<0·001$). The temporal IR patterns for Jasmine rice and Basmati rice differed considerably between the ethnic groups (Figs. 3 and 4). Time point analyses showed that the IR for Jasmine rice at 45, 60, 90 and 120 min (Fig. 3) were significantly higher for Asian-Indians than for Chinese and Malay. For basmati rice, the IR at 15, 30, 45, 60 and 90 min (Fig. 4) were also significantly higher for Asian-Indians compared with Chinese and Malays.

As shown in Table 2, the GI of Jasmine rice ($P=0·957$) and Basmati rice ($P=0·277$) did not differ between the ethnic groups. The overall mean GI was 91·1 (SD 20·7) (high GI) for Jasmine rice and 59·3 (SD 15·1) (medium GI) for Basmati rice. The GI values for both rice varieties were not significantly different between the ethnic groups after adjusting for body fat or waist circumference. Similarly, the II for Jasmine rice ($P=0·681$) and Basmati rice ($P=0·197$) were not different between the ethnic groups. The overall mean II for Jasmine rice was 75·8 (SD 25·9) and Basmati rice was 56·7 (SD 23·8). Adjustment for body composition (body fat or waist circumference) made no difference to the II values between the ethnic groups.

Correlation analyses indicated that GI and II for Jasmine rice and Basmati rice were not related to any of the body composition measurements (waist circumference and percentage of body fat) or metabolic indices (HOMA-IR and HOMA-B) ($P>0·05$). These results demonstrate that GI and II were independent of individuals' body composition and metabolic status, making them valid properties of food.

Between rice varieties, we observed that Jasmine rice showed consistently higher GR and IR as well as significantly higher GI ($P=0·001$) and II ($P<0·001$) values (Table 2) compared with Basmati rice for each ethnic group (Figs. 5 and 6).

Discussion

The present study was the first to examine the role of three ethnicities in a multi-ethnic Asian environment on postprandial GR and IR as well as GI and II values to two commonly consumed rice varieties. We observed no ethnic variations in GR to Jasmine rice and Basmati rice; yet, there were considerable differences in IR.

Even though there were no differences in fasting glucose and fasting insulin levels, Asian-Indians mounted significantly higher insulin excursions for both Jasmine rice and Basmati rice compared with Chinese and Malays, even after adjustment for percentage of body fat or waist circumference. These differences in IR were probably due to a compensatory hyperinsulinaemia in the presence of greater insulin resistance among the Asian-Indians to maintain fasting normoglycaemia. Genetically, Asian-Indians have a high prevalence of insulin

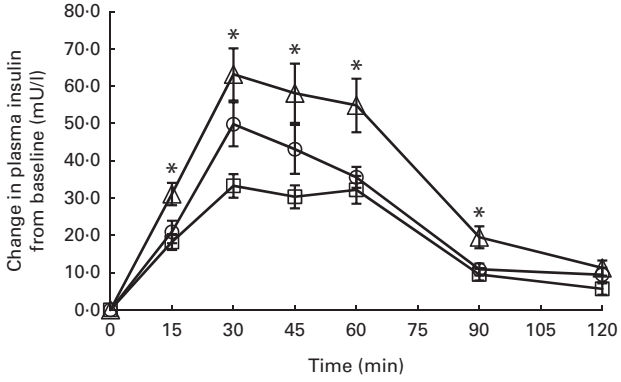

Fig. 4. Temporal plasma insulin response curves for Basmati rice among Chinese (-□-), Malays (-○-) and Asian-Indians (-△-). Data are represented as change in insulin response from baseline over 120 min following the consumption of Basmati rice. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different at a specific time point ($P<0·05$). Repeated-measures ANOVA with *post hoc* Bonferroni multiple-comparison tests revealed an overall difference between ethnic groups ($P < 0·001$). To convert insulin from mU/l to pmol/l, multiply by 6·945.

British Journal of Nutrition

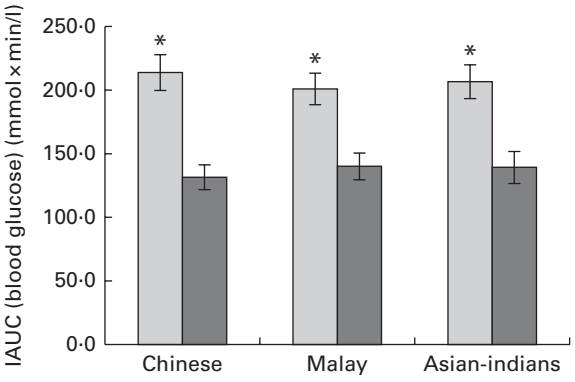

Fig. 5. Comparison of incremental AUC (IAUC) for glycaemic response between ethnic groups following consumption of Jasmine rice (□) and Basmati rice (■). Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different in IAUC glycaemic response between rice varieties for each ethnic group ($P < 0{\cdot}05$).

resistance[29] that may underlie their greater-than-normal tendency to develop diabetes and CVD compared with Chinese and Malays[22,30,31]. In the pathogenesis of T2DM, both β-cell dysfunction and decreased insulin sensitivity play key roles[32]. If insulin sensitivity decreases, insulin secretory response of pancreatic β cells must increase to preserve normal glucose tolerance. This was evidenced by significantly higher HOMA-IR (a steady-state measure of insulin sensitivity) and HOMA-B (a measure of pancreatic β-cell function) among the Asian-Indians compared with Chinese and Malays. Chronic elevated insulin levels lead to impaired action and an exhaustion of pancreatic β cells[33]. Further deterioration of β-cell function and insulin sensitivity will result in worsening of glucose tolerance and risk for T2DM[33,34,35]. Although the GI values for each rice variety were similar between the ethnic groups, the same rice variety presented different stress to Asian-Indians and their pancreatic β islet cells, resulting in higher compensatory IR for both Jasmine rice and Basmati rice, when compared with the Malays and Chinese. This may thus be an important contributing factor to the higher risk of T2DM in this ethnic group.

We did not find any significant differences in GI and II values to Jasmine rice and Basmati rice between Chinese, Malays and Asian-Indians. The present study lends support to the findings of previous research concerning the influence of ethnicity in GI values of foods. In the UK, there were no difference in GI values of biscuits and breakfast cereals between Asian-Indians and Caucasians[3]. Using the standard classification for low (≤55), medium (56–69) and high GI (≥70)[36–38], the overall GI for Jasmine rice was classified as high GI, while Basmati rice was considered medium GI in the present study. While it is known that Jasmine rice, with its low amylose content (11–18%)[39], elicits a greater GR and a corresponding greater IR, it was unexpected that Basmati rice, with an intermediate amylose rice content (20–25%)[40] and claimed low GI on its packaging, was classified as medium GI in the present study (overall group mean as well as ethnicity mean). In Singapore, there is a lack of GI database for locally tested foods, and thus, there is a pressing need to uncover rice varieties that are truly low GI since rice in the major carbohydrate staple in this country[7].

In the present study, we observed no differences in II values of both rice varieties despite ethnic differences in IR. The IR is a biological response to fluctuations in blood glucose levels. The II, however, is an index of the insulinaemic effects of food. The computation of II normalises an individual's IR to a test food against a reference food, similar to the computation of the GI. Therefore, while IR may differ, II, in contrast, remained consistent between individuals. The present study showed that both GI and II are properties of food, not influenced by the anthropometric measures and metabolic status of the participants. We showed that both GI and II were similar between ethnic groups regardless of their degree of insulin sensitivity and adiposity. Recent evidence has also shown that II values of rice were not different between healthy, hyperinsulinaemic and T2DM individuals, although the II was correlated with metabolic status[41].

Our findings and existing evidence have shown that consumption of a high-GI food such as Jasmine rice leads to higher postprandial GR, more rapid decline in glucose levels from 60 min onwards and a correspondingly augmented IR compared with a lower-GI food[42]. A recent study has demonstrated that consumption of a high-GI meal led to a rapid decline in blood glucose levels[43,44]. In addition, brain activity is increased in regions related to food intake, reward and craving in the late postprandial phase[45]. This triggered excessive hunger and a preference for foods, such as high-GI foods that rapidly restored blood glucose to normal[46,47]. This could lead to overeating and contribute to problems such as overweight and obesity. These findings have considerable potential clinical significance given the global epidemic of obesity and T2DM and the especially high rates in Asian countries[1], where white rice is the major contributor to the overall glycaemic load of the diet[7–10]. There was growing evidence that higher white rice consumption (≥300–420 g/d) had been associated with a significantly increased risk of T2DM, especially in Asian populations[9,10,12]. Despite the positive findings of rice consumption and increased

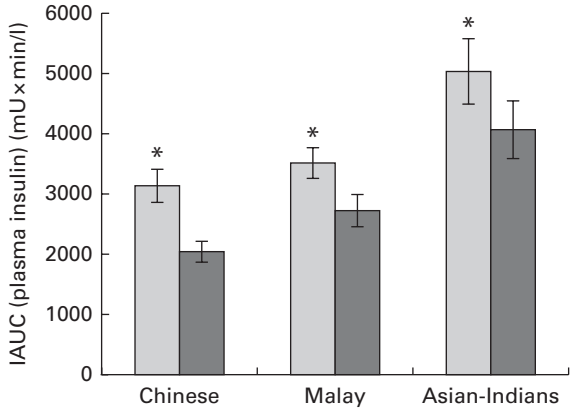

Fig. 6. Comparison of incremental AUC (IAUC) for plasma insulin response between ethnic groups following consumption of Jasmine rice (□) and Basmati rice (■). Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different in IAUC insulin response between rice varieties for each ethnic group ($P < 0{\cdot}05$). To convert insulin in mU/l to pmol/l, multiply by 6·945.

British Journal of Nutrition

risk of T2DM, rice intake was not associated with diabetes in a Chinese population[48] and added no additional risk of CVD in Japanese men and women[49]. Therefore, evidence is still equivocal on rice intake and risk of T2DM in Asian populations.

Nonetheless, rice is a major dietary staple in Asian populations, and it appeared that Asian populations were more susceptible to the adverse effects of high intakes of white rice compared with the Western populations[10]. In Singapore, the mean rice intake among the population was 361·8 (SD 5·7) g/d, with Chinese, Malay and Asian-Indians consuming 364·9 (SD 6·5), 348·1 (SD 15·5) and 352·7 (SD 19·2) g, respectively[7]. This puts the population as being high consumers of white rice. It is, therefore, prudent to advice on consumption of rice with a lower GI that may reduce the glycaemic load of the diet. Additionally, a lower IR can help to improve long-term metabolic control in individuals who are insulin resistant and thereby reducing the future risk of T2DM.

The International Tables of the Glycaemic Index[50] reported a wide range of GI values for different rice varieties, due to differences in the ratio of amylose to amylopectin, physical and chemical characteristics, other botanical structures and processing methods[51–55]. GI values vary even when considering the same variety of rice. For example, the GI values for Jasmine rice range from 48 to 109 and for Basmati rice from 43 to 69[50,56]. As the composition of rice and other carbohydrate-containing foods may vary according to where they are grown, the recommendation that the GI of such foods are best tested locally[36]. In the present study, we have used two locally available and commonly consumed rice varieties among the ethnic groups. The cooking method used was as per the manufacturers' instructions and represented how the population would cook these two rice varieties. There is currently no cut-offs nor classification for II values to food. Future studies can thus focus on establishing the corresponding II values when determining the GI of foods for greater clinical utility.

The strengths of the present study are that it measured the GR of the three ethnic groups fed identical foods within a common environmental milieu. We standardised the cooking method by using the exact amount of water to cook each rice variety in the same rice cooker to ensure consistency in rice texture. Using a standardised cooking method also allows a valid and unbiased comparison to be made between ethnic groups by removing the confounding effects of cooking methods. Moreover, we simultaneously measured the corresponding IR to gain a better understanding of the interplay between postprandial GR and IR as well as to determine both the GI and II values. However, we recognised that the limitation of the present study was that we have only tested two rice varieties and it may not be feasible to extrapolate these findings to all carbohydrate foods. However, these rice varieties were the most commonly consumed among the ethnic groups in Asia and are the major contributors to the overall glycaemic load of the Asian diet.

In summary, the present study has shown that there were no significant differences in the GI and II values to two commonly eaten rice varieties between the three ethnic groups. This suggests that GI values of Jasmine rice and Basmati rice determined in the present study are applicable to all the three ethnic groups in Singapore. Although there are currently no cut-offs for II values, the present results showed that II is a property of food, independent of the metabolic status of the individuals. Future studies testing the GI of foods may include testing the concurrent IR to derive the II for greater clinical utility in the prevention and management of T2DM. Despite similar GI values between the ethnic groups, the same rice presented greater pancreatic β-cell stress to Asian-Indians, resulting in higher compensatory IR, and possibly conferring a higher risk of T2DM in this ethnic group. Preserving β-cell function in at-risk populations is a critical factor in the prevention of T2DM onset[57]. Therefore, therapeutic prevention strategies should focus on encouraging the consumption of rice varieties with lower GI especially among the high-risk groups to maintain glucose homeostasis.

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The authors' contributions are as follows: V. M. H. T., C. J. H. and Y. S. L. contributed to the conception and design of the study; V. M. H. T. and T. W. carried out the study; V. M. H. T. was responsible for the analysis of the blood samples, carried out the statistical analysis and drafted the manuscript. All authors critically reviewed the content of the manuscript and approved the final manuscript.

None of the authors has any conflict of interest to declare.

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ORIGINAL CONTRIBUTION

The role of digestive factors in determining glycemic response in a multiethnic Asian population

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Abstract
Purpose There are wide inter-individual differences in glycemic response (GR). We aimed to examine key digestive parameters that influence inter-individual and ethnic differences in GR in healthy Asian individuals.
Methods Seventy-five healthy male subjects (25 Chinese, 25 Malays, and 25 Asian-Indians) were served equivalent available carbohydrate amounts (50 g) of jasmine rice (JR) and basmati rice (BR) on separate occasions. Postprandial blood glucose concentrations were measured at fasting (−5 and 0 min) and at 15- to 30-min interval over 180 min. Mastication parameters (number of chews per mouth and chewing time per mouthful), saliva α-amylase activity, *AMY1* gene copy numbers and gastric emptying rate were measured to investigate their relationships with GR.
Results The GR for jasmine rice was significantly higher than for basmati rice ($P < 0.001$). The median number of *AMY1* gene copies was 6, with a range of 2–15. There was a significant positive relationship between *AMY1* copy number and α-amylase activity ($P = 0.002$). There were no significant ethnic differences in GR. For both rice varieties, the number of chews per mouthful was positively associated with the GR (JR, $P = 0.011$; BR, $P = 0.005$), while chewing time per mouthful showed a negative association (JR, $P = 0.039$; BR, $P = 0.016$). Ethnicity, salivary α-amylase activity, particle size distribution, gastric emptying rate and *AMY1* gene copy numbers were not significant contributors to GR ($P > 0.05$).
Conclusion Mastication parameters contribute significantly to GR. Eating slowly and having larger food boluses before swallowing (less chewing), both potentially modifiable, may be beneficial in glycemic control.

Keywords Glycemic response · Mastication · Salivary amylase · *AMY1* · Gastric emptying

Abbreviations

BR	Basmati rice
ER	Gastric emptying rate
GI	Glycemic index
GR	Glycemic response
IAUC	Incremental area under curve
JR	Jasmine rice
T2DM	Type 2 diabetes mellitus

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Introduction

Postprandial glycemia has been linked to several chronic diseases such as obesity, type 2 diabetes mellitus (T2DM) and heart disease [1, 2]. A consistent observation in many studies is the wide inter-individual variations in glycemic response (GR) [3–5]. This suggests that variations in physiological digestive factors may contribute to these differences in GR. The influence of ethnicity on GR has been reported extensively showing that Asians display a larger GR compared with Caucasians [6–9]. In Singapore, the differing prevalence of obesity and T2DM among the three main ethnic groups suggests the possibility of ethnic differences in susceptibility; Malays have the highest prevalence of obesity, while the Asian-Indians have the highest prevalence of T2DM [10] compared to the Chinese. No studies to date have examined how physiological factors among the ethnic groups impact the GR. In this study, we wish to investigate key digestive parameters that influence and impact inter-individual and ethnic differences in GR.

Recent evidence has shown that rice elicited the greatest inter-individual variations in GR compared to spaghetti and carbohydrate drinks [11]. Rice is an intrinsic staple for many Asian diets, providing up to two-thirds of daily energy intake in Southeast Asian countries [9]. This makes rice a major contributor to the overall glycemic load of most Asian diets [12–14]. A local study has recently demonstrated that two commonly consumed rice varieties, jasmine rice and basmati rice, have high and medium GI, respectively [15]. As diabetes rates are increasing at alarming proportions in Asian countries [16], it is important to examine and understand key digestive parameters affecting the digestion of this carbohydrate staple.

The first step in the digestion process involves mastication. Recent evidence has shown that mastication plays a crucial role in determining the postprandial GR [17, 18]. Furthermore, studies have described large variations in chewing parameters between individuals, such as the number of chews per mouthful and chewing time per mouthful [19, 20]. This potentially contributes to the variability in GR.

Saliva is an important lubricant secreted by the parotid, submandibular and sublingual glands in the mouth and plays a vital role in mastication and swallowing [21]. An earlier study showed that postprandial blood glucose concentrations following ingestion of carbohydrate foods were higher if foods were chewed first, mixed with saliva and then swallowed rather than swallowed whole [22]. This highlighted the importance of salivary α-amylase in the initial starch hydrolysis process in the oral cavity [23]. There has been recent interest in copy number variation (CNV) of the *AMY1* gene and its association with obesity via carbohydrate metabolism [24]. An individual can carry between 2 and 16 diploid copies of the *AMY1* gene on chromosome 1p21, which codes for salivary α-amylase [25, 26]. The CNV of *AMY1* is strongly related to the amount of α-amylase in saliva [25–27]. Low levels of *AMY1* CNVs are associated with decreased levels of α-amylase in the saliva and an increased risk of obesity [24]. With large inter-individual variations in *AMY1* gene copy numbers, we wish to explore whether salivary factors are important determinants of the GR.

Gastric emptying is an important determinant of blood glucose concentrations by controlling the delivery of carbohydrate to the small intestine [28–30]. Gastric emptying accounts for about 35 % of the variance in the GR to oral glucose and/or carbohydrate-containing meals in health [29] and T2DM [31] and is therefore one of the key digestive processes that we wish to examine in this study.

To our knowledge, there are no studies to date that have investigated numerous digestive factors contributing to inter-individual variability in GR and possible inter-ethnic differences in GR. We therefore aim to focus on mastication, salivary α-amylase activity, *AMY1* gene copy numbers and gastric emptying rate that contribute to the variations in GR.

Subjects and methods

Subjects

Seventy-five healthy male participants, consisting of 25 Chinese, 25 Malays and 25 Asian-Indians, were recruited for the study. We choose to study these three ethnic groups as they represent the main ethnic groups in Singapore and verified each participant's ethnicity by ensuring both parents and grandparents were of the same ethnicity. We have chosen to study only men in this study in order to reduce the potential confounding effects of gender as well as the menstrual cycle on metabolic parameters. The participants underwent a screening visit to assess their eligibility, where anthropometric measurements (Table 1) were taken and a health questionnaire (relating to medical history, smoking habits, history of any illness and use of any medications) was administered. All anthropometric measurements were made at least 2 h after food. Height and weight were measured without shoes by using a digital scale cum stadiometer (SECA 763, Germany). A digital blood pressure monitor (Omron HEM-907, Japan) was used to obtain blood pressure readings. Waist circumference was measured using a standard measuring tape (SECA tape measure, Germany) at the midpoint between the coastal margins of the ribs and the upper margin of iliac crest and the hip circumference at the widest level of the greater trochanters on both sides.

Table 1 Baseline characteristics of participants

Characteristics	Mean (%)
Ethnicity	
Chinese	25 (33.3)
Malay	25 (33.3)
Asian-Indians	25 (33.3)
	Mean (SD)
Age (years)	23.4 (2.0)
Weight (kg)	66.1 (7.7)
Height (cm)	173.2 (6.7)
BMI (kg/m^2)	22.0 (1.8)
Systolic blood pressure (mmHg)	125 (9)
Diastolic blood pressure (mmHg)	74 (9)
Mean fasting glucose (mmol/L)	4.52 (0.29)
Waist circumference (cm)	75.3 (5.9)
Body fat (%)	18.1 (6.2)

Percentage body fat was measured using air displacement plethysmography method (BodPod, Cosmed, USA). Physical activity was quantified using the Baecke questionnaire [32], and only those not participating in competitive sports and endurance events were included.

Participants who fulfilled all acceptable criteria (BMI 18.5 to <25 kg/m^2; age 18–45 years; blood pressure 110–120/70–90 mmHg; fasting blood glucose 4–6 mmol/L; not on prescription medication; non-smoking; no genetic or metabolic diseases) were included in the study. On the day before the test visit, participants were reminded to avoid intake of alcohol, restrict caffeine-containing drinks, refrain from physical activity and consume their usual meals. This was to ensure standardized conditions on the day of each test visit. Participants were requested to fast overnight for 10–12 h before each test visit.

This study complied with the Helsinki Declaration as revised in 1983 and was approved by the National Healthcare Group Domain Specific Review Board, Singapore. The study was registered at clinicaltrials.gov as NCT01804738, and all participants provided written informed consent prior to their participation in the study.

Study procedures

Test food

A randomized, crossover, within-subjects, repeated-measure non-blind design was adopted. Participants arrived at the research center between 8:30 and 9:00 am after 10- to 12-h overnight fast. Following a 10-min rest, two fasting blood samples were obtained 5 min apart for baseline blood glucose and insulin levels. After which, they were given either jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand) or basmati rice (Dreamrice™, Singapore) containing 50 g of available carbohydrates (difference between total carbohydrates and dietary fiber) to consume at a comfortable pace within 15 min. This amounted to 63.6 and 66.5 g of uncooked jasmine rice and basmati rice, respectively, cooked in individual portions in a rice cooker (Iona GL12 rice cooker, Singapore) and served with 250 ml of water. These two rice varieties were chosen as they were commonly consumed by the local population. jasmine rice was previously tested locally to be classified as high glycemic index (GI) [33], while basmati rice was tested in a local laboratory and certified low GI on its product packaging. Each rice variety was tested once by each participant in a randomized order with at least 2-day gap between measurements to minimize carryover effects. During the entire study visit, the participants remained rested in the research center.

Measurement of mastication rate

During rice consumption, mastication rate was measured using surface electrode electromyography (EMG), a method adopted from previous studies [5, 20]. Bipolar surface electrodes were attached lengthwise along the left and right masseter muscles. Mastication data were captured and recorded using a data acquisition unit that was attached to the electrodes (DataLOG model P3X8; Biometrics Ltd, UK). Participants were instructed to consume spoonfuls of the rice as they usually would. The number of chews per mouthful and the time taken for each mouthful were quantified for each rice variety.

Blood collection

After the commencement of eating, further blood samples were collected at 15, 30, 45, 60, 90 and 120 min. The protocol used to measure blood glucose response was adopted from that described by Brouns et al. [34] and is in line with the FAO/WHO recommendations [35]. Blood was obtained by finger-prick using sterile, single-use lancing device (Accu-check Safe-T-Pro Plus, Roche, Germany). The first two drops of expressed blood were discarded, and the next drop was used for testing. Blood glucose was measured using a glucose dehydrogenase method (HemoCue 201 RT, Sweden).

Salivary α-amylase determination

Baseline saliva was collected in the fasted state and immediately after each rice variety has been consumed for the measurement of salivary α-amylase activity. In preparation for passive saliva collection, participants were asked to empty their mouths by swallowing all saliva and to rinse

their mouth with water. They were then instructed to place a pre-weighed cotton swab (Salivette®, Sarstedt, Germany) in their mouth for 2 min to absorb passive saliva. The saturated cotton swab was re-weighed and then stored at −80 °C until further analysis of salivary α-amylase activity. Upon thawing, the Salivette® tube was centrifuged for 10 min at 1500 g to yield clear saliva sample. The α-amylase activity was measured using the Phadebas® Amylase test (Magle AB, Lund, Sweden) following the manufacturer's instruction.

AMY1 *gene copy number*

The method used to determine diploid *AMY1* gene copy numbers was adapted from previously published work [25]. DNA was extracted from whole blood using QIAamp® DNA Mini Kit (Qiagen, Netherlands) as described by the manufacturer. Extracted DNA was standardized to 5 ng/μL. A TaqMan Copy Number Assay for *AMY1* (Assay ID Hs07226362_cn) and RNAse P reference assay (44003328, Applied Biosystems, Foster City, CA) were used with TaqMan Genotyping Master Mix (Applied Biosystems), according to product literature. Twenty microliter reactions (final DNA concentration of 1 ng/μL) were run in quadruplicate on an ABI Prism 7500 Real-Time PCR System (Applied Biosystems). Values extracted following analysis with the SDS software were analyzed using Copy Caller software v2.0 (Applied Biosystems). *AMY1* diploid copy number was estimated using a standard curve constructed from a reference DNA sample NA18972 (Coriell Cell Repositories, Camden, NJ) previously determined to have 14 *AMY1* diploid copies by qPCR and Fiber FISH [26].

Gastric emptying rate

Gastric emptying rate was determined using an ultrasound method described previously [36, 37]. Ultrasonographic measurements (LOGIQ P5, GE Healthcare, Korea) were performed at 0, 30, 45, 60 and 90 min immediately after blood sampling. The abdominal aorta and the left lobe of the liver were used as internal landmarks in each measurement of the gastric antrum to obtain the same standardized scanning level consistency. The participants were examined in the supine position, but were allowed to sit up between examinations. At each examination, the longitudinal and antero-posterior diameters (D1 and D2, respectively) were measured 3 times, and the mean values were used to calculate the cross-sectional area of the gastric antrum (A_{Antrum}) as follows:

$$A_{\text{Antrum}} = \pi \times D_{1\text{mean}} \times D_{2\text{mean}}/4$$

Gastric emptying rate (GER) was expressed as the percentage reduction in antral cross-sectional area from 15 to 90 min, calculated as follows:

$$\text{GER} = [(A_{\text{Antrum90min}}/A_{\text{Antrum15min}}) - 1] \times 100$$

Particle size distribution of masticated rice

At the end of each study visit, the participants were given a 100-g portion of the same cooked rice to determine the particle size distribution of masticated rice samples. The participants had to chew each mouthful of rice to the point that they need to swallow. Instead of swallowing, they were instructed to expectorate the chewed contents into the pre-weighed disposable plastic container provided. To recover all chewed rice, they were asked to wash out their mouth between mouthfuls with the 150 ml water provided and to expectorate the contents into the plastic container. All participants were advised not to swallow any rice if possible. Total masticated sample weight was calculated by difference (weight of masticated rice + container + water − weight of plastic container − weight of water). Particle size analysis was carried out within 10–15 min of obtaining the samples, with the method used adapted from previously published work [17]. Briefly, each sample was washed through a set of three sieves of different mesh sizes (2000, 1000 and 500 μm) under running water for 10 min. The retained particles on each sieve were transferred into individual pre-weighed drying dishes and placed in an oven at 105 °C overnight. This was to ensure constant weight was achieved. The weight of dried matter on each dish was calculated as a percentage of the total sample weight. Particle sizes determined were >2000, <2000 to >1000, <1000 to 500 and <500 μm (calculated by difference of weights). Based on previous in vitro study [17], only the percentage of particles >2000 μm and less than 500 μm were used for analyses as these represented the greater proportions of the masticated food.

Statistical analysis

The total glycemic response (GR) over 180 min for each rice variety was expressed as the incremental area under curve (IAUC) ignoring the area under the baseline using the trapezoidal rule [34, 35]. Differences in IAUC between jasmine rice and basmati rice were analyzed using paired t tests. Correlation analyses and trend analyses were performed to determine the relationships between particle size distribution and mastication parameters as well as saliva α-amylase and *AMY1* copy numbers. The linear regression procedure was used to determine the relationship between the digestive factors and the IAUC for each rice variety. Statistical analyses were conducted using the SPSS version

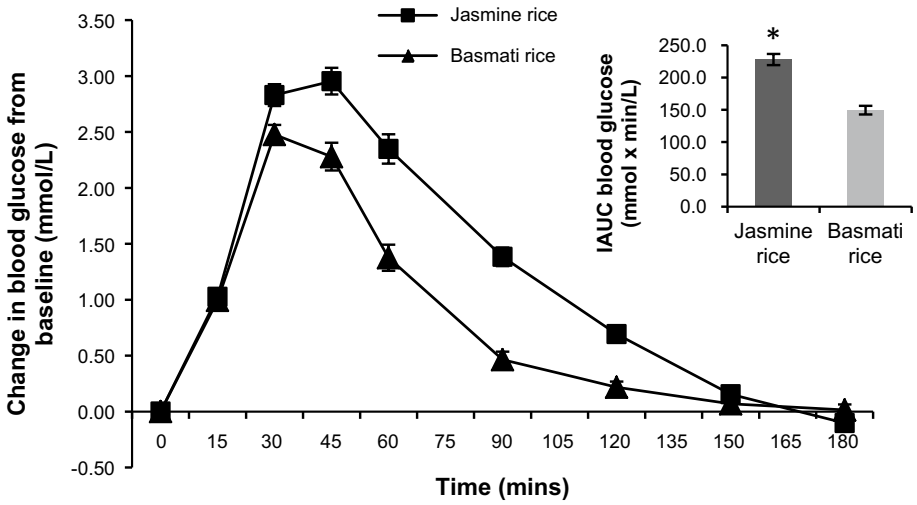

Fig. 1 Comparison of incremental change in the blood glucose concentration following consumption of 50 g available carbohydrate of jasmine rice and basmati rice ($n = 75$). *Error bars* are standard errors. There is a significant difference in incremental area under the curve (IAUC) for blood glucose response between jasmine rice and basmati rice ($P < 0.001$)

19 (SPSS Inc, Chicago, Ill). Data were presented as means (standard deviation) where appropriate.

One study on ethnic differences in postprandial glycemia [7] between 34 UK Caucasians and 13 Asian-Indians observed at least a 59 mmol min^{-1} L^{-1} difference in glycemic response between the two groups with standard deviations of 79 and 47 mmol min^{-1} L^{-1}, respectively. At the 5 % level of significance with power of 80 %, a minimum sample size of 21 subjects for each group would be required. As we were also investigating ethnic differences, a sample size of 25 from each ethnic group was considered adequate for the current study, with a total sample size of 75.

Results

The anthropometric measurements of the participants were within the acceptable normal limits for BMI, fasting blood glucose and blood pressure (Table 1).

Figure 1 illustrates the change in GR following the consumption of Jasmine and basmati rice. The IAUC for jasmine rice was significantly higher than for basmati rice ($P < 0.001$). For jasmine rice, peak glucose was observed at 45 min, while basmati rice peak GR was observed earlier at 30 min. The GR for jasmine rice and basmati rice were not associated with other baseline characteristics except body fat percentage.

The median number of *AMY1* gene copies was 6, with a range of 2–15 (online resource 1). In Fig. 2, we observed that salivary α-amylase activity increased as *AMY1* gene copy number increased ($P = 0.002$). However, fasting salivary α-amylase activity and *AMY1* gene copy numbers were not correlated with postprandial GR for jasmine rice (salivary α-amylase activity: $R = -0.032$, $P = 0.791$; *AMY1:* $R = 0.072$, $P = 0.542$) and basmati rice

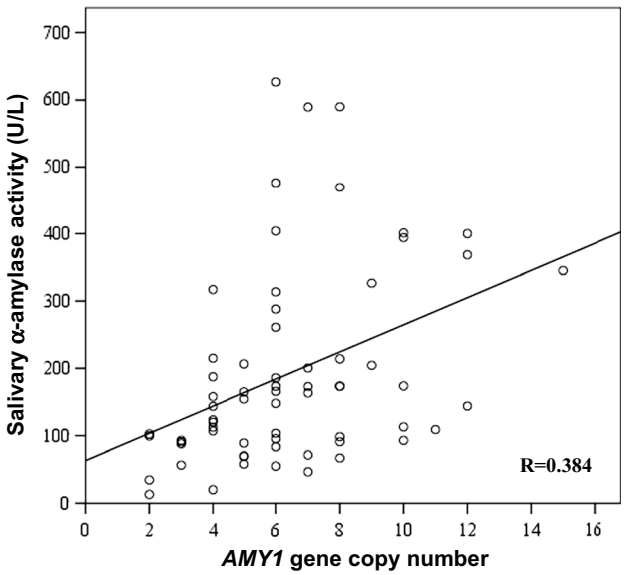

Fig. 2 Correlation of salivary α-amylase activity and *AMY1* gene copy numbers. There was a significant positive relationship between *AMY1* copy number and amylase activity ($P = 0.002$)

(salivary α-amylase activity: $R = 0.192$, $P = 0.117$; *AMY1:* $R = 0.091$, $P = 0.437$). We observed significant correlations between the mastication parameters and particle size distribution of the masticated rice (Table 2). For both rice varieties, particle size distribution was generally related to both chews per mouthful and chewing time, but more so for chews per mouthful. We found that the greater the number of chews per mouthful, the fewer the particle size of more than 2000 μm but a greater percentage of smaller particle sizes of <500 μm for jasmine rice (P for trend <0.001) and basmati rice (P for trend = 0.008). The relationships between chewing time and particle sizes were more pronounced for larger particle sizes of more than 2000 μm

Table 2 Correlation between mastication parameters and particle size distribution in masticated rice

Particle size distribution (%)	Jasmine rice				Basmati rice			
	No. of chews per mouthful		Chewing time per mouthful		No. of chews per mouthful		Chewing time per mouthful	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
>2000 μm	−0.405	<0.001	−0.292	0.011	−0.364	0.001	−0.236	0.042
>1000 to <2000 μm	0.423	<0.001	0.338	0.003	0.307	0.007	0.228	0.049
>500 to <1000 μm	0.514	<0.001	0.387	0.001	0.397	0.001	0.221	0.057
<500 μm	0.318	0.005	0.215	0.064	0.340	0.003	0.214	0.065
P for trend	<0.001		0.008		0.008		0.208	

Table 3 Regression analysis of determinants of glycemic response to Jasmine and basmati rice

Parameters	Jasmine rice			Basmati rice		
	β	*P* value	95 % CI	*β*	*P* value	95 % CI
Race (Malay)	0.035	0.838	−49.014, 60.221	0.185	0.243	−15.606, 60.259
Race (Asian-Indians)	0.067	0.659	−37.149, 58.304	0.172	0.266	−16.230, 57.761
No. of chews per mouthful	0.732	0.011	0.639, 4.841	0.944	0.005	0.730, 3.966
Chewing time taken per mouthful (s)	−0.531	0.039	−5.640, −0.150	−0.774	0.016	−4.877, −0.533
Saliva α-amylase activity (fasted) (U/L)	0.055	0.686	−0.088, 0.132	0.241	0.088	−0.014, 0.203
Particle size <500 μm	0.205	0.657	−4.363, 6.862	0.395	0.505	−3.832, 7.699
Particle size >2000 μm	0.345	0.481	−2.935, 6.158	0.656	0.281	−2.102, 7.095
Gastric emptying rate (%)	−0.276	0.057	−3.660, 0.57	−0.013	0.922	−1.213, 1.099
Diploid *AMY1* gene copy numbers	0.144	0.273	−3.148, 10.936	0.042	0.757	−4.664, 6.380

Race (Chinese) was used as the reference group

but less so for smaller particle sizes of <500 μm. Table 3 details the regression analyses of various determinants of GR to Jasmine and basmati rice. For both rice varieties, the number of chews per mouthful was positively associated with the GR, while chewing time per mouthful was negatively associated with GR. Adjustment for total adiposity did not alter the relationships between mastication parameters and GR. Ethnicity, fasting salivary α-amylase activity, particle size distribution, gastric emptying rate and *AMY1* gene copy numbers were not significant contributors to GR ($P > 0.05$).

Discussion

In this study, we examined the physiological processes involved in the postprandial regulation of carbohydrate metabolism following the consumption of two rice varieties.

Jasmine rice (Double FP Thai Hom Mali premium quality fragrant rice, Thailand), an aromatic low-medium amylose white rice (11–18 %) [38], is commonly consumed in Southeast Asia and was recently found in a local study to have a high GI value of 75.8 [15]. Basmati rice (Dreamrice™, Singapore) has a medium amylose content (20–25 %) [39] and has a medium GI value of 56.7 [15]. We showed that the glycemic response IAUC was significantly higher for Jasmine compared to basmati rice. Our findings reinforced existing evidence that the consumption of a high GI food such as jasmine rice led to higher postprandial GR [15, 17, 33] as well as a rapid decline in blood glucose levels [40, 41]. This can lead to excessive hunger [42, 43] and consequently overeating, in order to restore blood glucose to normal. This can contribute to problems such as overweight, obesity and increased risk of T2DM, especially in Asian populations where white rice is the major contributor to the overall glycemic load of the diet [12–14]. It is therefore prudent to advice on consumption of rice with a lower GI that may reduce the glycemic load of the diet to reduce future risk of T2DM.

Our findings showed that increased *AMY1* copy numbers were positively associated with salivary α-amylase activity. This is consistent with existing evidence illustrating that individuals with high α-salivary amylase activity had more *AMY1* gene copies within their genomes than did the low salivary α-amylase activity individuals [25, 27]. We did not

observe any associations between salivary α-amylase activity or *AMY1* copy numbers with postprandial GR. Research has shown that individuals with high endogenous salivary α-amylase activity had improved glycemic homeostasis than those with low salivary α-amylase activity [27], following the ingestion of a starch solution, which did not take into account individual differences in starch digestion. Findings from our study, using two commonly consumed rice varieties and measuring key digestive parameters, did not demonstrate this relationship with glucose homeostasis. This suggests that the genetic link between *AMY1* and carbohydrate metabolism may be modulated by other mechanisms other than through influencing GR. This warrants future investigation into possible biological explanations underpinning the link between *AMY1*, salivary α-amylase amounts and activity with carbohydrate metabolism.

The median *AMY1* copy number in this study (6 copies) was higher than that reported by Mandel et al. [25] (4 copies). Perry et al. [26] noted that individuals from populations with traditionally high-starch diets (7 copies) have on average more *AMY1* copies than those with low-starch diets (5 copies). We further investigated whether there were ethnic differences in *AMY1* gene copy numbers among the participants in this study. We would have expected ethnic differences in *AMY1* gene copies due to inherent differences in traditional cultural diets in a multiethnic Asian population. Unexpectedly, we found no ethnic differences in *AMY1* gene copy numbers between Chinese, Malays and Asian-Indians. The reason behind this is currently unclear. Further investigation in future studies is integral in view of recent evidence showing reduced *AMY1* copy number was associated with increased BMI and obesity risk [24]. Nonetheless, the results provided in this study provided an overview of the *AMY1* gene copy number variation in a multiethnic Asian population. This can serve as a reference for further genetic population studies, which may correlate these polymorphisms with other phenotypic features.

We found significant correlations between mastication parameters and the particle distribution of the mastication food. In this study, the number of chews per mouthful was consistently correlated with the particle size distribution, in which the greater the number of chews, the larger the proportion of smaller particle sizes (500 to <2000 μm). As an intact grain, the starch in rice needs to be released for digestion via mechanical breakdown [44]. Thorough chewing increases the bioaccessibility of nutrients to enzymatic activity due to increased breakdown of the food matrix [45] and increases digestion rate [46].

In this study, we observed that mastication parameters but not particle size predicted GR despite the close correlations between these two factors. For both rice varieties, our results showed a positive relationship between the number of chews at each mouthful and the GR. These findings were corroborated by a local recent study, which showed that the GR was attenuated by lesser chews per mouthful as a result of increased particle sizes [47]. Our results suggest that mastication rate may be a significant predictor of GR and that chewing less will result in a lower GR. We postulate that more mastication will result in finer particle size, which aid breakdown of the complex carbohydrate structure resulting in higher GR, though we are unable to show the significant correlation with particle sizes as measured by our method, which may have its limitation. Chewing time per mouthful or eating rate was inversely associated with GR. It was found that a longer oral processing time (eating slowly) led to a more pronounced anorexigenic gut hormone (PYY and GLP-1) response [48]. GLP-1 potentiates insulin secretion and regulates glucose homeostasis [49]. This then leads to a lower overall GR, which we postulate is a possible mechanism for the observed results in this study. Eating at a physiologically moderate pace has shown to produce a more pronounced anorexigenic gut peptide response (PYY and GLP-1) than eating very fast [48], which elicited a weaker anorexigenic gut hormone response. Collectively, evidence has shown that a shorter oral processing time or a fast eating rate can promote overeating [50–52]. As this study provided a fixed amount of rice for each participant, we hypothesized that eating fast could lead to greater consumption of food within a shorter period of time, thus resulting in a higher GR. Further studies are pertinent to determine the mechanisms involved in the satiety response between fast and slow eaters. Taken together, these results suggest that eating slowly and having larger food boluses before swallowing (less chewing) may be beneficial in glycemic control.

Gastric emptying rate was not associated with GR for basmati rice although there was a marginal relationship for jasmine rice. There is still not a consistent picture regarding the role of gastric emptying on the GR with studies suggesting that higher gastric emptying rate results in a lower GR [53, 54] or a higher GR [5, 28]. These discrepant results may be due to differences in the methods used in measuring gastric emptying or characteristics of the test meal such as macronutrient composition and food viscosity [54]. We are not aware of any studies that have examined the impact of rice consumption on gastric emptying between Asian ethnicities. These findings have thus provided evidence for similar gastric emptying capacity between Asian ethnicities, at least for two commonly consumed rice varieties.

The strengths of this study were that it measured GR together with key physiological digestive factors of three ethnic groups fed identical foods within a common environmental milieu. To our understanding, this was the first study that examined numerous digestive parameters simultaneously (salivary α-amylase, mastication rate, particle size distribution and gastric emptying) and its association

with glycemic response within the same individual and between ethnic groups. We have also provided an overview of the *AMY1* gene copy number variation in a multiethnic Asian population, which can serve as a reference for further genetic population studies among Asian ethnicities. Through our findings, we have understood the relationships between mastication parameters (chews per mouthful and chewing rate) and glycemic response, which are potentially modifiable eating behavior. Despite the difference in GI between jasmine rice and basmati rice, the association between *AMY1* copy numbers and salivary α-amylase activity as well as the correlation between mastication parameters and particle distribution has been previously shown, and they were reported in Caucasian populations. It is known that Asians and Caucasians have different glycemic response profile, but data in Asian population are lacking. Thus, we wish to demonstrate these findings in our multiethnic Asian population. However, we recognized that the limitation of this study was that we did not include intestinal transit time, which is another factor known to influence GR [55]. Mastication force was also not measured as chewing force is an important measurement when making inferences to the oral stage of digestion. We also did not measure appetite-related hormones, which was a limitation as these exert incretin effect and influences glycemia. However, we did not wish to hamper recruitment rate due to increased blood collection as well as budget constraint. Salivary α-amylase concentration was not determined in this study, but it has been found that higher *AMY1* gene copy numbers and higher α-salivary activity highly correlate with higher concentrations of salivary α-amylase [25, 27]. However, it might be possible to have two individuals with the same concentration of salivary α-amylase, with rather different levels of salivary enzymatic activity, and vice versa. We also consider low power as a potential explanation for the absence of relationship between postprandial GR with salivary α-amylase activity, *AMY1* copy numbers, gastric emptying rate as well as ethnic differences in *AMY1* copy numbers.

In summary, we have determined the contribution of key digestive parameters on GR. The mastication parameters showed a significant relationship with GR—the greater the number of chews at each mouthful and a shorter chewing time (faster eating rate), the higher the GR. We recommend that future work can focus on measuring the gut hormones profile, where further understanding of the satiety mechanisms will provide greater insight into the physiological factors influencing inter-individual and ethnic variability in GR.

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Compliance with Ethical Standards

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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ORIGINAL ARTICLE

Ethnic differences in insulin sensitivity and beta-cell function among Asian men

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BACKGROUND AND OBJECTIVES: Lean Asian Indians are less insulin sensitive compared with Chinese and Malays, but the pancreatic beta-cell function among these ethnic groups has yet to be studied in depth. We aimed to study beta-cell function in relation to insulin sensitivity among individuals of Chinese, Malay and Asian-Indian ethnicity living in Singapore.

SUBJECTS AND METHODS: This is a sub-group analysis of 59 normoglycemic lean (body mass index (BMI) $< 23\ kg\ m^{-2}$) adult males (14 Chinese, 21 Malays and 24 Asian Indians) from the Singapore Adults Metabolism Study. Insulin sensitivity was determined using fasting state indices (homeostatic model assessment—insulin resistance), the euglycemic-hyperinsulinemic clamp (ISI-clamp) and a liquid mixed-meal tolerance test (LMMTT) (Matsuda insulin sensitivity index (ISI-Mat)). Beta-cell function was assessed using fasting state indices (homeostatic model assessment—beta-cell function) and from the LMMTT (insulinogenic index and insulin secretion index). The oral disposition index (DI), a measure of beta-cell function relative to insulin sensitivity during the LMMTT, was calculated as a product of ISI-Mat and insulin secretion index.

RESULTS: Asian Indians had higher waist circumference and percent body fat than Chinese and Malays despite similar BMI. Overall, Asian Indians were the least insulin sensitive whereas the Chinese were most insulin sensitive. Asian Indians had higher beta-cell function compared with Chinese or Malays but these were not statistically different. Malays had the highest incremental area under the curve for glucose during LMMTT compared with Asian Indians and Chinese. However, there were no significant ethnic differences in the incremental insulin area under the curve. The oral DI was the lowest in Malays, followed by Asian Indians and Chinese.

CONCLUSION: Among lean Asians, Chinese are the most insulin sensitive whereas Asian Indians are the least insulin sensitive. However, Malays demonstrate higher postprandial glucose excursion with lower beta-cell response compare with Chinese or Asian Indians. The paths leading to type 2 diabetes mellitus might differ between these Asian ethnic groups.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is projected to affect nearly 600 million people worldwide by 2035,[1] with China and India projected to contribute more cases of T2DM than any other country in the world. The population of Singapore comprises three major ethnic groups; Chinese, Malays and Asian Indians. The prevalence of T2DM in Singapore has increased from 8.2% in 2004 to 11.3% based on the Singapore National Health Survey 2010.[2] The highest prevalence of T2DM has been seen in Asian Indians (17.2%), followed by Malays (16.6%) and Chinese (9.7%).[2]

Much of the work to understand ethnic differences in susceptibility to T2DM has focused on insulin resistance.[3–5] Recently, we showed that degree of insulin sensitivity differs between Asian ethnic groups and this ethnic difference is more prominent amongst lean individuals.[6] Among lean and young Singaporean males, Chinese and Malays are more insulin sensitive compared with Asian Indians.[6,7]

It is well established that the pathogenesis of T2DM involves both decreased insulin sensitivity and impaired beta-cell function.[8,9] Initially, decreased insulin sensitivity may be compensated by increased beta-cell response, and progression to T2DM is thought to occur when beta-cells fail to compensate to a sufficient degree.[10] Accumulating evidence demonstrates that insulin secretory defect may play a more important role than insulin sensitivity in the pathogenesis of T2DM, particularly in East-Asians.[11–13] However, studies comparing beta-cell function between Asian ethnic groups are limited. It is not clear whether Chinese, Malays and Asian Indians exhibit differences in beta-cell function, in addition to the differences in insulin sensitivity.

To gain further insights into the ethnic susceptibility to T2DM, the present study aimed to examine ethnic differences in beta-cell function among lean individuals of Chinese, Malay and Asian-Indian ethnicity, after accounting for differences in insulin sensitivity.

SUBJECTS AND METHODS

Subjects

This was a sub-group analysis of the Singapore Adults Metabolism Study (SAMS).[6] SAMS was a cross-sectional study that examined the associations

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between ethnicity, obesity and insulin resistance in 3 ethnic groups involving 100 Chinese, 80 Malays and 78 Asian-Indian males. A more detailed account of the selection procedure and the study participants was elsewhere.[6] The main study (SAMS) was registered at clinicaltrials.gov as NCT00988819.

Fifty-nine healthy lean (body mass index (BMI) $< 23\ kg\ m^{-2}$) adult males, comprising 14 Chinese, 21 Malays and 24 Asian Indians, who had full data from the euglycemic-hyperinsulinemic clamp procedure and the liquid mixed-meal tolerance test (LMMTT) were included in this study. We selected lean individuals as we have shown previously that ethnic differences in insulin sensitivity was observed among lean but not overweight or obese individuals.[6] All subjects had fasting blood glucose of $< 7.0\ mmol\ l^{-1}$ and had no prior history of hypertension or dyslipidemia. We excluded those with significant changes in diet or weight loss of more than 5%, a history of heart disease, epilepsy, insulin allergy, current smoking, a history of ingesting any drug known to alter insulin sensitivity (for example, corticosteroids), or any hospitalization or surgery during the 6 months before enrollment in the study. Ethics approval was obtained from the National Healthcare Group Domain Specific Review Board (Singapore) (approval code number C/2009/00022). All subjects provided written informed consent.

Clinical measurements

Demographic data, medical and drug history, and data on lifestyle factors were collected using interviewer-administered questionnaires. Height was measured using a wall-mounted stadiometer, and weight using a digital scale (SECA, model 803; Vogel & Halke, Hamburg, Germany). BMI was calculated using the weight (kg) divided by the square of height (m). Waist circumference was measured at the midpoint between the lower costal margin and iliac crest at mid-respiration. Body composition (percent body fat and lean body mass) was measured using a dual-energy X-ray absorptiometry scanner (Hologic Discovery Wi, Hologic, Bedford, MA, USA). All dual-energy X-ray absorptiometry measurements were performed within 1 week of the euglycemic-hyperinsulinemic clamp procedure and the LMMTT.

Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was assessed using the euglycemic-hyperinsulinemic clamp technique, after an overnight fast (10 h).[14] Insulin was infused at a fixed rate of $40\ mU\ m^{-2}$ body surface area/min for the duration of the clamp (120 min). Blood glucose was measured every 5 min using the glucose oxidase method (Yellow Spring Glucose Analyzer; YSI Life Sciences, Yellow Spring, OH, USA). The infusion rate of the dextrose 20% solution was adjusted to maintain a constant blood glucose level at about $90\ mg\ dl^{-1}$ ($5\ mmol\ l^{-1}$) throughout the clamp. The insulin sensitivity index (ISI-clamp) was calculated using the mean glucose infusion rate and steady-state insulin concentrations during the final 30 min of the clamp, adjusted for the lean body mass.

LMMTT procedure

On a separate day after the clamp procedure, the LMMTT was conducted after an overnight 10-h fast. As a mixed-nutrient load, the liquid mixed meal provides a more physiologic stimulus than glucose alone for assessing postprandial glucose and insulin responses,[15] dynamically reflects beta-cell function[16] and provides an appropriate stimulus for assessing insulin sensitivity.[17,18] Subjects were provided a liquid meal that consisted of two 200 ml servings of Ensure Plus (Abbott Laboratories, Columbus, OH, USA), each providing 300 kcal, 40.4 g carbohydrate, 9.8 g fat and 12.5 g protein. A single intravenous catheter was placed in the antecubital space for collection of venous blood. Blood samples were obtained from the indwelling catheter for plasma glucose and insulin concentrations at 0, 30, 60, 90, 120 and 240 min.

Biochemical analyses

Biochemical analyses were conducted at the National University Hospital Referral Laboratory, which is accredited by the College of American Pathologists. Plasma glucose concentrations obtained during LMMTT were analyzed using enzymatic methods (ADVIA 2400, Bayer Diagnostics, Tarrytown, NY, USA), and plasma insulin concentrations using a chemiluminescence assay (ADVIA Centaur Analyzer, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Derivatives of insulin sensitivity and beta-cell function

Using fasting indices, we computed homeostatic model assessment—insulin resistance (HOMA-IR) using the formula: (fasting insulin ($mU\ l^{-1}$) fasting glucose ($mmol\ l^{-1}$)/22.5). Homeostatic model assessment—beta-cell function (HOMA-B), a measure of beta-cell function was computed using the formula: ($20 \times$ fasting insulin ($mU\ l^{-1}$))/(fasting glucose ($mmol\ l^{-1}$)–3.5).

From the LMMTT, we calculated total area-under-the-curve (AUC) and incremental AUC (IAUC) for glucose and insulin responses using the trapezoidal rule.[19,20] The insulin secretion index, calculated as the ratio of total AUC insulin to total AUC glucose, provides information on the total insulin response following the LMMTT. The Matsuda insulin sensitivity index (ISI-Mat) was calculated as follows: 10 000/square root of (fasting glucose $\times$ fasting insulin) $\times$ ($IAUC_{glucose240} \times IAUC_{insulin240}$).[21,22] ISI-Mat is a measure of glucose disposal during the LMMTT, representing a composite of both hepatic and muscular tissue insulin sensitivity.[21]

The insulinogenic index was used as a marker of early-phase insulin response and was calculated as follows: (Insulin30–Insulin0)/(Glucose30–Glucose0). The oral disposition index (DI), a measure of beta-cell function relative to insulin sensitivity during the LMMTT, was calculated as a product of ISI-Mat and insulin secretion index.

Statistical analyses

Data are presented as mean (SE) unless otherwise stated. Analysis of Variance was used for comparisons of continuous variables, with post hoc Bonferroni corrections applied for group comparisons. The incremental changes in the post-meal plasma glucose and insulin concentrations

Table 1. Baseline characteristics of study subjects by ethnicity

	Chinese (n = *14*)	*Malay* (n = *21*)	*Asian Indians* (n = *24*)	P-*value (age adjusted)*
Age (years)	29.6 (1.7)[a,b]	25.2 (0.7)[a]	24.3 (0.7)[b]	—
BMI ($kg\ m^{-2}$)	21.5 (0.4)	20.9 (0.3)	21.6 (0.3)	0.212
Waist circumference (cm)	75.8 (1.1)[a]	73.9 (1.0)[b]	78.7 (0.9)[a,b]	< 0.001
Percent body fat	19.4 (1.4)	17.5 (1.0)[a]	20.4 (1.0)[a]	0.024
Fasting glucose ($mmol\ l^{-1}$)	4.41 (0.09)	4.12 (0.10)	4.12 (0.07)	0.382
Fasting insulin ($mU\ l^{-1}$)	4.85 (0.65)	5.93 (0.90)	7.54 (0.92)	0.103
Total cholesterol ($mmol\ l^{-1}$)	5.10 (0.27)	4.60 (0.17)	4.43 (0.16)	0.513
LDL-cholesterol ($mmol\ l^{-1}$)	3.27 (0.24)	2.86 (0.15)	2.76 (0.12)	0.650
HDL-cholesterol ($mmol\ l^{-1}$)	1.34 (0.07)	1.34 (0.04)	1.33 (0.05)	0.974
Triglycerides ($mmol\ l^{-1}$)	1.08 (0.13)	0.86 (0.06)	0.76 (0.07)	0.284
Aspartate aminotransferase ($U\ l^{-1}$)	20.1 (1.5)	22.1 (1.3)	22.5 (1.0)	0.158
Alanine aminotransferase ($U\ l^{-1}$)	17.9 (2.1)	18.7 (1.4)	19.0 (1.1)	0.190

Abbreviation: BMI, body mass index. Data are presented as mean (SE). *P*-value for comparison among the ethnic groups adjusted for age. Mean values within each row with same superscript letters were significantly different ($P < 0.05$).

between ethnic groups were assessed using repeated-measures Analysis of Variance, with ethnic group set as between-subject factors and time as a within-subject factor. All analyses were carried out using the SPSS statistical analysis software version 19.0 (SPSS, Chicago, IL, USA) and adjusted for age. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

The mean (SE) for age and BMI was 25.9 (0.6) years and 21.3 (0.2) kg m^{-2}, respectively. Table 1 shows the baseline characteristics of study participants by ethnic groups. Chinese were significantly older than Malays and Asian Indians, thus all subsequent analyses were age adjusted. The BMI was not statistically different between the ethnic groups, but Asian Indians had significantly higher waist circumference and percent body fat compared with Chinese or Malays. Fasting glucose concentrations were similar between the ethnic groups, but fasting insulin concentrations appeared higher among Asian Indians compared with Chinese and Malays ($P > 0.05$). Other metabolic parameters (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, AST and ALT) were not statistically different between ethnic groups.

Glycemic and insulin response

The overall total and IAUC for glucose were greatest in Malays, followed by Asian Indians and Chinese ($P < 0.05$; Table 2). Figure 1 shows the incremental change in plasma glucose concentration following the LMMTT. The post-meal glycemic excursion displayed a time by ethnic group interaction ($P_{interaction} = 0.011$, adjusted for age).The plasma glucose concentrations were significantly higher in Malays compared with Chinese at 60 min and 90 min, and higher in Malays and Asian Indians compared with Chinese at 120 min.

There were no statistical differences in total or IAUC for insulin between the ethnic groups (Table 2), although Malays appeared to have lower insulin response compared with Chinese or Asian Indians. There was no significant difference in the post-meal plasma insulin responses between ethnic groups ($P_{interaction} =$ 0.164; Supplementary Figure 1).

Measures of insulin sensitivity

Asian Indians were the least insulin sensitive compared with Chinese or Malays, based on ISI-clamp and HOMA-IR (Table 2). The trend for ISI-Mat was similar to ISI-clamp, being highest in Chinese and lowest in Asian Indians. Similarly, adjusted ISI-clamp or ISI-Mat for waist circumference was lower in Asian Indians compared with Chinese. Insulin sensitivity was no different between Chinese and Malays based on the euglycemic clamp, but was significantly lower in Malays compared with Chinese based on the LMMTT derivative.

Measures of pancreatic beta-cell function

In parallel with HOMA-IR, Asian Indians showed a significantly higher HOMA-B compared with Chinese and Malays (Table 2), indicating a compensatory hyperinsulinemia in the presence of greater insulin resistance to maintain fasting normoglycemia. Chinese had higher insulinogenic index (marker of early-phase insulin response) than Malays or Asian Indians, but Asian Indians had higher insulin secretion index (total insulin response following the LMMTT) than Chinese or Malays. The ethnic differences in insulinogenic index and insulin secretion index however, did not reach statistical significance.

Relationship between insulin sensitivity and pancreatic beta-cell function

The oral DI was significantly higher in Chinese compared with Malays or Asian Indians (Figure 2). Figure 3 shows the hyperbolic relationship between beta-cell function (insulin secretion index)

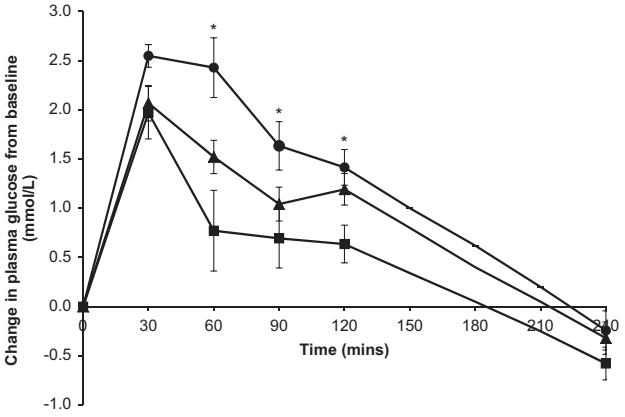

Figure 1. Incremental change in the plasma glucose concentration following LMMTT for Chinese (■), Malays (●) and Asian Indians (▲). P interaction for ethnic groups × plasma glucose response = 0.011 (adjusted for age). There were significant differences in the plasma glucose concentration at 60 min between Chinese and Malays ($P < 0.001$); at 90 min between Chinese and Malays ($P = 0.010$); at 120 min between Chinese and Malays ($P = 0.006$), and at 120 min between Chinese and Asian Indians ($P = 0.039$).

Table 2. Measures of insulin sensitivity and pancreatic beta-cell function

	Chinese (n = 49)	*Malay (n = 31)*	*Asian Indians (n = 29)*	P-*value (age adjusted)*
HOMA-IR	1.31 (0.08)[a]	1.40 (0.19)	2.00 (0.31)[a]	0.003
HOMA-B	133.0 (8.9)[a]	156.2 (21.0)[b]	284.6 (53.2)[a,b]	0.008
Euglycemic clamps derivative				
Insulin sensitivity index (ISI-clamp) per kg lean mass (mg kg^{-1} min^{-1} mU^{-1} l^{-1})	14.2 (1.2)[a]	12.9 (1.2)[b]	8.8 (0.6)[a,b]	0.001
LMMTT derivatives				
Incremental area under curve—glucose (mmol•min l^{-1})	157.0 (31.5)[a]	306.0 (29.5)[a]	224.6 (23.4)	0.002
Incremental area under curve—insulin (mU•min l^{-1})	11138 (1272)	9963 (1316)	13061 (1282)	0.188
Insulinogenic index	83.3 (41.7)	34.6 (7.1)	51.9 (5.6)	0.057
Insulin secretion index (mU mmol^{-1})	10.5 (1.2)	9.1 (1.2)	12.3 (1.1)	0.136
ISI-Mat	2.70 (0.49)[a,b]	1.69 (0.28)[a]	1.51 (0.16)[b]	0.029

Abbreviations: HOMA-B, homeostatic model assessment—beta-cell function; HOMA-IR, homeostatic model assessment—insulin resistance; ISI-Mat, matsuda insulin sensitivity index; LMMTT, liquid mixed-meal tolerance test. Data are presented as mean (SE). *P*-value for comparison among the ethnic groups was adjusted for age or waist circumference. Mean values within each row with same superscript letters were significantly different ($P < 0.05$).

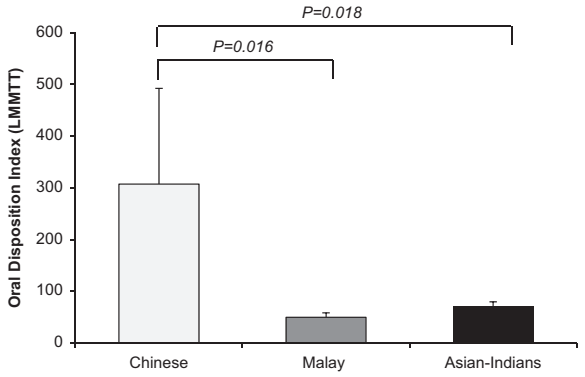

Figure 2. Mean (SE) oral DI derived from the LMMTT by ethnic group.

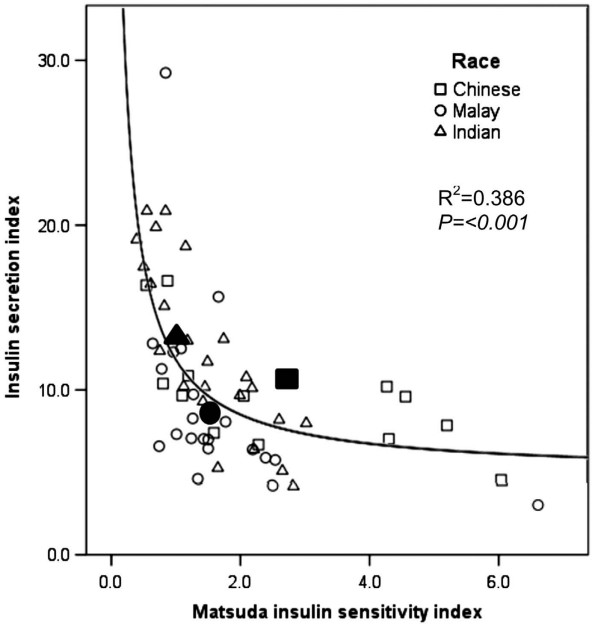

Figure 3. Relationship between insulin secretion index and ISI-Mat. Scatterplots of insulin sensitivity (measured by euglycemic-hyperinsulinemic clamp) vs insulin secretion index among Chinese (□), Malays (○) and Asian Indians (Δ). Filled symbols represent the mean values for each ethnic group. The non-linear regression line is shown for the hyperbolic fit of the data.

and insulin sensitivity (ISI-clamp). When we plotted the means of insulin secretion index and ISI-clamp by ethnicity, they were located at different points along the hyperbolic curve; Chinese and Asian Indians along the hyperbolic DI curve indicating appropriate beta-cell compensation for the prevailing insulin sensitivity, but Malays 'falling off the DI curve', indicating a lower beta-cell response relative to insulin sensitivity.

DISCUSSION

In this study, we examined insulin sensitivity and beta-cell function between lean individuals of Chinese, Malay and Asian-Indian ethnicity. The findings from this study add to the body of evidence that in addition to differences in insulin sensitivity, postprandial glucose response and beta-cell function differ between Asian ethnic groups.

Our study showed that, among lean Asians, Malays exhibited higher postprandial glucose response but lower postprandial insulin response when compared with Asian Indians or Chinese. The insulinogenic index, a marker for early-phase insulin secretion, trended highest amongst Chinese, followed by Asian Indians and was the lowest amongst Malays. It has been suggested that a robust insulin response at the early phase after a meal is crucial in promoting hepatic glycogen storage and for the suppression of hepatic glucose production.[23] A less robust insulin secretory capacity among the Malays might explain why this ethnic group displayed higher postprandial glucose excursion compared with Chinese or Asian Indians.

The euglycemic-hyperinsulinemic clamp directly measures whole-body glucose disposal and is regarded the gold standard against which all other measures of insulin sensitivity must be compared with. Surrogate markers of insulin sensitivity such as HOMA-IR reflects more of hepatic insulin sensitivity since fasting glucose is determined by hepatic glucose production, which itself is primarily regulated by insulin. The ISI-Mat is an insulin sensitivity index derived from a dynamic physiologic meal challenge in order to understand glucose-insulin responses.[21] Our findings from ISI-clamp, HOMA-IR and ISI-Mat supported previous results by showing that Asian Indians were the least insulin sensitive, whereas Chinese were the most insulin sensitive.[6,24] In addition, Asian Indians had higher total adiposity (in particular abdominal adiposity) compared with the other two Asian ethnic groups. When we adjusted for waist circumference, the difference in insulin sensitivity between Chinese and Asian Indians remained significant (data not shown). An earlier study reported that among Japanese American men who developed T2DM, insulin insensitivity, increased insulin secretion and increased intra-abdominal fat were already present before the onset of glucose intolerance.[25] This may help to explain why Asian Indians are more prone to developing T2DM and other cardio-metabolic diseases at a lower BMI and at younger age.[26]

Several studies reported that decreased insulin sensitivity and defective insulin secretion precede the onset of dysglycemia.[27,28] As long as the insulin sensitivity is matched by insulin secretion, normoglycemia is preserved, and a mismatch between these two parameters will therefore results in dysglycemia. This close relationship between insulin sensitivity and insulin secretion follows a hyperbolic curve, and the multiplication product of these two parameters is known as the DI.[9] The DI therefore reflects beta-cell function relative to the prevailing insulin sensitivity,[29] and predicts incident diabetes beyond fasting and 2-h glucose levels.[19,20] In prospective epidemiology studies, individuals who progressed from normoglycemia to T2DM exhibited the 'falling off the DI curve' phenomenon, indicating a failure of insulin secretion to compensate for the degree of insulin resistance.[20]

In this study, we showed that the Chinese had the highest oral DI compared with Malays or Asian Indians. Malays exhibited lower beta-cell function (falling off the curve) compared with Chinese despite having similar insulin sensitivity. This suggests that Malays might have inadequate beta-cell secretory ability to compensate for their prevailing insulin sensitivity. We have further ascertained whether family history of diabetes might explain the lower beta-cell function amongst the Malays. The prevalence of first-degree relatives with diabetes mellitus in our study participants was 28.6% among Chinese, 19.0% among Malays and 16.7% among Asian Indians (data not shown), indicating that family history of diabetes does not explain why Malays have lower beta-cell function compared with Chinese. Several candidate gene regions for T2DM have been discovered by genome-wide association studies and confirmed in various populations worldwide.[30,31] These candidate gene regions are likely to influence beta-cell function. One of them is KCNQ1 gene polymorphism, in particular a risk allele r2283228 has been associated with a 1.7 times higher odds of developing diabetes mellitus among Malaysia Malays.[32] Whether genetic polymorphism in the KCNQ1 gene or other

diabetes candidate genes may account for the ethnic differences in beta-cell function warrants further investigations.

To our knowledge, there was no prior publication that compared beta-cell function in relation with insulin sensitivity between Malay ethnicity and other ethnic groups. The reason why Malays have lower beta-cell function compared with Chinese despite similar insulin sensitivity is not clear. The finding of low beta-cell function among Malays has important implications. Based on the National Health Survey in Singapore, the age-standardized prevalence rate of obesity is the highest among Malays and has increased from 19.1% in 2004 to 24.0% in 2010.[2] Countries like Indonesia and Malaysia, where the majority population consists of Malay origin, have seen a rapid increase in the prevalence of obesity.[33,34] It is well established that insulin resistance is higher with greater obesity. Thus, with a background of compromised beta-cell function, it is probable that this select (Malay) population will see a greater rise in the prevalence of impaired glucose tolerance and diabetes mellitus with increasing obesity. A recent population forecast using an individual-level simulation model, based on Markov chain Monte Carlo methods demonstrated that the rising prevalence of obesity will double the prevalence of diabetes mellitus among Singaporeans by 2050, with Malays and Asian Indians being the most affected.[35]

Our study has several strengths. We studied three major ethnic groups (Chinese, Malays and Asian Indians) that represented the majority of ethnic groups living in Asia, a region where the prevalence of T2DM and cardiovascular disease are expected to increase over the next several decades.[36] Our subjects were young, healthy and lean males, which allow us to determine the metabolic responses following a liquid mixed-meal challenge prior to the onset of chronic diseases. We have used the gold standard for measurement of insulin sensitivity using the euglycemic-hyperinsulinemic clamp technique.[37–39] We also carried out the liquid mixed-meal challenge to investigate insulin secretory capacity instead of using an oral glucose tolerance test. As a mixed-nutrient load, the liquid mixed meal provides a more physiologic stimulus for assessing glucose and insulin homeostasis.[15–18] Furthermore, the liquid mixed meal does not require chewing, thus it will not be a potential confounding factor in the post-meal glucose or insulin response.[40]

There are also limitations in this study. We recognize that the number of subjects in this study was small, however they were well-matched for age, BMI and other metabolic parameters. The insulin sensitivity, as determined by hyperinsulinemic-euglycemic clamp study, agrees with previous publications that showed a statistical difference between Chinese and Asian Indians. Nonetheless, future study with a larger sample size and inclusion of lean females is needed to validate these results. Differences in the nutrient absorption might also contribute to the ethnic differences in the post-meal glucose response. All of our subjects did not have any history of malabsorption nor was there evidence of lactose intolerance, thus we believe that differences in nutrient absorption play a minimal role in our study findings. Future studies should look into differences in nutrient absorption and the role of stool metagenomics in mediating the ethnic differences in postprandial glucose and insulin response. We did not measure incretin responses, which might contribute to the differences in the post-meal insulin response. However, one study showed that the higher early insulin response in African Americans compared with European Americans was not due to differences in circulating incretin concentrations.[41] Interpretation of insulin secretion and insulin sensitivity must also take into account hepatic insulin extraction and hepatic insulin sensitivity, both of which were not examined in this study.

In summary, we have shown that, in addition to ethnic differences in insulin sensitivity, lean Chinese, Malays and Asian Indians also exhibit differences in beta-cell function. While lean Chinese and Asian Indians show appropriate beta-cell function in relation to insulin sensitivity, lean Malays exhibit lower beta-cell function relative to their prevailing insulin sensitivity. With this background, Malays may face a rapid increase in the incidence of diabetes with rising prevalence of obesity, and that measures to maintain healthy body weight would be a key strategy to mitigate the development of T2DM in this population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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EST, MKSL and YSL designed the research; MKSL, KV, EYHK and CMK conducted the research; VMHT and CMK analyzed the data; VMHT and CMK wrote the paper; CMK had primary responsibility for final content. All authors read and approved the final manuscript. This study was funded by the National Medical Research Council Translational and Clinical Research Flagship Programme Grant (NMRC/TCR/004).

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