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EFFECT OF DILUTION IN GLYCEMIC TESTING

Master of Science, 2000
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ABSTRACT

To assess the effect of dilution on postprandial glycemia and its consequences for
glycemic testing and diabetes screening, 25g oral sucrose, glucose, and fructose tolerance tests
and 75g oral glucose tolerance tests (75g-OGTT) were administered to nondiabetic subjects at
different dilutions in three experiments. In the first study, glycemic responses to the three 25g
sugar meals were significantly increased by a 3-fold dilution from 200ml to 600ml (P<0.05). The
same was found in the second study: a 3-fold dilution of a 75g-OGTT from 300ml to 900ml
significantly increased glycemia (P<0.05). Increases however occurred only at intermediate time
points, not at the diagnostically relevant 2h-point. In the third study, replicated 75g-OGTTs at
identical levels of dilution were found to have no or the opposite effect on glycemia in a larger
cross-section of subjects. Furthermore, no meaningful effect was observed on reproducibility of
results, expressed as the intra-subject coefficient-of-variation. It was concluded that dilution
might raise glycemia with some ambiguity upon repeat testing. This effect may account for some
of the variability in glycemic testing, although it will likely not confound diabetes screening
using the 75g-OGTT.
ACKNOWLEDGEMENTS

There are several people to whom I am indebted and must acknowledge. First, I thank my family and Denise for their unconditional love, patience, and understanding. These great gifts gave me the strength to persevere and pursue my passions.

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The same is true for the third group that I would like to thank, my committee members, Drs. Jenkins and Josse. Their contributions to my project and thesis expanded my thinking and improved the work. Their validation of my ideas and constant encouragement also provided for an incredibly stimulating master's experience.

Fourth, I thank my past professors from Ryerson Polytechnic University who gave me the tools and confidence to excel at the Master's level. Specifically, I thank Dr. Mendelson for her examples of consumer professionalism, teaching proficiency, and support; Patricia Jensen for administrative excellence and ever-present reinforcement; Dr. Sue Wilson for stirring my passion for statistics; and Dr. Janet Chapple for her wonderful model of sympathy and dedication.

Finally, my friends in the department who shared in the graduate experience deserve great thanks: Mark for film schooling, recreational diversions, challenging debates, and history lessons; Elizabeth for strong advice when I needed it; Cyril for political guidance and diving adventures;
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Without the contributions of these great people, this work would not have been possible.
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RELEVANT PUBLICATIONS

A. Published and Accepted Articles


B. Submitted Articles

B1. Sievenpiper JL, Jenkins DJA, Josse RG, Vuksan V: Dilution of the 75g oral glucose tolerance test improves overall acceptability but not reproducibility in subjects with different body composition. Submitted to *Diabet Med*, July 1999


B3. Sievenpiper JL, Jenkins DJA, Josse RG, Leiter LA, Vuksan V: Body composition does not affect the reproducibility of the 75g oral glucose tolerance test (OGTT). Submitted to *CJDC*, December 1999

C. Published and Accepted Abstracts

C2. Sievenpiper JL, Welch SF, Jenkins DJA, Josse RG, Vuksan V: Dilution of the 75g oral glucose tolerance test (OGTT) does not affect the reproducibility of diagnostic results. Diabetes 48(Suppl 1):A88-A89, 1999


D. Submitted Abstracts


D2. Sievenpiper JL, Jenkins DJA, Josse RG, Leiter LA, Vuksan V: Reproducibility of the 75g oral glucose tolerance test (75g-OGTT) is not influenced by body composition. American Diabetes Association conference 2000 in San Antonio, TX, January 2000
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>2hPG</td>
<td>two-hour plasma glucose</td>
</tr>
<tr>
<td>4S</td>
<td>Scandinavian Simvistatin Survival Trial</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BF</td>
<td>Body fat</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardio vascular disease</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
</tr>
<tr>
<td>DSP</td>
<td>Diabetes screening product</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GAD</td>
<td>Glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GI</td>
<td>Glycemic index</td>
</tr>
<tr>
<td>HC</td>
<td>Hip circumference</td>
</tr>
<tr>
<td>IAA</td>
<td>Insulin autoantibody</td>
</tr>
<tr>
<td>ICA</td>
<td>Islet cell autoantibody</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired fasting glucose</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
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xvi
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IMT</td>
<td>Intima medial thickness</td>
</tr>
<tr>
<td>IRAS</td>
<td>Insulin Resistance Atherosclerosis Study</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>MI</td>
<td>Myocardial infarction</td>
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<tr>
<td>min</td>
<td>Minutes</td>
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<tr>
<td>ml</td>
<td>Milli-litres</td>
</tr>
<tr>
<td>mmol/L</td>
<td>Milli-moles per litre</td>
</tr>
<tr>
<td>MRFIT</td>
<td>Multiple Risk Factor Intervention Trial</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>NDDG</td>
<td>National Diabetes Data Group</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Noninsulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor 1</td>
</tr>
<tr>
<td>pmol/L</td>
<td>Pico-moles per litre</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RPG</td>
<td>Random plasma glucose</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acids</td>
</tr>
<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
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<td>vWF</td>
<td>Von Willebrand factor</td>
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CHAPTER 1.

INTRODUCTION
1. INTRODUCTION

1.1 Background

Numerous dietary factors have been found to affect postprandial glycemia. These include type (1-3) and amount of carbohydrate (3-5); food form (6-10); processing (11-16); ripeness (17,18); and fibre (19-41), protein (42-47), fat (48-53), and antinutrient (54-58) content. Evidence indicates that dilution may also be a factor. Schwartz et al found that a 3-fold dilution from 150ml to 450ml of a 50g-OGTT significantly raised peak blood glucose by 10% in pregnant women (59). Similar observations were made by Young and Wolever (60) and Torsdottir and Anderson (61) after increasing the volume of water taken with solid test meals both in nondiabetic and in well-controlled diabetic subjects respectively. In contrast, neither Torsdottir and Anderson (61) nor Gregerson et al (62), using a similar protocol, noticed any effect in poorly controlled and mixed diabetic subjects respectively. Together these data are inconclusive for people with diabetes but suggest that in nondiabetic subjects dilution may increase glycemia.

Such an effect of dilution on glycemia might have implications for glycemic testing methodology. The volume of test meals can vary between laboratories. For example, meals can range in volume from 240mL to 950mL within the same study (63) and from 240ml (63) to 750ml (64) between different studies. Furthermore, the volume administered is not always controlled (45,51,65-67). The resultant differences in the dilution of test meals between and within laboratories may affect postprandial glycemia, leading to some variability in results.

A blood glucose raising action of dilution might also have implications for diabetes screening using the 75g oral glucose tolerance test (75g-OGTT). Because of the increasing prevalence of diabetes (68,69) and the importance of early diagnosis (70,71), there is a need for a robust diagnostic test. To this end, the American Diabetes Association (ADA) (70) and Canadian Diabetes Association (CDA) (71) have encouraged the use of fasting plasma glucose (FPG) over 2h plasma glucose (2hPG) following a 75g-OGTT. Poor reproducibility is a major reason for
this recommendation (70). The intrasubject variation reported for 75g-OGTT outcome based on 2hPG is from approximately 11% (72) to 18% (73), while it is from 5% (72) to 12% for FPG (73). Reasons for this high variability are not clear. One explanation may be inconsistencies and deficiencies in the established protocols for the test. For example, it is possible for the 75g-OGTT to be given at different volumes. There is a discrepancy in the instructions for the dilution of the test between the 1985 World Health Organization (WHO) (74) guidelines, endorsed by the ADA (70), and the 1979 National Diabetes Data Group (NDDG) guidelines (75), endorsed by the CDA (71). In addition, because poor palatability of the test is a major complaint (76,77), recipients will often request additional water with the test to increase its acceptability. If such alterations in the dilution of the 75g-OGTT are able to amplify postprandial glycemia, then skewed diagnoses and poor reproducibility may be a consequence.

These concerns of dilution's possible confounding role in glycemic testing and oral glucose tolerance testing remain to be addressed. Whether the evidence for an effect of volume is repeatable in a liquid test meal format with different substrates or when the dose of oral glucose is the 75g required by the OGTT is unclear. We therefore studied the effect of dilution in the glycemic testing of 25g oral glucose, sucrose, and fructose solutions and in diabetes screening using the 75g-OGTT.

1.2 Objectives

1.2.1 To assess the effect of a 3-fold dilution from 200ml to 600ml of 25g oral sucrose, glucose, and fructose tolerance tests on postprandial glycemia

1.2.2 To assess the effect of a 2-fold or 3-fold dilution of a 300ml 75g-OGTT on postprandial glycemia and diagnostic outcome based on 2h blood glucose

1.2.3 To assess whether the same dilutions of a 75g-OGTT will affect reproducibility of results as assessed by the intra-subject coefficient of variation (CV) of three repetitions at each volume in a cross-section of people that include lean, normal, and obese.
1.2.4 To assess which dilution of the 75g-OGTT has the best tolerability profile as assessed by 7-point bipolar scales of palatability, acceptability, satiety, dizziness, and nausea in this same cross-section of people.

1.3 Hypotheses

1.3.1 A 3-fold dilution from 200ml to 600ml of 25g oral sucrose, glucose, and fructose tolerance tests will increase postprandial glycemia

1.3.2 A 2-fold or 3-fold dilution of a 300ml 75g-OGTT will increase postprandial glycemia such that it affects diagnostic outcome based on 2h blood glucose

1.3.3 The same increases in dilution of a 75g-OGTT will also decrease the intrasubject CV of three repetitions at each volume in the three groups of subjects studied.

1.3.4 The 600ml 75g-OGTT will have the best tolerability profile as assessed by 7-point bipolar scales of palatability, acceptability, satiety, dizziness, and nausea in the subjects.
CHAPTER 2.

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1 Diabetes Classification

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. There are several etiologically based classifications of this disorder. These include type 1, type 2, gestational, and other specific types of diabetes. Prediabetic intermediate classifications of this disorder include impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) (70).

2.1.1 Type 1 diabetes

This form of diabetes, formerly known as insulin dependent (IDDM), type (roman numeral) I, or Juvenile onset diabetes mellitus, comprises those diabetes that are prone to ketoacidosis and result from a destruction of pancreatic ß-cells by either an autoimmune process or a process for which an etiology is not known. It does not include cases to which non-autoimmune-specific causes can be assigned, such as cystic fibrosis. Diagnosis is by hyperglycemia and its consequent classic symptoms (polyuria, polydipsia, ketonuria, rapid weight loss, polyphagia, and blurred vision) in the presence of markers of immune destruction of ß-cells. These markers include autoantibodies to islet cells (ICAs), insulin (IAAs), glutamic acid decarboxylase (GAD), and tyrosine phosphatases (IA-2 and IA-2β). For those cases where the first two diagnostic criteria are met and there are no signs of autoimmunity and the etiology of the destruction is not known, the diabetes is classified as idiopathic type 1 (70).

2.1.2 Type 2 diabetes

This form of diabetes, formerly known as non-insulin dependent (NIDDM), type (roman numeral) II, or adult onset diabetes mellitus, results from insulin resistance combined with an insulin secretory defect (70). The combination is such that insulin secretion in these patients is unable to compensate for the resistance of cells to insulin,
causing a relative insulin deficiency and hyperglycemia. The specific etiology of this disease process is unclear. However unlike type 1 diabetes, it has no autoimmune component and ketoacidosis seldom occurs. Most patients who present with this form of diabetes are obese or may have an android distribution of fat (78,79). Obesity itself is a cause of some degree of insulin resistance (80,81) and increases the likelihood of developing type 2 diabetes (69,82,83). Risk also increases with age, lack of exercise (69,82,83), hypertension, dyslipidemia, black, Hispanic, and aboriginal ethnicity (69,82,83), increased glycemic load of the diet (84,85) and prior incidence of gestational diabetes mellitus (GDM) (69,83). Diagnosis is by fasting, casual, or postprandial hyperglycemia and classic symptoms. Type 2 diabetes, however, often goes undiagnosed. The reason is that the hyperglycemia that characterizes this type of diabetes develops slowly and initially is often not severe enough for the patient to notice any of the classic symptoms.

2.1.3 Gestational diabetes

This form of diabetes is defined as any degree of glucose intolerance that results from pregnancy or is first diagnosed during pregnancy (70). It does not exclude cases where the intolerance occurred before or persists after pregnancy. Reclassification of these and all other cases, however, is required six weeks postpartum. This screening shows that type 2 diabetes, IGT, or IGF may develop, but in the majority of cases, normal glucose homeostasis returns. Unlike other forms of diabetes, diagnosis is made only by postprandial blood glucose. Blood glucose is obtained one hour after a 50g oral glucose screening test and used to make an initial assessment. If positive, then it must be confirmed by a subsequent larger 100g oral glucose challenge. Excluded from routine screening are women who are <25 years of age, of normal body weight, have no family history of diabetes, and are not a member of an ethnic group (Hispanic, Native American,
Asian, or African American), as they are considered to be at a low risk. Reclassification diagnosis is different. It is by the standard procedure for diabetes mellitus. The specific methods of this procedure, including the 75g oral glucose tolerance test (OGTT) will be discussed below (70).

2.1.4 Other specific types of diabetes

This classification represents eight different groups of diabetes whose etiology does not involve immune destruction of β-cells, a combination of insulin resistance and secretory defect, or pregnancy. Included are those diabetes caused by: (i) genetic defects of β-cells, such as defects of chromosome 12 (HNF-1α), 7 (glucokinase), and 20 (HNF-4α); (ii) genetic defects in insulin action, such as Leprechaunism; (iii) diseases of the exocrine pancreas, such as cystic fibrosis; (iv) endocrinopathies, that is diseases which result in an excess production of insulin antagonising hormones, such as Cushing’s syndrome; (v) drug or chemical induction, such as from Vacor; (vi) infections, such as Congenital rubella; (vii) uncommon forms of immune-mediated diabetes, such as “Stiffman” syndrome; and (viii) genetic syndromes associated with diabetes, such as Down’s syndrome. Diagnosis of these forms of diabetes is by hyperglycemia and presentation of symptoms from the underlying disorder (70).

2.1.5 Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)

Both of these classifications are not considered to be clinical entities unto themselves, but instead stages intermediate to all above-mentioned types of diabetes. They are characterized as glucose regulation between that of diabetes and normal homeostasis. Diagnosis is by fasting hyperglycemia for IFG and postprandial hyperglycemia following an OGTT for IGT. By these diagnoses, not all IFG and IGT cases are the same. Many individuals who present with IGT may have normal glycosylated hemoglobin concentrations (86) and be euglycemic at fasting (87). As a
result, hyperglycemia will be observed only after an oral glucose challenge and not a fasting measurement in these individuals (70).

2.2 Epidemiology of Diabetes

2.2.1 Prevalence

Diabetes in its many forms is considered a major health concern. The most recent data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) show that undiagnosed (2.7%) and diagnosed diabetes (5.1%) combined affect 7.8% of adults over 20 years of age in the US (68). Added to this prevalence are those affected by IFG (6.9%) and IGT (11.9%) (68). In people between 40-74 years of age, these percentages increase to 12.3% for diabetes, 9.6% for IFG, and 15.6% for IGT. According to Harris et al, from the last NHANES (1976-1980) (88), these percentages represent an approximately 28%, 33%, and 0% increase respectively (68). If we consider the entire data set of people 18-74 years of age, then the increases are smaller but striking: 13% and 6% respectively for diabetes and IGT (68,88). Similar trends have been noticed for Canadians despite a lack of comparable data (69). The overall suggestion is that diabetes and intermediate categories of hyperglycemia are on the rise in North America.

2.2.2 Complications

The consequences of this situation are very serious. There are numerous vascular complications that can result from diabetes. Complications with microvascular foci include nephropathy, retinopathy, neuropathy, and ulceration. Within these diseases, diabetes is the leading cause of endstage renal disease (diabetic nephropathy) (89) and blindness (diabetic retinopathy) (90) in North America and Europe. It is also responsible for half of all nontraumatic lower limb amputations (diabetic neuropathy/ulceration) in the U.S. (91). Macrovascular complications include coronary heart disease (CHD), cardiovascular disease (CVD), and cerebral vascular disease. The insulin resistance
Atherosclerosis study (IRAS) showed that people with diabetes have significantly greater intimal-media thickness (IMT) of the common carotid artery than people without diabetes (92). This marker represents a surrogate endpoint of cardiovascular disease, in which thickness is positively associated with increased risk (93). More definitive data from a Finnish cohort showed that people with diabetes are at equal or greater risk for myocardial infarction (MI) and stroke as those who have had a prior MI (94). That is, their risk for cardiovascular events is the same as people with already established CHD.

The relative abundance of cardiovascular risk factors in diabetes may explain some of these vascular complications. In addition to hyperglycemia, abnormalities in lipids (95,96,97), blood pressure (96,97), coagulation/fibrinolytic factors, clotting factors, glycation end products, endothelial function (98), and body fat distribution (99,100) have been found to be more prevalent in the people with diabetes compared to the general population. The most notable abnormalities include hypertriglyceridemia; decreased HDL (95,96,97); hypertension (96,97); elevated plasminogen activator inhibitor 1 (PAI-1), a fast acting inhibitor of fibrinolysis; elevated fibrinogen; elevated von Willebrand factor (vWF), a marker of endothelial damage; decreased lipoprotein lipase activity; increased lipoprotein oxidation; increased lipoprotein and platelet protein glycation (98); and greater visceral adiposity (99,100). The clustering of these risk factors around the insulin resistance that underlies diabetes has been termed the insulin resistance syndrome, multiple metabolic syndrome, or syndrome X (101). It is this syndrome that is thought to increases atherogenesis and the "basal risk" of coronary artery disease in people with diabetes (101).

2.2.3 Economics

Coupled with the management of diabetes, these risk factors and the complications they precipitate come at a considerable economic cost. Medical expenditures for people
with diabetes in the US in 1997 were $10,071 compared to $2,669 for people without (102). This cost difference is reflected in hospital data from the UK which showed that out of people admitted for vascular disease, infection, neuropathy, or ulceration, those with diabetes were approximately 7 times more likely to be admitted, 31 times more likely to receive a surgical procedure, and stayed twice as long as those without (103).

2.2.4 Mortality

There is also a considerable cost in mortality. More than 178,000 deaths result from the disease each year (104) with diabetic subjects having a higher mortality rate, lower survival rate, and lower median life expectancy than those without diabetes (104,105). Diabetes and level of glycemia also have been confirmed to be independently associated both with increased CVD and all-cause mortality (105,106). An increased risk of death from cardiovascular causes relative to non-diabetic subjects is another observation. In people who have had a prior MI, incidence of death was noticed to be more than 3-fold greater in diabetic than nondiabetic individuals. In people who did not have a history of prior MI, the difference increased to 8-fold (94). These findings are corroborated by recent NHANES data that demonstrate that people with diabetes have benefited far less from declines in CHD deaths than their nondiabetic counterparts. Men with the disease have experienced a significantly smaller decrement while the incidence in diabetic women has increased (107). Together these data make a very compelling case for the early diagnosis of IFG, IGT, and diabetes.

2.3 Diagnosis of diabetes

To diagnose new cases of diabetes and intermediate stages of hyperglycemia in nonpregnant individuals, the 75g-OGTT is one of four methods used. The other three methods include overt classic symptoms (polyuria, polydipsia, ketonuria, rapid weight loss, polyphagia, and blurred vision), fasting blood glucose, or random plasma glucose. Unlike these other
methods, the OGTT measures postprandial hyperglycaemia. In practice, it is typically used to assess diabetes in epidemiological research and also in a clinical setting when overt classic symptoms are not present and there is no confirmation from fasting blood glucose or random plasma glucose. There are two sets of internationally accepted guidelines for its use: the NDDG (75) and WHO (74) guidelines. With some changes to the diagnostic criteria, these provide the basis for the newer CDA (71) and ADA (70) guidelines respectively.

2.3.1 NDDG 1979 Guidelines

The NDDG (75) guidelines were published in 1979 and arose out of a need for a consensus in the criteria for interpreting OGTT results. They instructed that following three days of unrestricted diet (≥150g carbohydrate) and non-water restricted 10-16h fast, a 75g glucose dose (1.75g/kg ideal body weight for children, up to 75g) should be given. This was an amount midway between the 50g dose widely used in Europe and the 100g dose often used in the U.S. up to that point. The reasons given for this change in dose were: (i) to achieve standardization; (ii) to be consistent with a number of major research centres and the national survey of glucose tolerance in the U.S. who were already using 75g doses; (iii) to overcome problems associated with the two other doses - a 50g dose was shown not to be provocative enough while a 100g was shown to cause nausea, and (iv) to eliminate significant differences observed between the results from 50g and 100g doses. They also instructed that the concentration of this dose should not exceed 25g glucose/dl water or 75g/300ml, allowing for test meals to be given at volumes above 300ml. The test meal should be consumed in about 5 minutes and blood samples should be taken at fasting, and then 30, 60, 90, and 120 minutes after the start of the test meal. The criteria they provided for diagnosis are shown in Table 2.1 and were to be satisfied by two separate tests.
Table 2.1A - NDDG OGTT diagnostic criteria for diabetes mellitus in nonpregnant adults

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Glucose @ fasting</th>
<th>Glucose Level @ 30, 60, or 90 minutes</th>
<th>Glucose Level @ 120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>&lt; 7.8mmol/L (140mg/dl)</td>
<td>≥ 11.1mmol/L (200mg/dl)</td>
<td>≥ 11.1mmol/L</td>
</tr>
<tr>
<td>Venous whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>≥ 10.0mmol/L (180mg/dl)</td>
<td>≥ 10.0mmol/L</td>
</tr>
<tr>
<td>Capillary whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>≥ 11.1mmol/L (200mg/dl)</td>
<td>≥ 11.1mmol/L</td>
</tr>
</tbody>
</table>

Table 2.1B - NDDG OGTT diagnostic criteria for IGT in nonpregnant adults

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Glucose @ fasting</th>
<th>Glucose @ 30, 60, or 90 minutes</th>
<th>Glucose @ 120 minutes minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>&lt; 7.8mmol/L (140mg/dl)</td>
<td>≥ 11.1mmol/L (200 mg/dl)</td>
<td>7.8-11.1mmol/L</td>
</tr>
<tr>
<td>Venous whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>≥ 10.0mmol/L (180 mg/dl)</td>
<td>6.7-10.0mmol/L</td>
</tr>
<tr>
<td>Capillary whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>≥ 11.1mmol/L (200 mg/dl)</td>
<td>7.8-11.1mmol/L</td>
</tr>
</tbody>
</table>

Table 2.1C - NDDG OGTT diagnostic criteria for normal glucose levels in nonpregnant adults

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Glucose @ fasting</th>
<th>Glucose @ 30, 60, or 90 minutes</th>
<th>Glucose @ 120 minutes minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>&lt; 6.4mmol/L (115mg/dl)</td>
<td>&lt; 11.1mmol/L (200 mg/dl)</td>
<td>&lt; 7.8mmol/L</td>
</tr>
<tr>
<td>Venous whole blood</td>
<td>&lt; 5.6mmol/L (100mg/dl)</td>
<td>&lt; 10.0mmol/L (180 mg/dl)</td>
<td>&lt; 6.7mmol/L</td>
</tr>
<tr>
<td>Capillary whole blood</td>
<td>&lt; 5.6mmol/L (100mg/dl)</td>
<td>&lt; 11.1mmol/L (200 mg/dl)</td>
<td>&lt; 7.8mmol/L</td>
</tr>
</tbody>
</table>

2.3.2 WHO 1985 Guidelines

The WHO (74) guidelines were published in 1985 and are quite similar. Like the NDDG guidelines, they instructed that after three days of unrestricted diet (≥150g carbohydrate) and non-water restricted 10-16h fast, a 75g glucose dose (1.75g/kg ideal body weight for children, up to 75g) should be given, the meal should be consumed over 5 minutes, and blood should be collected at fasting and 120 minutes after the start of the
test meal. Also, diagnostic values for glucose values at baseline and 120 minutes provided by WHO are the same as those given by the NDDG (Table 2) and must be satisfied by two tests to confirm the diagnosis. Unlike the NDDG guidelines however, the WHO stipulated that the volume of the test meal should be 250-300ml. This change in protocol does not allow for volumes above 300ml to be given. In addition, the additional collection of blood samples at 30, 60, and 90 minutes is optional and diagnostic values for these time points are not included in their interpretation of results.

Table 2.2A - WHO OGTT diagnostic criteria for diabetes mellitus in nonpregnant adults

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Glucose @ fasting</th>
<th>Glucose @ 30, 60, or 90 minutes</th>
<th>Glucose @ 120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>≥ 7.8mmol/L (140mg/dl)</td>
<td>-</td>
<td>≥ 11.1mmol/L (200mg/dl)</td>
</tr>
<tr>
<td>Venous whole blood</td>
<td>≥ 6.7mmol/L (120mg/dl)</td>
<td>-</td>
<td>≥ 10.0mmol/L (180mg/dl)</td>
</tr>
<tr>
<td>Capillary whole blood</td>
<td>≥ 6.7mmol/L (120mg/dl)</td>
<td>-</td>
<td>≥ 11.1mmol/L (200mg/dl)</td>
</tr>
<tr>
<td>Capillary plasma</td>
<td>-</td>
<td>-</td>
<td>≥ 12.2mmol/L (220mg/dl)</td>
</tr>
</tbody>
</table>

Table 2.2B - WHO OGTT diagnostic criteria for IGT in nonpregnant adults

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Glucose @ fasting</th>
<th>Glucose @ 30, 60, or 90 minutes</th>
<th>Glucose @ 120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>&lt; 7.8mmol/L (140mg/dl)</td>
<td>-</td>
<td>7.8-11.1mmol/L (140-200mg/dl)</td>
</tr>
<tr>
<td>Venous whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>-</td>
<td>6.7-10.0mmol/L (120-180mg/dl)</td>
</tr>
<tr>
<td>Capillary whole blood</td>
<td>&lt; 6.7mmol/L (120mg/dl)</td>
<td>-</td>
<td>7.8-11.1mmol/L (140-200mg/dl)</td>
</tr>
<tr>
<td>Capillary plasma</td>
<td>-</td>
<td>-</td>
<td>8.9-12.2mmol/L (160-220mg/dl)</td>
</tr>
</tbody>
</table>

2.3.3 75g-OGTT Criticisms

There have been several recent criticisms of the OGTT, whether administered by either guideline. First, the test is considered to have poor palatability (76,77). In our clinic, when patients are given the 75g glucose, 300ml test meal, it is often poorly
received. Patients report that it has poor taste, irritates their throat slightly, and sometimes causes nausea. As a result, they usually request additional water to improve the palatability. Other investigators' findings support these observations. Elks (108) and another group (59,109) report that a significant proportion of patients feel nauseated, dizzy, or otherwise ill during the test. In addition, Court et al (110) Katz at al (111) in the screening of a group of pregnant women and Wolever et al (112) in non-pregnant adults found that the OGTT had a significantly lower preference rating and caused significantly more side-effects (nausea and vomiting) than a substitute meal test.

Second, the test is considered to be patient and practitioner unfriendly (76). As mentioned, the test must be done twice, takes a minimum of two hours to administer and requires that the patient be fasting before the test, give multiple blood samples in the case of the NDDG guidelines, not eat or drink during the test with the exception of the glucose solution provided, and remain seated throughout. The result is that it is often perceived as burdensome, time consuming, inconvenient, and difficult to schedule.

Third, the test is considered to have poor reproducibility (76,77). Several studies have shown high intra-subject variability of the OGTT compared to fasting plasma glucose (FPG). Cummings and Fraser (72) in 14 healthy subjects and Feskens et al (73) in 237 mixed subjects (136 healthy and 111 diabetic subjects) found the intra-individual CV for 2hPG were 11.0% and 17.7% respectively after a 75g OGTT compared to 4.7% and 12.0% respectively for FPG. Similarly, Mooy et al (113) found the intra-individual variation for 2hPG - as assessed by the standard deviation of the test-retest differences were 1.3 in 246 healthy subjects, 1.8 in 198 IGT subjects, and 2.3 in 80 Type 2 diabetic subjects after a 75g OGTT compared to 0.4, 0.5, and 0.7 respectively for FPG in these same subjects. This translates into a CV of 16.7% for 2hPG and 6.4% for FPG in all subjects (n=524). Other studies have also shown higher variability in the OGTT relative
to substitute test meals. Wolever et al in 10 healthy subjects (114) and then later in 36 mixed subjects (112) found that the intra-individual CV for 2hPG after a 75g OGT was significantly greater than that after a diabetes screening test bar (12.9% versus 4.7% and 12.7% versus 10.5%, respectively). Overall, these findings question the reliability of this test.

Finally, the specificity of the OGT is considered to be no greater than that of FPG. NHANES III showed that both FPG and 2hPG are strongly but similarly associated with the development of retinopathy (68). This finding is confirmed by studies in other populations. Both McCance et al (115) in 960 Pima Indians and Engelgau et al (116) in 1018 Egyptians found that FPG and 2hPG were equally strong predictors of retinopathy and, in the former case, equally strong predictors of nephropathy. Together these studies suggest that the OGT is no more specific for diabetes than FPG as measured by its ability to predict microvascular complications. If we consider its ability to predict macrovascular complications a measure of specificity, then the same is also true. Beksa et al (117) in 564 caucasians found that FPG and 2hPG were both significantly associated with any arterial disease. Likewise, Jackson and colleagues (118) in 223 subjects found the same. When this lack of difference in specificity is coupled with the FPG’s easier administration and higher reproducibility, the OGT’s utility as a diagnostic test is severely diminished.

2.3.4 CDA 1998 and ADA 1997 revised guidelines

Due to this series of criticisms, both the CDA (71) and ADA (70) in their most recent guidelines abandoned the 75g-OGTT. The new guidelines state that diabetes may be diagnosed by (i) presentation of classic symptoms plus random blood glucose, (ii) FPG, or (iii) OGTT and subsequent confirmation by any one of these three. The diagnostic criteria for each method are provided in Table 2.3. They recommend,
however, that the OGTT should not be used for routine clinical use or as an estimate of diabetes prevalence and incidence in epidemiological studies. In the diagnosis of stages of glucose regulation intermediate to diabetes (IFG and IGT) the same applies.

Table 2.3A - CDA and ADA diagnostic criteria for diabetes mellitus in nonpregnant adults

<table>
<thead>
<tr>
<th>Diagnosis Method</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Symptoms + Random Plasma Glucose (RPG)</td>
<td>Symptoms: Polyuria, polydipsia, unexplained weight loss, and others RPG: ≥ 11.1mmol/L (200mg/dl)</td>
</tr>
<tr>
<td>2. Fasting Plasma Glucose (FPG)</td>
<td>≥ 7.0mmol/L (126mg/dl)</td>
</tr>
<tr>
<td>3. 2Hpg during an OGTT</td>
<td>≥ 11.1mmol/L (200mg/dl)</td>
</tr>
<tr>
<td>performed by NDDG protocol for CDA and WHO protocol for ADA</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3B - CDA and ADA diagnostic criteria for IGT in nonpregnant adults

<table>
<thead>
<tr>
<th>Diagnosis Method</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fasting Plasma Glucose (FPG)</td>
<td>≥ 6.0mmol/L (110mg/dl) and &lt; 7.0 mmol/L (126mg/dl)</td>
</tr>
<tr>
<td>2. 2hPG during an OGTT</td>
<td>≥ 7.8mmol/L (140mg/dl) and &lt; 11.1mmol/L (200mg/dl)</td>
</tr>
<tr>
<td>performed by NDDG protocol for CDA and WHO protocol for ADA</td>
<td></td>
</tr>
</tbody>
</table>

FPG alone, however, may not be as effective. The ADA expert committee conceded that people with IGT often only manifest with hyperglycemia when challenged with an OGTT (11). In other words, people with IGT may not present with IFG, suggesting that the OGTT will identify more people as having an intermediate level of hyperglycemia. The ADA advises therefore that it is imperative investigators always report the test used (11). This limitation has been confirmed by population (119) and cohort (120) based studies in which 1997 FPG criteria were observed to be less sensitive than OGTT criteria, missing 71% and 81% of the IGT patients respectively. It has also been noticed that CDA (71) and ADA (70) FPG criteria may under
estimate the prevalence of diabetes compared to OGTT criteria by approximately 40% (121,122). An argument is made for the continued use of the OGTT.

2.4 Prevention/Management of Diabetes

In addition to early diagnosis by the means described above, prevention/management of diabetes and its complications is a key goal. The Diabetes Control and Complications Trial (DCCT) showed that an increase in glycemic control will decrease the onset and progression of diabetic microvascular complications in type 1 diabetes. This effect was shown with insulin (123). The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated parallel results both with intensive oral hypoglycemic and insulin treatment in type 2 diabetes. Oral hypoglycemic regimens included insulin secretagogues (sulfonylureas) and sensitizers (metformin) alone or in combination (124,125). Additional treatment of concomitant risk factors that cluster with diabetes may also be important. The conclusion both of the Multiple Risk Factor Intervention Trial (MRFIT) (126) and the Insulin Resistance Atherosclerosis Study (IRAS) (92) was that diabetic subjects should have their cardiovascular risk factors treated aggressively. Haffner and coworkers further concluded that their risk factors should be treated as though they have established CHD (94). Support for this strong position is offered by subgroup analyses from the Scandinavian Simvastatin Survival Trial (4S) (127) and the Helsinki Heart Study (128), both of which reinforced the benefit of lipid lowering therapy in diabetic subjects with simvastatin and gemfibrozil respectively. Combined these data illustrate the necessity for concerted pharmaceutical management efforts in diabetes.

2.4.1 Diet plus exercise

A strong case can also be made for the use of nutritional hygienic and lifestyle strategies. One strategy that has been investigated with some promise includes a combination of diet and exercise. In the Da Qing study, a group of 126 subjects who consumed 55-65% of calories from carbohydrate in combination with exercise were observed to have
significantly reduced glucose tolerance deterioration and delayed conversion from IGT to type 2 diabetes over 6 years (129). Subsequently, Stefanick and coworkers (130) found that a similar intervention reduced LDL cholesterol significantly among men (13%) and women (9%) with high risk lipoprotein levels. When the high-carbohydrate/low-fat diet was given without exercise in both cases, these same benefits, however, were observed to be either not as strong (129) or absent (130). This has suggested to some that these diets may be inadequate when given alone. Support is offered by other studies that have looked at the effect of high-carbohydrate/low-fat National Cholesterol Education Program (NCEP) diets, in which only very modest reductions in LDL cholesterol have been observed (131), with no benefit to lipid ratios (132).

2.4.2 Diet enriched with monounsaturated fat

Another strategy that has emerged involves a diet enriched with monounsaturated fat. Because of the perceived inadequacy of high-carbohydrate/low-fat diets, there has been a recent shift toward the advocacy of high monounsaturated fat diets. For example, the ADA in their 1999 nutrition recommendations advised the sharing of calories between monounsaturated fat and complex carbohydrate at the expense of saturated fat to achieve nutritional goals (133). Diets arising from this strategy have been noticed to have effects comparable to high-carbohydrate plus exercise interventions. High monounsaturated diets have been shown to decrease glycemia, reduce fasting plasma triglycerides and maintain HDL, without adversely affecting LDL cholesterol (134,135). These diets have not however been shown to improve glycemic control as measured by glycosylated hemoglobin (135). In individuals with the insulin resistance syndrome, the benefits have been used as justification for recommending diets high in monounsaturated fat over the use of high carbohydrate/low fat diets (136). The main criticism against this recommendation is that these diets represent a concentrated source of energy that could lead to increased obesity.
2.4.3 Diet enriched with soluble fiber

Low-fat/high carbohydrate diets may still have promise as a therapeutic approach. An alternative strategy that has been investigated is supplementation of these diets with soluble fiber. Added guar gum, pectin, oat products, and psyllium have been shown to improve total and LDL cholesterol significantly, with no improvement to triglycerides and slight or no adverse effects on HDL (137). Both guar gum (138) and konjac mannan fibre (139-141) supplementation have also been shown to improve other risk factors, including glycemia and blood pressure. These last data lead to support for their use in the treatment of the insulin resistance syndrome (138,140). Evidence further suggests that supplementation with these soluble fibers may augment concurrent drug therapy. Improvements in these assorted risk factors following supplementation have been noticed beyond what was achieved by drugs alone in subjects receiving hypolipidemic (138,142), hypoglycemic (138,143), and hypotensive (138,144) medications. The overall suggestion is that low fat/high carbohydrate diets supplemented with soluble fiber may confer additional benefits over diet alone, benefits that are comparable to strategies using monounsaturated fat or high-carbohydrate diet plus exercise.

2.4.4 Diet with a low Glycemic Index

Use of low glycemic index (GI) foods is an additional strategy. It also offers a means for augmenting traditional high carbohydrate/low fat diets. The GI is a tool that provides a physiological assessment of foods, comparing foods based on their blood glucose raising ability (145). It works on the premise that because different foods elicit different glycemic responses, if one is able to identify and limit the consumption of those with a large blood glucose raising ability while emphasizing the consumption of those with the opposite, then an increase in glycemic control may be possible. Clinical studies by Wolever et al (146) and Brand et al (147) have shown that a low GI diet improves overall
blood glucose control. Furthermore, two recent epidemiological cohort studies from Harvard (88,89) showed that a reduction in glycemic load of the diet, as measured by GI, could significantly decrease the risk of developing diabetes.

This concept of a classification system based on glycemic response is not unique to GI. Otto and coworkers (148), in their 1973 study of diabetic patients, were the first to undertake such a classification. They compared the glycemic response of various carbohydrates. A group from Stanford (21) followed in 1976 with a similar effort that confirmed differences between carbohydrate foods observed by Otto and coworkers. The principal objective of these studies was to develop food tables of biological equivalence from which diabetic diets could be constructed (149). The suggestion was that traditional diabetic exchange lists based only on the amount of carbohydrate might not reflect the actual physiological effect of the foods contained on these lists. Jenkins and colleagues in 1981, built on their work, publishing the first GI classification of 62 foods (145).

This method of classification was distinct from that first developed by Otto et al. Instead of using just the glycemic response to a food (an absolute value) the GI used a "relative" blood glucose value as a basis of comparison. It was defined as the positive incremental area under the blood glucose response curve (AUC) for a test food expressed as a percent of the AUC for a reference food (white bread or glucose), where both contain the same amount of biologically available carbohydrate (typically 50g) and are given with the same volume of water (145). Expressed as a formula, the definition was as follows (149):

\[
GI = \frac{(AUC \text{ for test food})}{(AUC \text{ for reference food})} \times 100
\]

Values generated allowed for the systematic classification and rank ordering of foods based on their blood glucose raising potential. The expectation was that they could be used in the
construction of low-GI therapeutic diets for diabetes (145,146) and hyperlipidemia (150,151).

Since its introduction however, there have been some criticisms over the use of GI in this role. The Stanford group were one of the first to question whether GI could be used to predict the glycemic response to mixed meals (152). They contended that expected differences between foods in glycemic response did not persist in the context of mixed meals (152). They also suggested that the clinical utility and effectiveness of the index was not equivalent to other dietary related manoeuvres, such as weight control, saturated fat reduction, and regulation of the relative amounts of carbohydrate and fat (153). This view was supported by the ADA. In their 1994 position statement, the utility of the GI was dismissed and amount of carbohydrate was indicated as more important than type of carbohydrate (154). The implication, in this latter case, was that all types of carbohydrate were equal in their effect and thus GI was superfluous.

Wolever, in defence of the GI, suggested that these criticisms were based on misinterpreted data, misquoted literature, and misused statistics (155). He argued that conclusions by the Stanford group regarding the application of GI in mixed meals were based on total rather than incremental area under the curve, did not take into account all carbohydrate foods in the meal, ascribed an incorrect GI value to one of the foods, and did not consider the possibility of a type II error where expected differences were small (155,156). In addition, the conclusions by the ADA regarding the same matter were only based on three studies, ignoring the results of other better designed studies in this area that showed a significant correlation between expected and observed glycemic responses to mixed meals (157-159). Other evidence not considered by the ADA were several longterm studies that demonstrated a glycemic control increasing effect of low GI-diets (146,147,161-165).
Despite the controversy, the GI still enjoys credibility and popularity. The method has been endorsed in the management of diabetes by the CDA (165), WHO (166), and Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes (167). In addition, more than 100 published studies have used and applied the GI method, firmly establishing its importance.

A 1995 study by Oster-Powell and Miller consolidated the results from 79 of these studies to produce an “International table of glycemic index” which contained 600 food entries (168). From this comprehensive table some characteristics of the index become evident. First, it shows that there is large variability in GI values among different foods. The values, for example, can vary from as low as 10 for peanuts to as high as 150 for maltose, a 15-fold difference.

Second, this table shows the freedom the index allows for pooling the results from diverse sources. It has been established that GI results are reproducible. Both the results of different investigators and those from people with varying glucose tolerances (healthy versus diabetic) have been found to agree well with each other (149), making comparisons between different foods from different sources possible. This table draws on the findings of many different studies from around the world in which normal, well or poorly controlled type 2, or type 1 diabetic subjects make up the samples.

Finally, this table shows that the glycemic index to a food cannot be predicted with certainty simply by its carbohydrate content. Not all sugars have the same GI. This is also true for legumes, nuts, grains, cereals, fruits, and vegetables. These examples show that differences in blood glucose-raising potential can exist between foods belonging to the same basic food groups and possessing similar macronutrient profiles.
2.5 Dietary Factors and GI

To understand the role of GI in the prevention/management of diabetes, it is important to consider its dietary determinants. The effect of different food factors and their interaction on the postprandial glycemic response is very complex. Some generalizations, however, can be made about the effect certain constituents and processes have. Several of these follow in the next seven sub-sections.

2.5.1 Type and Amount of Carbohydrate

An effect of type of carbohydrate on glycemia has been demonstrated. Crapo et al. were one of the first to show (1) and then confirm (2) that different complex carbohydrate foods possess different responses. They found the response to baked potato was higher than to an identical amount of cooked rice (1) and subsequently to identical amounts of cooked rice, corn, and bread (2). These differences were corroborated by GI testing, showing the GI of potato as 121 compared to 101, 78, 72, and for bread, corn, and rice respectively (168). Wolever and Bolognesi further demonstrated a significant independent effect of type of complex carbohydrate, where potato, bread, spaghetti, and barley were the sources studied (3). Source of complex carbohydrate was found to explain 46-64% of the variability in glucose and insulin responses. Similar variations in glycemic response have been observed between simple carbohydrates. The difference in GI between the lowest fructose (GI=32) and highest maltose (GI=150) is more than four-fold. Intermediate are lactose (GI=65), sucrose (GI=92), and glucose (GI=138) (168). Whether comparing within or between complex and simple carbohydrates, these data show that different types are not equal in their responses.

Different quantities of carbohydrates are likewise not equal in their response. In addition to an effect of type of carbohydrate, Wolever and Bolognesi (3), in the same study cited above, showed a significant independent effect of amount of carbohydrate. It
was found to explain 47-57% of the variability in glucose and insulin responses. Others have corroborated this strong relationship between amount of carbohydrate and blood glucose response. In non-diabetic subjects, the blood glucose curve has been shown to increase nearly linearly as the dose of white bread or glucose was increased from 0 to 50g. This response curve flattens, however, as the dose is increased from 50 to 100g creating a curvilinear response profile (4). The relationship for insulin appears to be linear over this entire dosage range (4). A similar dose-dependent effect has been shown both in type 1 (169) and type 2 (170) diabetic subjects after the consumption of starch-rich meals containing 20, 40, and 60g of carbohydrate, with no evidence of flattening.

The reason both type and load of carbohydrate affect postprandial glyemia is thought to involve substrate delivery. In the case of the former, the reason for this phenomenon has largely been ascribed to the speed with which carbohydrate is delivered and made available. That is, the rate of absorption (149) and proportion and rate of hepatic conversion of non-glucose sugars to glucose are determinants of glycemic response, whereby starches and sugars that are more quickly absorbed and converted have higher responses (5). The conversion of nonglucose sugars applies principally to sucrose and its subunit fructose and lactose and its subunit galactose, all of which, as indicated have lower GIs than glucose (168). On the other hand, the reason that the load of carbohydrate affects glyemia is more a function of the absolute quantity of carbohydrate delivered. The greater the amount consumed, the greater the amount that is absorbed and enters circulation, with hepatic release of carbohydrate in nondiabetic subjects presumably being a limiting factor for carbohydrate intake beyond 50g.

2.5.2 Food Form and Processing

Food form has also been shown to affect glyemia. Wolever and co-workers (6) noticed that the glycemic responses to pastas were variable. Macaroni was found to have
a significantly higher GI (GI=68) than spaghetti (GI=45) with the GI of star pastina (GI=54) intermediate. The same variability has been shown extensively between different varieties of rice. Both Larsen et al. (7) and Miller JB et al (8) showed that there were differences in GI between rices with different amylose content, where low-amylose rice had a significantly higher GI than high-amylose rice. Panlasigui and co-workers (9) showed the same effect in different rice varieties with similar amylose content, where IR42, a high amylose rice, had a significantly higher GI (GI=91) than IR62 (GI=61), another high-amylose rice. Wolever and colleagues (10) also showed that regular long grain rice had a significantly higher GI (GI=83) than both parboiled (GI=67) and instant rice (GI=65). O'Dea and colleagues (11), however, showed no significant difference between white and brown rice. Various Breads have also been noticed to effect glycemia differently. The tables of GI (168) showed the GI of white flour wheat bread as 101 compared to 92 for Rye flour bread, 71 for Rye kernel bread, 64 for mixed grain bread, and 49 for barley kernel bread.

Possible factors that may be responsible for this observed effect of food form include starch structure and gelatinization. These attributes are determined both by the amylose content and other physiochemical properties. Because of the linear structure of amylose, starch granules with a high amylose to amylopectin ratio are thought to form more hydrogen bonds and thus a more crystalline structure that less readily swells and gelatinizes. The effect is to slow digestion and thus decrease glycemia (7, 8,149). Rices with the same amylose content nevertheless can have different responses as indicated above. Other physiochemical properties such as a high gelatinization temperature, low amylograph consistency, high cooking time, and low expansion volume following cooking are therefore also important determinants of slow absorption and a low glycemic response (9).
It may be speculated that differences in processing is another reason for some of these observed effects of food form. Jenkins and co-workers (12) showed that drying boiled-blended lentils for 12h at 250 °C prior to consumption significantly enhanced the glycemic response relative to standard (20min) boiled lentils. There was no significant effect of either boiling them for an additional 40mins or blending them. Others, however, have noticed an effect of boiling. Collings and colleagues (13) found that boiled cornstarch significantly increased the glycemic response compared to raw cornstarch and Wolever and co-workers found that an additional 10 minutes of boiling (from 5 to 15 minutes) had a significant blood glucose raising effect with rice (10), but not pasta (6).

Processes similar to blending that physically break down foods have also been shown to increase blood glucose. Jenkins and co-workers (14) demonstrated that breads made from milled wheat or rye flours resulted in a significantly higher GI than unmilled (whole) wheat or rye grains or bread made from these (pumpernickel). Similarly, both O’Dea et al. (11) and Collier et al. (15) found that ground white (11) and brown (11,15) rice had significantly higher glycemic responses than the same unground rice.

Finally, when numerous of these processes are combined this blood glucose raising effect remains. Brand and colleagues (16) showed that factory prepared foods which undergo multiple processes (instant rice, Rice Bubbles, corn chips, Cornflakes, instant potato, and potato chips) had a significantly higher GI than their respective conventionally prepared counterparts (boiled rice, sweet corn and potato). The exception was potato crisps versus boiled potato. The overall suggestion is that an increase in the degree of processing as cooking, drying, milling, grinding, mashing, or combinations of these, may increase the glycemic response.

An explanation for the effect of processing again involves rate of absorption. Because processing generally results in degradation, it can have the effect of preempting
some degree of digestion, increasing surface area, enhancing accessibility/susceptibility to pancreatic enzymes, and/or decreasing food viscosity (6, 9,10,12). The effect of these outcomes is to increase the rate of absorption and thus glycemia.

2.5.3 Fibre Content and Composition

The evidence for an effect of fibre content on glycemia is equally compelling. Since Burkitt’s early observations linking fibre consumption to a decreased risk for various diseases (171), it has been shown to have a protective effect in diabetes (84,85), as indicated. It has also been shown to have a protective effect in hyperlipidemia (172,173); coronary heart disease (174,175); hypertension (138,176); colon cancer (177,178); and other intestinal disorders (179,180). A reduction in blood glucose is thought to be a possible mediator of this effect in some of these disorders (84,85,181). Wolever (19) in 1990 confirmed that a decrease in postprandial blood glucose was associated with the consumption of certain unpurified fibre components contained in food. Prior to this study such a determination was not possible as detailed information about the fiber content and composition of foods did not exist until Anderson and Bridges (182) analysis of 60 foods in 1988. Relating their data to the known GIs for 25 foods. Wolever noticed that the content of uronic acid in insoluble fiber, cellulose, and total fiber was significantly negatively correlated with blood glucose response, where these variables explained 34%, 25%, and 21% of variation in GI respectively. The same, however, was not found for soluble fibre (19). Subsequent findings by Guevin and co-workers (20) supported this lack of effect of unpurified soluble fiber in food. They reported that an increase in the total fibre of a meal decreased postprandial blood glucose, but altering the proportion of insoluble to soluble fibre, via the use of different unpurified food sources, had no effect on it.
Although an effect of unpurified fiber on blood glucose was demonstrated, the implication that insoluble fibre may be a stronger determinant than soluble fibre was unexpected. Evidence has yet to be presented for a blood glucose lowering effect of cellulose when given in purified form as a supplement. It has been shown that the response to 50g glucose supplemented with purified cellulose is no different than that to the 50g glucose alone (20). Others have shown the same lack of effect with purified cellulose where the glucose dose was increased to 100g (21). The effect of purified uronic acid, which is derived principally from hemicelluloses, is also unknown (19). On the other hand, evidence for an effect of purified soluble fibers on glycemia has been very strong. Pectin (22-24), psyllium (25-27), beet-fibre (28,29), konjac mannan (26,30), beta-glucan (31,32) derived from oat bran, and mixtures (33-35) of these have all been shown to decrease postprandial blood glucose when given as a purified supplement. The same is also true for various soluble fibre containing gums: guar (23,36-38), xanthan (26,39), oat (40,41), and locust bean gum (39).

Reasons why unpurified soluble fibres do not appear to affect postprandial glycemia while purified do are unclear. One possibility may be that when naturally occurring in foods, certain fiber components may have their effect on glycemia altered by other factors present. One interfering factor may be viscosity differences between purified and nonpurified sources. An increase in the viscosity of the digesta is considered to be the main mechanism by which soluble fibre has its glycemic lowering effect (26,183). It would be expected that purified forms would have a greater viscosity and thus a greater effect. Alternatively, some of the other dietary factors discussed above and below, as well as fibre-nutrient interactions may be responsible. All of these factors can influence the rate of digestion and, therefore, may explain differences between the action of unpurified and purified fibres.
2.5.4 Protein Content

The protein content of food has been shown to have a variable affect on postprandial glycemia. In the 1960s, it was well reported that protein and amino acid ingestion stimulated insulin secretion both in diabetic (184,185) and normal subjects (186-189). The extrapolation was that protein might thus affect postprandial blood glucose in both these cohorts. This was confirmed for diabetic subjects by subsequent studies. It was shown by the same group twice: once in 1984 (42) and again in 1992 (43), that the addition of either 50g of protein as lean beef (42) or 25g of protein as cottage cheese or egg white (43) to a 50g glucose load had a synergistic effect on insulin, in type 2 diabetic subjects. The effect was such that the insulin response for the combination of the protein and glucose was greater than for the sum of their individual responses. On both occasions the result was a significant decrease in blood glucose. Others and subsequently this same group showed a similar effect with different sources of protein and carbohydrate. Both Gulliford at al (44) and Gannon et al (45) noticed a significant decrease in blood glucose after the consumption of carbohydrate (glucose or mashed potatoes, respectively) mixed with 25g of protein as either tuna fish (44) or turkey, gelatin, or cottage cheese (45) compared to the carbohydrate source taken alone. Together the results of these studies suggest that various proteins attenuate the glycemic response to carbohydrate in type 2 diabetic subjects.

The same cannot be said for nondiabetic subjects. Westphal and co-workers (190) observed that the addition of 50g protein to 50g glucose significantly increased postprandial insulinemia compared to the glucose taken alone, but had no effect on glycemia in nondiabetic subjects. Also neither blood parameter was affected by the addition of smaller doses of protein of 30g or 10g. Similarly, Day and coworkers (191) found that there was no significant difference in the glucose and insulin concentrations
between protein coingested with glucose and glucose taken alone in nondiabetic subjects. There is however some disagreement about the effect of protein where this cohort is concerned. Spiller and coworkers (46) found that protein added to a carbohydrate mixture produced significantly higher blood insulin and lower blood glucose responses than this mixture taken alone in healthy subjects. Hofman and colleagues (47) also found that one hour after exercise, the ingestion of 6g protein added to a sports drink with 69g carbohydrate significantly increased insulin and reduced blood glucose compared to the drink taken alone in nondiabetic trained athletes.

An explanation for these last two exceptions to the lack of effect of protein on the glycemic response to ingested carbohydrate in nondiabetics may be differences in the type of carbohydrate used. Those groups that found no effect of protein in nondiabetic subjects used straight glucose, whereas the last two groups introduced additional carbohydrates in the form of fructose and lactose (46) and sucrose (47). The interplay between these carbohydrates and protein may be different than that between glucose and protein, such that a decrease in the blood glucose response to these carbohydrates is the outcome. In the case of the last study, another reason may be an interactive effect between protein and exercise, where protein potentiates the insulin -sensitivity-increasing -effect of exercise that has been previously shown in other studies (192), thereby decreasing blood glucose. These explanations, however, are unproved.

Also speculative is the reason why protein appears to have an effect in type 2 diabetic subjects but not in nondiabetic subjects. Protein is considered a rather potent insulin secretagogue in people with type 2 diabetes compared to those without. It has been suggested that a relative difference between the two groups in the β-cell sensing of a rise in glucose and amino acid concentrations may offer an explanation. Alternatively, differences in incretin hormone activity may be a contributor. Incretin hormones that
include gastric inhibitory peptide (GIP), glucagons-like peptide 1-7-36-amide (GLP-1), and cholecystokinin (CCK) are released from intestinal mucosal cells in response to ingested food and bind to β-cells in the pancreas. Their effect is either to stimulate insulin secretion directly or potentiate glucose or protein stimulated insulin secretion. It is possible that greater secretion of these hormones and/or β-cell sensitivity to them in response to the coingestion of carbohydrate and protein occurs in people with diabetes compared to those without (50).

2.5.5 Fat Content

Fat affects the glycemic response to carbohydrate, without the discrepancies between nondiabetics and diabetics. Rasmussen and coworkers (48) found that the addition of 100g of margarine to approximately 53g of carbohydrate as potato significantly reduced the glycemic response compared to potato alone in 12 diabetic subjects. Similarly, Gulliford et al (49) noticed that the addition of 25g of butter to 25g of carbohydrate as potato and 25g of protein as tuna fish significantly reduced glycemic response compared to potato and tuna fish alone in 6 diabetic subjects. This same effect of fat appears true in individuals without diabetes. A group from the University of Minnesota thrice reported that 50g of butter significantly reduced the glycemic response to 50g of carbohydrate as potato in healthy nondiabetic subjects (50-52). This was also shown earlier by Collier and coworkers using the same test foods and a similar protocol (193).

These studies used butter or margarine as the fat source. It is unclear whether these results would be true for all sources. Not all fats appear to be equal in their effect: Different fats affect glycemic response to carbohydrates differently. Rasmussen and coworkers (48) found that although 100g of butter added to 53g of carbohydrate reduced postprandial glycemia, the same amount of olive oil did not. This suggested differential
effects of saturated (SFA) and monounsaturated fat (MUFA) in diabetic subjects. In nondiabetic subjects, similar observations were made in the context of mixed meals. Joannic et al (53) observed that in matched mixed meals where the carbohydrate source was parboiled rice, the addition of polyunsaturated fat (PUFA) decreased glycemic response more than MUFA. Lardinois et al (194) further noticed that the glycemic response to mixed meals enriched either with SFA or omega-6 PUFA were smaller than to mixed meals enriched with the less saturated omega-3 PUFA. Overall, these data suggest that the degree of saturation may affect glycemia with more saturated fats eliciting lower postprandial responses.

Mechanisms by which fat may exert these differential effects on glycemia are not known. One suggestion involves an insulin secretagogue effect similar to that described above for protein. In three of the above studies in which a glycemia lowering effect of added fat was demonstrated, insulin was increased in a concomitant fashion (48,49,52). These increases were after the addition of SFA (48,49,52) but not MUFA (48). Findings for C-peptide supported the direction of these findings (52). The remaining studies either showed no effect (50,51,193) on insulin secretion or the opposite (53,194). Another mechanistic option involves an acute insulin sensitizing effect of fat. Both Collier et al (193) and Lardinos et al (194) observed that although insulin was unchanged by the addition of SFA, GIP was increased relative to carbohydrate alone and a meal enriched with PUFA respectively. In addition to being implicated as possessing inulinotropist (50,52,193) properties, this incretin hormone is thought also to be an insulin sensitizere (195). It is possible that a SFA glucose lowering effect might therefore be secondary to a GIP increasing effect that mediates greater glucose disposal (52). A final possibility to explain the observed reductions comes back to an effect on rate of carbohydrate absorption. It has been shown repeatedly that high fat mixed meals of varying degrees of
saturation have slower gastric emptying than high carbohydrate mixed meals (196-198). Delayed and prolonged mesenteric blood flow following high fat meals compared to high carbohydrate meals has also been demonstrated (198). Both effects would likely contribute to slower carbohydrate absorption and hence lower glycemia when fat and carbohydrate are coingested (196,197).

2.5.6 Antinutrient content

Antinutrients are another factor that affects the postprandial glycemic response to carbohydrate. These compounds include phytic acid, polyphenols (tannins), organic acids, and lectins.

Phytic acid has repeatedly been shown to decrease postprandial glycemia. The same group demonstrated that endogenous phytic acid in 15 different carbohydrate foods (55) and exogenous phytic acid added to navy bean starch (56) significantly decreased the glycemic response to these foods. Similarly, Trout and coworkers (57) observed that phytic acid concentration in 18 foods was negatively correlated with their known GIs. In all three cases, slowed digestion was implicated as the mechanism. It was speculated that phytic acid due to its reactivity at neutral or alkaline pH could bind either with starch via phosphate linkages or the protein that is closely associated with starch in food, physically hindering access of amylolytic enzymes or bind with the amylase enzyme itself, rendering it inactive (55-57). Alternatively, it could complex with Ca²⁺, an amylase cofactor, inhibiting the activity of amylase and thus slowing digestion. This last hypothesis was supported by in-vitro work that demonstrated that the addition of Ca²⁺ reversed reductions in digestion resulting from phytic acid (55,56).

Polyphenolic compounds that are commonly referred to as “tannins” have been found to have very similar effects. Thompson and coworkers (58) in an analysis of 13 foods found a significant negative correlation between GI and concentration or total
intake of polyphenols. These findings were supported by the observation of Gin and coworkers (199) that the addition of 2 glasses of red wine significantly reduced the glycemic response to a mixed meal in the first 150 minutes in 10 type 2 diabetic subjects. Because red wine contains tannins and phytic acid, it was hypothesized that they may have played a mechanistic role (199). The role tannins play however is unclear. Possibilities may be shared with phytic acid. Tannins are powerful reducing agents that can interact with starch, the protein closely associated with starch, proteolytic enzymes, and/or amylase directly. The effect of these interactions may render starch inaccessible to enzyme attack and/or the enzymes themselves less active (58). Either outcome would slow digestion, thereby reducing postprandial glycemia.

Organic acids, namely propionate and lactate, have also been shown to have glycemia lowering effects. Three studies in which propionate was incorporated into bread observed that it reduced postprandial glycemia compared to bread alone (200-202). In the second study, incorporation of lactate was also found to have the same effect (201). Adding to the evidence for a glycemia lowering action of organic acids, sourdough bread, a rich source of organic acids, was twice observed to lower postprandial glycemia and insulinemia compared to ordinary bread (202,203). The mechanisms involved are speculative. One suggestion is that propionate may delay gastric emptying. Liljeberg and coworkers (201) observed that paracetamol, a marker of gastric emptying added to the breads, was significantly decreased in blood following bread incorporated with propionate, indicating a lowered gastric emptying rate that may be mediated by a pH receptor mechanism. The same however was not found for bread incorporated with lactate. Another suggestion is that organic acids may interfere with amolotytic enzyme activity. Todesco and coworkers observed that the addition of sodium propionate to white bread in vitro resulted in inhibition of salivary amylase (200), although this could not be
replicated (202). Again either hypothesis, if true, would slow digestion, thereby reducing postprandial glycemia.

Finally, lectins may be responsible for additional blood glucose lowering. Although effects of lectins on the glycemic response to carbohydrate have not been assessed directly, lectin intakes have been found to correlate negatively with the blood glucose response (204). Lectins have also been noticed to reduce the rate of in-vitro starch digestion (149,205), suggesting a postprandial glycemia lowering effect.

2.5.7 Ripeness

Ripeness may be another determinant of the glycemic response to carbohydrate. Both Hermansen et al (17) and Wolever et al (18) observed that under-ripe bananas have a significantly lower GI than over-ripe bananas. The GIs were 43 and 59 for under-ripened bananas versus 74 and 69 for over-ripened bananas respectively. These data are supported by in vivo and in vitro findings that suggest banana starch granules are poorly digestible but that banana digestibility increases with ripening. Englyst and coworkers (206) showed that 69-91% of the starch in bananas passed through the small intestine unabsorbed in three people with ileostomys and that approximately 54% of the starch in raw bananas resisted enzymatic hydrolysis when incubated with α-amylase. This resistant starch however was shown to decrease with ripening, undergoing conversion to sugars that are readily absorbed: sucrose, glucose, and fructose largely. The implication was that as the banana level of ripeness increases, so too does its digestibility and hence ability to raise glycemia. In contrast, Ercan and coworkers (207) noticed no such effect of banana ripeness on glycemia.

2.6 Dilution and Postprandial Glycemia

In addition to the seven established factors discussed above, evidence suggests that dilution may affect postprandial glycemia. Research in this area has been done both in solid and
liquid meals. Three studies have been undertaken that investigated an effect of dilution using solid meals. The first by Torsdottir and Andersson (61) found that the addition of 300 mL of water to a mixed meal significantly increased the postprandial glycemic response both in healthy patients and well-controlled type-2 diabetic patients. This effect, however, was not seen in poorly controlled type 2 diabetic subjects. The findings of the second study by Gregersen and coworkers (62) were at variance with those of the first for healthy and well-controlled diabetic subjects. They found that an increase in the water volume consumed with a solid meal from 90ml to 600ml did not affect the postprandial glycemic response to the meal in their cohort of mixed (both well and poorly controlled) type-II diabetic patients. Although not in disagreement, the third study by Young and Wolever (60) noticed only modest effects of dilution in 12 nondiabetic subjects. Blood glucose was significantly higher for a solid diabetes-screening product (DSP) taken with 500ml than 50ml water at 15 minutes and 500ml and 750ml than 50ml, 250ml at 30 minutes. There was however no effect on AUC and no apparent dose response. The reverse was also true when 1000ml was added to the DSP: the glycemic response was lower following the addition of 1000ml than 500ml or 750ml at 30 minutes. And at 90 and 120 minutes the glycemic response to the DSP taken with low volumes was significantly higher than to it taken with high volumes. Overall, the data from these three studies is somewhat ambiguous for an effect of dilution, especially in diabetic subjects.

When using liquid meals the results have been more consistent. Two studies have observed an effect of water volume on glycemia with liquid meals. Wong and coworkers (26) noticed an effect indirectly in liquid control meals in a study in which alterations in water volume were used to adjust the viscosity of different fibers. They observed that the glycemic response to a 20g oral glucose control meal at 600ml was significantly higher than to the identical control meal at 300ml and that the glycemic response to both was significantly higher than to this meal at 75ml. Similarly, Schwartz and coworkers (59) noticed that a 3-fold dilution from 150ml to
450ml of a 50g-OGTT significantly raised peak blood glucose by 10% in pregnant women. Both studies suggested that dilution might increase glycemia.

2.7 Possible Mechanism of Dilution

Dilution may share a mechanism similar to other dietary factors in that an effect on rate of absorption is thought to mediate its effect on glycemia. Specifically, the proposed mechanism for this observed effect of water on glycemia is thought to involve rate of gastric emptying (61). Several studies have shown a relationship between gastric emptying and both water volume and osmolality in healthy subjects. A six fold increase in the volume of a phenol red solution (50ml to 600ml) (208) and a two fold increase in the volume of a liquid polycose meal (300ml to 600ml) (209) have been shown to significantly increase the rate of gastric emptying. Similarly, various decreases in the osmolality of carbohydrate solutions have also been shown to have the same effect (210,211). Another set of studies has in turn shown a relationship between gastric emptying and glycemic response. Both Mourot et al. (212) and Torsdottir et al. (213) in studies in healthy adults, found that the postprandial glycemic response to various starchy meals was positively correlated to their rate of delivery to the duodenum: the faster their rate of gastric emptying, the higher the response elicited. It was also twice observed that the faster an OGTT is emptied from the stomach, the higher postprandial glycemia (214,215). The extrapolation can be made from these data that an increase in the water volume or decrease in the osmolality of a meal may result in an increase in the rate of gastric emptying, which may in turn result in an increase in glycemic response (Figure 3.1).

Because this hypothesis is presumably dependent upon normal gastric emptying, differences observed between nondiabetic and diabetic subjects in the effect of volume may involve gastric function. Both type 1 and type 2 diabetic patients have been shown to have a high incidence of disturbed gastric emptying. Gastroparesis, or delayed gastric emptying of food, has been reported in 40-50% patients (216,217). In addition, abnormalities in gastric and small
intestinal contractility patterns have been observed in diabetic patients. Malagelada and co-workers (218) found that sporadic motor activity was significantly reduced in type 1 diabetic patients with gastroparesis. Jebbink and colleagues (219), in type 1 diabetic patients held at a normoglycemic level, observed a shortening of sporadic motor activity, increased burst activity in the duodenum, and hypomotility in the gastric antrum after a meal. The sum effect of these irregularities may be to negate the effect of volume on gastric emptying in diabetic patients, negating its proposed effect on postprandial glycemia in this cohort. In this regard, a study of Levitt et al (220) noticed that while viscous fibre reduced the postprandial glycemic response in type 2 diabetic patients without autonomic neuropathy, it produced no further flattening in patients with gastroparesis.

Figure 2.1 - Schematic of the dilution-gastric emptying hypothesis
2.8 Possible Therapeutic Implications of Dilution

An effect of dilution on glycemia may have therapeutic implications. If the blood glucose raising potential of various foods can be reduced by decreasing volume, then volume may prove to be a tool for regulating blood glucose levels. As indicated, a reduction in GI was shown to decrease the risk of developing diabetes in two recent epidemiological cohort studies (84,85). The investigators reported that in men (84) and women (85) the relative risk of type 2 diabetes was 1.37 when comparing the lowest and highest GI quintiles. The difference in GI between these quintiles (17% for women and 18% for men) was comparable to the 20% difference in postprandial blood glucose noticed by Tosdottir and Andersson (61) following the addition of 300ml water. In healthy individuals with poor blood glucose control, the long-term effect of volume regulation may be therefore to improve glycemic control, possibly preventing or delaying the onset of type 2 diabetes.

There may also be promise for people with already established diabetes. Improvement in glycemic control with oral hypoglycemics and insulin was shown to decrease the development and progression of microvascular complications complications (124,125). The same might be accomplished with GI. Again as indicated, the consumption of diets with a low GI have been shown to improve glycemic control, as measured by HbA1c, compared to high GI diets in type 2 diabetic subjects (146,147). If such a decrease in the GI of the diet could be affected by dilution, then dilution may prove a useful adjunct to the conventional treatment of diabetes.

Limitations of such an approach nevertheless exist. It is unlikely that it would be practical. Limiting water consumption at meals could potentially lead to dehydration. In addition, recent research in the area of dilution and hunger/appetite management is at odds with this modality. Lappalainen and coworkers (221) noticed that addition of 2 glasses of water with a breakfast significantly decreased subjective feelings of hunger and increased satiety. This was supported by two subsequent studies by Rolls and coworkers (222,223) that reported that
increasing the water volume of a preload significantly reduced hunger and subsequent energy intake. Together these data suggest that increasing not decreasing the level of dilution may be beneficial.

**2.9 Possible Methodological Implications of Dilution in Glycemic Testing**

Glycemic testing methodology is another area for which there may be implications of dilution. The literature shows that laboratories are inconsistent in the amount of water they give with or in their test meals. In the testing of oral glucose, for example, meals can range in volume from 240mL (63) to as high as 750mL (64). Other investigators studying an assortment of foods also vary in terms of the volume of the test meals they give: Gulliford et al. (46) and Rasmussen et al. (48) gave 250ml of tap water with test meals; Wolever et al. (114) 550ml; Walker et al. (224) and Jenkins et al. (145) 600ml; Gannon et al. (63) 950 ml; Gannon et al (45), in another study, ad libitum; and Bukar et al. (65) Ercan et al. (51), Hughes et al. (66), and Westphal et al. (67) did not state the amount of water/beverage, they served with their test meals or the absolute volume of their test meals. These examples demonstrate that volume is not standardized in glycemic testing and sometimes not controlled. If dilution is able to increase postprandial glycemia, then this situation might contribute to variability in results. Researchers may want to consider giving test meals of equal volume and exercise caution when comparing the glycemic testing results of other researchers, who do not control for meal volume in their study design or who give meals at a non-standardized volume. These concerns may be less relevant for GI testing, in which foods are tested against a standard of equal volume (145).

**2.10 Possible Diagnostic Implications of Dilution in Diabetes Screening**

Dilution may also have implications for diabetes screening using the 75g-OGTT. As indicated, there is considerable controversy regarding the poor reproducibility of this test (76,77,114,225). Several factors may be contributing to this problem. Administering the test at different phases of gastric emptying (215) has been shown to be a determinant of outcome and
may offer a partial explanation. Irregular gastric emptying resulting from the high osmolarity of the test has also been speculated to be a possible contributor (59,109,226). Another reason may involve dilution. There is a discrepancy between the established guidelines for its use. The WHO (74) and ADA (70) instruct that the 75g-OGTT should be administered at a dilution of 250-300ml, while the NDDG (75) and CDA (71) instruct that it should be administered at a minimum dilution of 300ml. This last case allows for it to be administered at a range of volumes above 300ml. In addition, because of the test's poor palatability (76,77,110-112) and side-effect profile (109,108-112), subjects may be compelled to request additional water with the test to improve its tolerability. Either situation may result in dilution differences. Provided a glycemia raising effect of dilution is true, this situation may lead to variability in results, incorrect diagnoses, and skewed comparisons of data derived from an OGTT at one volume with data derived from another.
CHAPTER 3.

EFFECT OF MEAL DILUTION ON THE POSTPRANDIAL GLYCEMIC RESPONSE:

IMPLICATIONS FOR GLYCEMIC TESTING
3. EFFECT OF MEAL DILUTION ON THE POSTPRANDIAL GLYCEMIC RESPONSE: IMPLICATIONS FOR GLYCEMIC TESTING

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3.6 Abstract

Objective - To investigate the effect of varying the volume of sugar meals on the postprandial glyemic response.

Methods - On six separate occasions, following an overnight fast, blood glucose concentrations were measured in eight healthy subjects (34±4 years of age, BMI: 22.9±0.9 kg/m²) after the consumption of 25g glucose, sucrose, or fructose dissolved in either 200ml or 600ml of water. Blood was obtained at fasting and then at times 15, 30, 45, 60, and 90 minutes after the start of the test meal.

Results - The postprandial glyemic response was found to be influenced by carbohydrate type (p<0.001). Mean response areas (min·mmol/L) to the three sugars were statistically different (p<0.05). Glucose had the highest response area 90.0±8.1, followed by sucrose 61.3±5.0, and fructose 14.7±2.8. Independent of this effect, the postprandial glyemic response was also found to be influenced by volume dose (p<0.01). By tripling meal volume from 200ml to 600ml, response areas were significantly increased for all three sugars: glucose (79.3±10.3 vs. 100.8±12.0, p=0.035), sucrose (52.6±5.5 vs. 70±7.4, p=0.0094) and fructose (11.0±3.8 vs. 18.4±3.9, p=0.012). Where the effects of time (p<0.05) and dose (p<0.05) were determined to be independent (interaction non-significant) for all three sugars, this increase in volume also significantly increased glycemic concentrations at 15 minutes, for glucose (p=0.033) and sucrose (p=0.026), suggesting that changes in gastric emptying time may be a mechanism of action.

Conclusion - Varying the volume of liquid sugar meals alters postprandial glycemia. Understanding this concept may help to reduce variability both in the glycemic testing of foods and oral glucose tolerance testing.

3.7 Key words

Volume, glucose, fructose, sucrose, glyemic response, OGTT
3.8 Introduction

Poor blood glucose control over the long-term may contribute to the development of non-insulin dependent diabetes mellitus and its associated complications (84,85). Because tight glycemic control may reverse this process (123), the effect of various dietary factors on postprandial glycemia has been investigated. Factors found to have an effect include type of carbohydrate (1-3); fibre (19-41), protein (42-47) and fat (48-53) content; food form (6-10); and processing (11-16). Evidence suggests that water volume may also a factor. Torsdottir and Andersson (61) found in both normal and well-controlled type II diabetic subjects that the addition of 300mL of water to a solid meal significantly increased the postprandial glyemic response.

This latter finding may prove of particular importance in the glycemic testing of food. The volume of test meals can vary between laboratories. In the testing of oral glucose, for example, meals can range in volume from 240mL (63) to as high as 750mL (64). The suggestion is that such a 3-fold change in meal volume may affect postprandial glycemia, leading to some variability in results. Whether this finding holds true in the case of a liquid meal, such as glucose is unknown. The objective of the present study was, therefore, to investigate if a similar 3-fold increase in the water volume of glucose, sucrose, and fructose test meals would have an effect on the postprandial glyemic response in healthy subjects.

3.9 Methods

3.9.1 Participants

Five men and three women (mean age 34±4 years) recruited from faculty and students at Ryerson Polytechnic University and University of Toronto took part in the study, which was approved by the Research Ethics Committee at St. Michael’s Hospital (Appendix V). All were healthy and normoglycemic and gave informed written consent
(Appendix I) prior to undertaking the study. The participants mean fasting blood glucose was \(4.3 \pm 0.1 \text{mmol/L} \) and mean BMI was \(22.9 \pm 0.9 \text{kg/m}^2\).

### 3.9.2 Test meals

Each participant received six test meals in random order. The meals consisted of 25g of sucrose (Redpath sugar, Toronto, Canada), fructose (Flora Distributors Ltd., Burnaby, Canada), or d-glucose (Regal Pharmaceuticals, Burlington, Canada) powder dissolved in 200ml or 600ml of tap water. These three sugars were selected as they represent a wide range of physiological responses (168) and, thus, increase the potential application of the study results to a diversity of other foods.

### 3.9.3 Protocol

Participants attended the Risk Factor Modification Centre at St. Michael's hospital on six separate mornings following a 10-12 hour overnight fast. They were instructed to maintain the same dietary and exercise patterns the evening before each test. They were also instructed to avoid exercise on test mornings, so as to avoid any possible effects of preprandial exercise on fasting blood levels (227). To ensure that these instructions were followed, participants completed a questionnaire detailing information about their diet and lifestyle patterns during the 16 hours prior to the test. Upon commencement of the test, subjects gave an approximately 250μL fasting finger-prick capillary blood sample, using a *Monoejector Lancet device* (Owen Mumford Ltd., Woodstock, Oxon, England). A test meal was then given with instructions to drink it over a period of exactly 10 minutes. Additional blood samples using the same technique were obtained at 15, 30, 45, 60, and 90 minutes after the start of the meal. All blood samples were collected in tubes containing fluoride oxalate and immediately frozen at \(-20^\circ \text{C}\) for no longer than three days pending analysis.
3.9.4 Blood Glucose Analysis

The glucose concentration of each sample was determined by the glucose oxidase method using an YSI 2300 Stat glucose/L-lactate analyzer, model 115 (Yellow Springs, Ohio).

3.9.5 Statistical Analysis

Blood glucose concentrations at each time point were graphed and the positive incremental AUC was calculated geometrically for each participant, ignoring areas below the basal blood glucose value (156). Results were expressed as mean±SEM. Statistical analyses were then performed using the SAS statistical program (SAS Institute, Cary, NC, USA). Repeated measures two-way ANOVA assessed the independent interactive effects for volume dose (200ml and 600ml) and carbohydrate type (glucose, sucrose, and fructose) on AUC Carbohydrate type being calculated as the average between the 200ml and 600ml tests of each sugar. This same statistic also assessed the independent and interactive effects for volume dose (200ml and 600ml) and time (0, 15, 30, 45, 60, and 90 minutes) on changes in blood glucose concentrations. Between the three types of carbohydrate, differences in AUC were assessed by one-way repeated measures ANOVA, adjusted for multiple comparisons with the Newman-Keuls procedure. Finally, within each sugar, between the 200ml and 600ml doses, differences in AUC and changes in blood glucose concentrations at each time point were assessed by student’s t-test for paired data. In all cases, differences were considered statistically significant if $p<0.05$.

3.10 Results

All participants were able to consume all test meals in the time allotted and there were no complaints about the volume or palatability of any of the tests. Also, no symptoms of fructose malabsorption were recorded. One subject, however, had to repeat both the glucose 200ml and
600ml meals for failing to comply with the study protocol requirement of no exercise on test mornings.

3.10.1 Area Under the Curve

Figure 1 shows the mean AUCs (min·mmol/L) to the six test meals. The results of the repeated measures two-way ANOVA applied to these data demonstrated a significant difference in the effect both for volume dose (p=0.0081) and type of carbohydrate (p=0.0001). This two-way analysis also demonstrated no significant interaction (p=0.56) between these two factors, indicating the effects of type of carbohydrate and dose are independent. Pair-wise comparisons between the types of carbohydrate showed the mean AUCs (min·mmol/L) to all three sugars to be significantly different (p<0.05, repeated measures ANOVA with Newman-Keuls). Glucose had the highest area (90.0±8.1), followed by sucrose (61.3±5.0), and then fructose (14.7±2.8). Similarly, pair-wise comparisons with-in each sugar showed mean AUCs between the two volume doses to be significantly different in all three cases. The mean areas (min·mmol/L) to the 600ml meals were found to be significantly greater than those to the 200 ml meals for glucose (100.8±12.0 versus 79.3±10.3, p=0.035), sucrose (70.0±7.4 versus 52.6±5.5, p=0.0094), and fructose (18.4±3.9 versus 11.0±3.8, p=0.012) by paired t-test. Percent differences between these high and low volume meals were 19.8±7.5%, 23.6±6.3%, and 42.5±11.7% respectively.
Figure 3.1 - Comparison of glycemic response areas between the 200ml and 600ml meals for (A.) glucose, (B.) sucrose, and (C.) fructose in 8 healthy subjects. Values are mean±SEM. P values represent differences between the 200ml and 600ml test meals (Repeated measures one-way ANOVA adjusted for multiple comparisons with the Newman Keuls procedure).
A. GLUCOSE

- Incremental changes in blood glucose after the ingestion of 25g glucose at 200ml and 600ml.

B. SUCROSE

- Incremental changes in blood glucose after the ingestion of 25g sucrose at 200ml and 600ml.

C. FRUCTOSE

- Incremental changes in blood glucose after the ingestion of 25g fructose at 200ml and 600ml.

Values are mean±SEM. Asterisks indicate significant differences (p<0.05) between the 200ml and 600ml meals at 15 minutes for glucose and sucrose (paired t-test).
3.10.2. Incremental Glycemia

Figure 2 shows the incremental changes in blood glucose over 90 minutes after the consumption of the 200ml and 600ml meals of glucose, sucrose, and fructose. In the case of glucose and sucrose, the results of the repeated measures two-way ANOVA demonstrated a significant difference in the effect both for dose (p=0.044 and p=0.021 respectively) and time (p=0.0001 and p=0.0001 respectively) with no interaction (p=0.25 and p=0.21 respectively). This indicated the effects of dose and time on blood glucose concentrations are independent. Pair-wise comparisons further showed the incremental change in blood glucose to the 600ml meal to be significantly greater than that to the 200ml meal at 15 minutes (p<0.05, paired t-test) for these two sugars. Differences at all other time points, however, were shown to be insignificant, including at 30 minutes, the time of peak blood glucose rise for all meals. For fructose, the results of the repeated measures two-way ANOVA likewise demonstrated a significant difference in the effect both for dose (p=0.0084) and time (p=0.0001) with no interaction (p=0.78). Pair-wise comparisons between the 200ml and 600ml meals of this sugar, however, showed that differences in incremental changes in blood glucose were insignificant at all time points.

3.11 Conclusions

Present results suggest that meal volume influences the postprandial glyemic response in healthy subjects. A 3-fold decrease in volume from 600ml to 200ml resulted in a significant reduction in AUCs and had a significant reducing effect on incremental changes in blood glucose concentrations in all three sugars.

Previous findings in this area both support and challenge these results. Torsdottir and Anderson’s (61) finding that the addition of 300 ml of water to a solid meal significantly increased the postprandial glyemic response, in both normal and well controlled type II diabetic subjects, supports the findings of this study. In contrast, their finding that the same addition of
water had no significant effect on the postprandial glyemic response, in poorly controlled type II diabetics, does not. The subsequent findings of Gregersen and co-workers (62), are also at variance with the results of the present study. They found that the postprandial glyemic response to a solid meal taken with 90ml of water was statistically no different than that to the same meal taken with 600ml of water, in undifferentiated type II diabetic subjects. Overall, when the results of these two previous studies and those of the present study are viewed together, they indicate differences among the cohorts investigated. Specifically, they suggest an effect of water volume in healthy subjects, but are ambiguous for type II diabetics. The possible mechanism of action of water volume on postprandial glycemia may offer some insight into why these differences exist.

3.11.1 Mechanism of action

The proposed mechanism for this observed effect of water on glycemia is thought to involve gastric emptying (61). Several studies have shown a relationship between water volume and both gastric emptying and osmolality in healthy subjects. A six fold increase in the volume of a phenol red solution (50ml to 600ml) (208) and a two-fold increase in the volume of a liquid polyose meal (300ml to 600ml) (209) have been shown to significantly increase the rate of gastric emptying. Similarly, various decreases in the osmolality of carbohydrate solutions have also been shown to have the same effect (210,211). Another set of studies has in turn shown a relationship between gastric emptying and the postprandial glyemic response. Both Mourot et al. (212) and Torsdottir et al. (213) in studies on healthy adults, found that the postprandial glyemic response to various starchy meals was positively correlated to their rate of delivery to the duodenum: the faster their rate of gastric emptying, the higher the response elicited. It can be extrapolated from these two separate sets of studies that an increase in the water volume or decrease in the osmolality of a meal may result in an increase in the rate of gastric emptying, which may in turn result in an increase in glycemic response.
In the present study, the observed difference in the blood glucose concentrations between the 600ml (4.2% concentration) and 200ml (12.5% concentration) meals for glucose and sucrose would seem to support this theory. Significant differences in blood glucose concentrations between these meals occurred only at the first time interval, 15 minutes. Here, the blood glucose rise resulting from the 600ml meals was higher than that resulting from the 200ml meals. There were no significant differences at other time intervals. This finding may be interpreted as being consistent with a faster rate of gastric emptying for the 600ml meals, owing to their higher volume and lower osmolality.

That being said, the key to the observed differences between healthy and nondiabetic subjects may involve gastric function. Both IDDM and NIDDM patients have both been shown to have a high incidence of disturbed gastric emptying. Gastroparesis, or delayed gastric emptying of food, has been reported in 40-50% patients (216,217). In addition, abnormalities in gastric and small intestinal contractility patterns have been observed in diabetic patients. Malagelada and co-workers (218) found that sporadic motor activity was significantly reduced in IDDM patients with gastroparesis. And Jebbink and colleagues (219), in IDDM patients held at a normoglycemic level, observed a shortening of sporadic motor activity, increased burst activity in the duodenum, and hypomotility in the gastric antrum after a meal. The sum effect of these irregularities may be to negate the effect of volume on gastric emptying in diabetic patients, negating its proposed effect on the postprandial glyemic response in this cohort. In this respect, a study of Levitt et al (220) noticed that while viscous fibre reduced the postprandial glyemic response in NIDDM patients without autonomic neuropathy, it produced no further flattening in patients with gastroparesis.

Whether water volume has an effect on the postprandial glyemic response only in healthy subjects and not in diabetic subjects is debatable. Other studies have shown
higher rates of gastric emptying in newly diagnosed NIDDM relative to healthy controls (228,229). There is a need for more research in this area. In particular, the effect of volume must be investigated further in impaired glucose tolerance and all types of diabetes.

3.11.2 Type of Carbohydrate

The present study results also point to an effect of type of carbohydrate on glycemia. Previous research in this area has demonstrated that different mono and disaccharides elicit different postprandial glyemic responses (145,230). The reason for this phenomenon has largely been ascribed to rate of absorption and to the proportion and rate of conversion of non-glucose sugars to glucose, with sugars that are more quickly absorbed and converted having higher responses (5). According to the international tables of GI (168), the response to glucose is approximately 33% higher than that to sucrose and the response to sucrose approximately 65% higher than that to fructose. The present results are in close agreement with these data. Expressed as relative proportions, they show similar differences.

3.11.3 Implications

The effect of water volume we observed may have clinical applications. If the blood glucose raising potential of various foods can be reduced by decreasing volume, then volume may prove to be a tool for regulating blood glucose levels. A reduction in GI was shown to decrease the risk of developing diabetes in two recent epidemiological studies (84,85). The investigators reported that in men (84) and women (85) the relative risk of NIDDM was 1.37 when comparing the lowest and highest GI quintiles. The difference in GI between these quintiles (17% for women and 18% for men) was comparable to the 19.8, 23.6, and 42.5% reductions in the postprandial glyemic response in the present study. In healthy individuals with poor blood glucose control, the long-
term effect of volume regulation may, therefore, be to improve glycemic control, possibly preventing or delaying the onset of NIDDM. It is unlikely, however, that such a modality would be practical. Limiting water consumption at meals could potentially lead to dehydration.

An effect of water volume may also have implications for glycemic testing. Foods can be processed to have different amounts of water. For example, sugar solutions, soups, sauces, drink mixes, frozen juices, hot cereals, and some meal replacement drinks can be eaten either concentrated or diluted. Different amounts of water can also be consumed with a meal. The results of the present study suggest that such alterations will likely skew the postprandial glycemic responses to foods. Meals of a lower substrate concentration or consumed with more water may elicit a higher the postprandial glycemic response than identical meals of a lesser volume. The three fold change in GI among fruits supports this possibility. The tables of GI (168) report the GI of watermelon, a fruit with a low carbohydrate concentration (7%) (231), as 103 and cherries, a fruit with a higher carbohydrate concentration (29%) (231) as 32. Likewise, some food products manufactured with high glucose concentration have a low GI such as Ultracil (Mead Johnson, Evansville, IN) (GI=55.4).

This possible effect of meal volume should be considered in the methodology of glycemic testing. The literature shows that laboratories are inconsistent in the amount of water they give with or in their test meals (45,46,48,63-67,114,145,224). This list of examples is not exhaustive, but demonstrates that volume is not standardized in glycemic testing and that, on occasion, it may not be controlled for at all. The results of the present study indicate that this situation might be a source of potential invalidity in glycemic testing: differences observed in the postprandial glycemic response between different test meals consumed with varying amounts of water may reflect an effect of meal volume as
opposed to an effect of the actual food. This situation may also be a source of potential unreliability: variations observed within and between subjects in the postprandial glyemic response to identical meals may be attributable to changes in the water volume consumed with or as a part of these meals. The consequence may be that changes to glyemic testing methodology are justified. Future researchers may want to consider giving test meals of equal volume and exercise caution when comparing the glyemic testing results of other researchers, who do not control for meal volume in their study design or who give meals at a non-standardized volume. This situation is important when single foods are tested. When foods are tested against a standard of equal volume as in glyemic index testing (145), these concerns may be less relevant.

Control of volume would seem to be of particular importance in oral glucose tolerance testing. A reliable and valid diagnostic test for diabetes is important. The OGTT does not appear to have met this mandate. There has been a cloud of controversy surrounding the value of this test (76,77,114,225), with much of the debate centering around poor reproducibility. It is possible that water volume may offer a partial explanation. There is a discrepancy between the WHO (74) and NDDG (75) published protocols for OGTT. The WHO (74) stipulates that subjects “should drink 75g of glucose in 250-300ml of water...”, where as the NDDG (75) stipulates that subjects consume 75g glucose in a minimum of 300ml of water (maximum concentration: 25g glucose/dl water). In this latter case, health professionals may choose to give the glucose in a range of volumes above 300ml. The result may be to cause variability between their own results and those of other health professionals following this protocol or that of the WHO. Incorrect diagnoses may also be a result. If an OGTT is given at a larger volume in relation to the reference test to which it is being compared, then an erroneously high reading and false positive may be produced. Conversely, the opposite is also true.
An effect of volume is clear. Before precise recommendations can be made regarding glycemic testing methodology, more studies in this area are required. It should be determined if there is a possible dose dependant effect of water on the postprandial glyemic response and, as suggested above, it should be determined in which groups does an effect of meal volume exist.
CHAPTER 4.

DILUTION OF THE 75g ORAL GLUCOSE TOLERANCE TEST INCREASES POSTPRANDIAL GLYCEMIA: IMPLICATIONS FOR DIAGNOSTIC CRITERIA
4. DILUTION OF THE 75g ORAL GLUCOSE TOLERANCE TEST INCREASES POSTPRANDIAL GLYCEMIA: IMPLICATIONS FOR DIAGNOSTIC CRITERIA

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4.5 Duality of interest

JLS and VV have both received travel grants from MuscleTech Research and Development

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4.7 Abstract

**Background** - Dilution has been noticed to increase the glycemic response to various sugars, including glucose. This effect may contribute to the poor reproducibility of the oral glucose tolerance test (OGTT). To test this hypothesis, we initially assessed the effect of diluting a 75g-OGTT on two-hour postprandial blood glucose based diagnostic outcome, incremental glycemia, and area under the glucose curve.

**Methods** - On three separate occasions, 10 subjects (age:40±2 years, BMI:27.2±0.6kg/m²) without previously diagnosed dysglycemia were randomly administered a 75g-OGTT meal at 300ml, 600ml, or 900ml. The protocol followed American Diabetes Association guidelines. Finger-prick capillary blood samples were obtained at fasting and then 15, 30, 45, 60, 90, and 120 minutes after the start of the test.

**Results** - Area under the curve for the 900ml-meal (404±57 min.mmol/L) was significantly higher than for the 600ml-(331±51 min.mmol/L) and 300ml-meals (280±48 min.mmol/L) (p<0.05). Incremental glycemic concentrations were significantly higher for the 900ml-meal (4.9±0.4, 5.1±0.6, and 4.6±0.8mmol/L) than both the 600ml- (4.0±0.3, 4.16±0.6, and 3.6±0.7mmol/L) and 300ml-meals (3.8±0.5, 3.9±0.5, 3.2±0.6) at 30, 45, and 60 minutes respectively (p<0.05). The same was true for peak incremental blood glucose irrespective of time (p<0.05). No other significant differences were observed, including in 2h postprandial glucose.

**Conclusions** - Dilution of the 75g-OGTT will likely not confound current screening criteria that use 2h postprandial glucose as the basis for diagnosis. Because intermediate time points appeared sensitive to alterations in volume, it may however confound the interpretation of older criteria that rely on these points.

4.8 Key words

OGTT, screening, diagnosis, diabetes, IGT, volume, postprandial, glycemic response
4.9 Introduction

Diabetes and intermediate classifications of hyperglycemia are on the rise (68,69). It is important to have a reliable and valid test to diagnose new cases. Both the Canadian (71) and American (70) Diabetes Associations in their most recent reports recommended the preferential use of fasting plasma glucose in this role. Use of the OGTT, which had also been previously recommended, was discouraged. One of the reasons cited for this decision was the poor reproducibility of the test compared to fasting plasma glucose.

Alterations in water volume may offer an explanation. The WHO (17) and ADA instruct that the OGTT meal be given as 75g glucose dissolved in 250mL to 300mL of water, whereas, the NDDG (18) and CDA (11), instruct that it be given as 75g glucose in a minimum of 300mL. This last case allows for it to be given at any volume above 300mL. In addition, because poor palatability (76,77,110-112) of the test and resulting nausea and dizziness (109,108-112) are major complaints, our patients will often request additional water with the test to increase its overall acceptability.

Glycemia may be affected by these differences. We recently demonstrated that a three-fold increase in the volume of a 25g oral glucose meal increased glycemia by 19.8% (232). Others using a 50g dose of glucose found that a three-fold increase in volume raised peak blood glucose significantly by 14% in pregnant women (59). Whether these results apply where the glucose dose is 75g is unknown. In a preliminary study, we therefore chose to investigate the effect of a two- and three-fold increase in the volume of a 300ml 75g-OGTT on glycemic concentrations, 2h postprandial glucose based diagnostic outcome, and area under the blood glucose curve. Such large variations in volume above 300ml were chosen in order to cover a large physiological range.
4.10 Methods

4.10.1 Participants

Ten participants (5 male, 5 female, age: 40±2 years, BMI: 27.2±0.6 kg/m², fasting glucose: 4.9±0.2 mmol/L) recruited from faculty and students at the University of Toronto and through hospital advertisements gave informed written consent to take part in this study, approved by the Research Ethics Committee at St. Michael’s Hospital. All were healthy and not taking medications with no previous diagnosis of dysglycemia. Two were smokers and six were overweight by BMI criteria (>27 kg/m²).

4.10.2 Test meals

Each participant received a total of three 75g glucose Glucodex® OGTT meals (Technilab Inc., Montreal, Quebec) in random order: one at 300ml (undiluted, osmolarity: 1.39 mol/L), 600ml (300ml of tap water added, osmolarity: 0.69 mol/L), and 900ml (600ml of tap water added, osmolarity: 0.46 mol/L).

4.10.3 Protocol

The protocol was designed to match the ADA guidelines for the OGTT. Participants attended St. Michael’s hospital on three separate mornings following a 10-16 hour overnight fast. They were instructed to maintain the same dietary and exercise patterns the evening before each test and consume a minimum of 150g of carbohydrate each day over the three days prior to the test. To ensure that these instructions were followed, participants completed a questionnaire detailing pre-session information about their diet and lifestyle patterns. Upon commencement of the test, subjects gave an approximately 250μL fasting finger-prick capillary blood sample, using a Monoejector Lancet device (Owen Mumford Ltd., Woodstock, Oxon, England). One of the three test meals was then given with instructions to drink it over a period of exactly 5 minutes. Additional finger-prick blood samples were obtained at 15, 30, 45, 60, 90, and 120
minutes after the start of the meal. No smoking or physical activity was permitted before or during the test.

**4.10.4 Blood Glucose Analysis**

All blood samples were collected in tubes containing fluoride oxalate, immediately frozen at -20° C, and analyzed within three days of collection. The glucose concentration of each was determined by the glucose oxidase method using a YSI 2300 Stat glucose/L-lactate analyzer, model 115 (Yellow Springs, Ohio).

**4.10.5 Statistical Analyses**

Blood glucose curves were plotted as the incremental change in blood glucose over time and the positive incremental area under the blood glucose curve was calculated geometrically for each participant, ignoring areas below the fasting value (156). Incremental glucose concentrations were used so as to control for baseline/fasting differences between the treatments. 2h absolute blood glucose values were compared to CDA (71) diagnostic criteria for IGT and diabetes. Criteria for venous plasma samples were used, as the same cut-off values are provided for both venous plasma and capillary whole blood glucose (74,75). Statistical analyses were then performed using the Number Cruncher Statistical System (NCSS statistical software, Kaysville, Utah). Repeated measures two-way ANOVA adjusted for multiple pair-wise comparisons with the Newman Keuls procedure assessed interactive and independent effects of volume dose (300, 600, and 900ml) and time (0, 15, 30, 45, 60, 90, and 120 minutes) on incremental change in blood glucose concentrations. Differences in peak blood glucose rise and area under the curve between the 300, 600, and 900ml OGTT meals were assessed using repeated measures one-way ANOVA adjusted for multiple pair-wise comparisons with the Newman Keuls procedure. All results were expressed as mean±SEM and considered statistically significant if p < 0.05.
4.11 Results

All participants were able to follow the study protocol without difficulty. Questionnaires revealed that evening dietary patterns and activities, amount of sleep, reported feelings of health and well-being, mode of transportation to the clinic, and weight remained consistent between the sessions within each subject. Subjects were also able to consume all test meals in the time allotted and there were no complaints about the volume of any of the tests. One exception was a subject who complained of a headache following the 300ml-OGTT. Furthermore, no differences emerged between men and women in response to the treatments: coefficient of variation in results was no different between the sexes (data not shown).

4.11.1 2h diagnostic values

Absolute two-hour postprandial blood glucose results for each of the 10 subjects following the three OGTT meals were compared to corresponding CDA (71) diagnostic glucose cut-off values for diabetes (≥11.1mmol/L), IGT (≥7.8 to <11.1mmol/L), and normal glucose tolerance (<7.8mmol/L). Subject 1's 900ml OGTT result (8.8mmol/L) was diagnostic for IGT, while their 600ml and 300ml result (7.5 and 4.7mmol/L respectively) were not. Similarly, subject 3's 600ml OGTT result was diagnostic for diabetes (11.8mmol/L), but this was not confirmed by the 900ml- and 300ml-tests, both of which were diagnostic for IGT (9.9 and 9.0 mmol/L respectively). The remaining eight subjects tested negatively for IGT or diabetes on all three tests. According to Canadian and American Diabetes Association criteria that require two abnormal glucose values to confirm a diagnosis, these data indicated that subject 3 had IGT while the rest had normal glucose tolerance.

4.11.2 Incremental Glycemia

Table 4.1 and Figure 4.1A show incremental changes in glycemic concentrations at 0, 15, 30, 45, 60, 90, and 120 minutes following the consumption of the 300ml, 600ml,
and 900ml OGTT meals. The results of the repeated measures two-way ANOVA demonstrated that the effects of dose (300, 600, 900ml) and time (0, 15, 30, 45, 60, 90, 120 minutes) were independent (p<0.05). Pairwise comparisons showed that incremental changes in glycemic concentrations meals at 30, 45, and 60 minutes for the 900ml-meal (4.9±0.4, 5.1±0.6, and 4.6±0.8mmol/L) were significantly higher than both the 600ml- (4.0±0.3, 4.16±0.6, and 3.6±0.7mmol/L) and the 300ml- (3.8±0.5, 3.9±0.5, 3.2±0.6 mmol/L) meals (p<0.05). Incremental peak blood glucose rise calculated irrespective of time was also found to be significantly higher for the 900ml meal (5.68± 0.3) than the 600ml- (5.03±0.3) and 300ml- (4.61±0.2) meals (p<0.05). No other significant differences in glycemic concentrations were observed at any other time interval, including the diagnostically relevant 2h time point.

Table 4.1 - Indices of blood glucose following a 75g-OGTT at three dilutions*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fasting Blood Glucose (mmol/L)</th>
<th>Incremental Blood Glucose (mmol/L)</th>
<th>2h Diagnostic Value (mmol/L)</th>
<th>Area under the curve (mmol/L.min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGTT-300ml</td>
<td>5.1±0.2</td>
<td>0 2.1±0.4 3.8±0.5 4.0±0.5 3.2±0.6 1.6±0.6 0.5±0.6</td>
<td>5.5±0.3</td>
<td>280.3±48.1</td>
</tr>
<tr>
<td>OGTT-600ml</td>
<td>4.9±0.7</td>
<td>0 2.7±0.4 4.0±0.3 4.2±0.6 3.6±0.7 2.6±0.8 0.6±0.7</td>
<td>5.5±0.4</td>
<td>331.1±51.0</td>
</tr>
<tr>
<td>OGTT-900ml</td>
<td>4.8±0.1</td>
<td>0 2.9±0.3 4.9±0.4† 5.1±0.6† 4.6±0.8† 3.1±0.7 0.7±0.7</td>
<td>5.4±0.4</td>
<td>404.3±57.2†</td>
</tr>
</tbody>
</table>

*Data are mean±SEM  
†Significantly different from other treatments within the same column (p<0.05, repeat measures ANOVA with Newman Keuls procedure)

4.11.3 Area Under the Curve

Table 4.1 and Figure 4.1B show the mean AUCs to the 300, 600 and 900ml OGTT meals. The results of the repeated measures one-way ANOVA applied to these data demonstrated a significant difference in mean AUC between all three (p=0.006). Pairwise comparisons indicated that the 900ml-meal (404±57min.mmol/L) elicited a
significantly higher AUC than both the 600ml- (331±51 min.mmol/L) and 300ml- (280±48 min.mmol/L) meals (p<0.05), with no significant difference in AUC between the 300ml- and 600ml-meals.

Figure 4.1 – Glycemic Responses to 75g-OGTTs at 300ml, 600ml, and 900ml in 10 subjects with previously undiagnosed dysglycemia: (A) Comparison of incremental changes in blood glucose and (B) comparison of area under the blood glucose curve between the volume doses. Values are mean±SEM. Points at the same time interval with different letters and bars with different letters are significantly different (repeated measures ANOVA with Newman Keuls procedure, p<0.05).
4.12 Discussion

This preliminary study suggests that both a 3-fold dilution of the 75g-OGTT from 300ml to 900ml and a 1.5-fold dilution from 600ml to 900ml significantly increase postprandial glycemia. The same is not true for a 2-fold dilution from 300ml to 600ml. These findings are consistent with those of our previous study in which we diluted 25g oral glucose, sucrose, and fructose solutions by three times (232). They are also in agreement with the findings of other studies in which both liquid (59) and solid test meals (61,62) were diluted by three-fold or greater.

The mechanism by which volume amplifies postprandial glycemia is likely similar to that described previously (61). The involvement of gastric emptying is a possibility, such that an increase in the volume (209) or decrease in the osmolality (210) of a meal results in an increase in the rate of gastric emptying, which in turn results in an increase in glycemia (212). We believe the timing of glycemic differences observed on the present study to be consistent with this hypothesis. Findings of other related studies in which the OGTT has been investigated offer further support. It was twice observed that the faster an OGTT is emptied from the stomach, the higher postprandial glycemia (214,215). Schwartz and co-workers also attributed a significant rise in glycemic concentrations at 30 minutes and decreased incidence of nausea following a diluted 50g-OGTT, as owing to a faster rate of gastric emptying (59).

Both the results of Schwartz et al (59) and our own have implications for the reproducibility of the OGTT. The 30%, 14%, and 19.8% differences in postprandial glucose noticed after dilution of 75g- (present study), 50g - (15), and 25g- (232) OGTTs respectively suggest that alterations in volume may be contributing to the reported poor reproducibility of the test. Our observations that the 900ml-meal in the case of subject # 1 and the 600ml-meal in the case of subject # 3 produced false positive 2h results for IGT and diabetes respectively may offer additional support. Differences in incremental changes however were seen only at peak blood
glucose rise and intermediate time intervals during the first half of the test: 30, 45, and 60 minutes. Alterations in volume also appeared to have the least effect on incremental change in glycemia at 2h (p=0.971). The likelihood therefore that dilution will confound the 2h based diagnostic criteria and lead to misdiagnoses seems low.

It is nevertheless possible that some of the earlier poor reproducibility of the test may be explained by an effect of volume. In addition to 2h glucose, the 1979 National Diabetes Data Group guidelines relied on intermediate glycemic values for diagnosis (75). As we eluded, these points appear more sensitive to changes in volume than 2hPG, indicating that a diagnostic vulnerability may have existed. Our data may be interpreted as lending support to the predicted rationale for abandoning the use of these values in subsequent established protocols for the test (70,71,74).

Before we can know with any confidence how much of the variation in the 75g-OGTT is explained by an effect of volume, further study is required. Investigations to assess whether the present findings hold true in groups with different glucose tolerance and whether one dilution has superior reproducibility compared to another should be undertaken. Others that further explore a gastric emptying link are also warranted.

4.13 Acknowledgements

We would like to thank MuscleTech Research and Development, Toronto, Canada for their financial support of this study and Technilab, Montreal, Quebec for supplying the Glucodex® test meals.
CHAPTER 5.

DILUTION OF THE 75g ORAL GLUCOSE TOLERANCE TEST IMPROVES OVERALL ACCEPTABILITY BUT NOT REPRODUCIBILITY IN SUBJECTS WITH DIFFERENT BODY COMPOSITIONS
5. DILUTION OF THE 75g ORAL GLUCOSE TOLERANCE TEST IMPROVES OVERALL ACCEPTABILITY BUT NOT REPRODUCIBILITY IN SUBJECTS WITH DIFFERENT BODY COMPOSITIONS

5.1 Citation

Diabet Med, submitted July 20, 1999

5.2 Running title

Dilution and reproducibility of 75g-OGTT

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5.5 Duality of interest

JLS and VV have both received travel grants from MuscleTech Research and Development

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5.7 Abstract

Background - Poor reproducibility of the 75g oral glucose tolerance test (75g-OGTT) is a major criticism. To test whether dilution might be a contributor, we studied the effect of administering it at 300ml, 600ml, and 900ml on the coefficient of variation (CV) and overall acceptability.

Methods - Each dilution was randomly repeated three times by 35 subjects: 11 lean (body fat [BF]:11.3±1.5%, BMI:24.1±0.8kg/m², age:33±3years), 12 normal (BF:24.2±1.2%, BMI:25.0±1.3 kg/m², age:32±3years), and 12 obese (BF:34.1±1.8%, BMI:34.5± 2.0kg/m², age:41±5years). The protocol followed American Diabetes Association guidelines with venous samples drawn at 0, 15, 30, 45, 60, 90, and 120minutes. Scales assessing palatability, acceptability, satiety, nausea, and dizziness were also completed.

Results - No differences were detected in CV between the three dilutions at the 2h-diagnostic-endpoint in any group. CV for glycemia however was lower for the 300ml-OGTT (10±2.1%) than either the 600ml- (17.9±2.1%) or 900ml-OGTT (19.9±4.4%) at 45minutes in the lean group (p<0.05). And CV for insulinemia was lower for the 300ml- (30.6±4.5%) and 600ml- (30.6±4.1%) OGTT than the 900ml-OGTT (53.7±7.9%) at 60minutes in the obese group (p<0.05). When affective ratings were compared, the 600ml-OGTT scored highest on palatability and acceptability (p<0.05).

Conclusions - Dilution of the 75g-OGTT improves overall acceptability but not reproducibility of results. Practitioners may choose to give it at the best-tolerated dilution.

5.8 Key words

Volume, OGTT, glucose, insulin, intrasubject variation, acceptability, palatability

5.9 Introduction

The ADA in 1997 made a shift away from the use of the 75g-OGTT in the diagnoses of diabetes and intermediate stages of hyperglycemia. They advised the preferential use of FPG
A shift had already begun in practice. A survey of 174 physicians in Pittsburgh showed that 70% never used the 75g-OGTT (233). Numerous articles (77) commentaries (76,108,115) and editorials (225) also appeared that discouraged and argued against the use of the 75g-OGTT. Several compelling criticisms formed the rationale for this movement. These included suggestions that the 75g-OGTT was inconvenient, slow, labor intensive, expensive, patient unfriendly, and less reproducible, while being no more specific for diabetes than FPG (70).

Recent debate regarding the merit of the test has kept the controversy alive. The ADA acknowledges that FPG will not capture all people with IGT, leading to underestimation of the prevalence of impaired glucose homeostasis (70). This is supported by population (119) and cohort (120) based studies in which 1997 FPG criteria were observed to be less sensitive than 75g-OGTT criteria, missing 71% and 81% of the IGT patients respectively. In addition, it has been noticed that 1997 FPG criteria may underestimate the prevalence of diabetes compared to 75g-OGTT criteria by approximately 40% (121,122). Studies that continue to investigate the 75g-OGTT therefore remain important.

Evidence suggests that the volume/osmolarity of the test might provide some explanation for some of its criticisms. A three-fold dilution of a 50g-OGTT from 150ml to 450ml was observed to decrease the incidence of nausea and vomiting significantly in 132 pregnant women (59). Three-fold dilutions of a 25g-OGTT (200ml to 600ml) (232), 50g-OGTT (150ml to 450ml) (59), and a 75g-OGTT (300ml to 900ml) (233) have also been noticed to increase postprandial glycemia by 19.8%, 14% and 30% respectively. These might be regarded as comparable to the 11% (72) to 17.7% (73) intra-subject variation that has been reported for the 75g-OGTT. Whether alterations in volume are contributing to the test’s poor reproducibility or one volume has superior patient acceptability or reproducibility is unclear. To test these questions, we undertook the present study to compare 300ml, 600ml, and 900ml 75g-OGTT meals on reproducibility and ratings of palatability, acceptability, satiety, dizziness, and nausea.
5.10 Methods

5.10.1 Participants

Forty subjects were recruited from faculty and students at the University of Toronto and from hospital and newspaper advertisements to participate in this study that was approved by the Research Ethics Committee at St. Michael’s Hospital. All were without previously diagnosed hyperglycemia and gave informed written consent prior to the commencement of the study. Of those recruited, 35 completed the study. They were stratified first by BMI and then by body fat (BF) into three groups: 11 lean (BMI >19 and \(\leq 27\, \text{kg/m}^2\), \(\text{BF} < 15\%\) for males and \(< 20\%\) for females), 12 normal (BMI >19 and \(\leq 27\, \text{kg/m}^2\); \(\text{BF} >15\%\) for males and \(> 20\%\) for females), and 12 obese (BMI >27kg/m\(^2\); \(\text{BF} >15\%\) for males and \(> 20\%\) for females). These strata were used to provide a wide range of insulin sensitivities related to body composition.

5.10.2 Test meals

Participants received three different liquid 75g Glucodex® OGTT meals (Technilab Inc., Chambly, Quebec) that differed only in their dilution: one at 300ml (undiluted, osmolarity: 1.39mol/L), one at 600ml (300ml of tap water added, osmolarity: 0.69mol/L), and one at 900ml (600ml of tap water added, osmolarity: 0.46mol/L). Each was repeated three times in random order for a total of nine tests.

5.10.3 Anthropometry

Participants submitted to various anthropometric measurements. Their fasting body weight was measured using a calibrated digital scale. Body circumferences were assessed according to standard techniques (234): WC was measured at the narrowest part of the torso between the lower rib and iliac crest and hip circumference (HC) at the level of greatest gluteal protuberance. Assessments of body fat (BF) were done by two methods. The first was by the infrared-interactance method using the FUTREX-5000® (FUTREX Inc., Gaithersburg,
MD). This procedure is considered reliable (235) and a reasonably valid alternative to underwater weighing (236). The second assessment was by the mean of three repeated skinfold caliper measurements at 5 sites: triceps, thigh, subscapular, iliac, and abdominal. Triceps and thigh were summed to provide an assessment of peripheral skinfold thickness and subscapular, iliac, and abdominal measurements were summed to provide an assessment of truncal skinfold thickness.

5.10.4 Protocol

The protocol was designed to follow the ADA guidelines for the 75g-OGTT. Participants attended St. Michael’s hospital on nine separate mornings following a 10-12 hour overnight fast. They were instructed to maintain the same dietary and exercise patterns the evening before each test and consume a minimum of 150g of carbohydrate each day over the three days prior to the test. To ensure that these instructions were followed, participants were provided with examples of what constituted 150g of carbohydrate and completed a questionnaire detailing pre-session information about their diet and lifestyle patterns. Upon commencement of the test, subjects had a catheter inserted into a forearm vein that was secured by tape and kept patent by saline. From this device a 7ml-blood sample was obtained in a tube containing fluoride oxalate. A randomly selected OGTT dilution was then given with instructions to drink it over a period of exactly 5 minutes. Additional blood samples were drawn using the same technique at 15, 30, 45, 60, 90, and 120 minutes after the start of the meal. Over the course of the blood collection, subjects filled out 7-point bipolar visual analogue scales, rating the test on “palatability”, described as the taste/smell/flavour/sensory characteristics of the test drink; “acceptability”, described as the adequacy/acceptability of the test drink and procedure; “satiety”, described as the level of fullness/absence of hunger/sufficiency experienced; “dizziness”, described as the level of disorientation/vertigo/
light-headedness experienced; and “nausea”, described as the level of gastric discomfort/malaise/stomach-upset experienced. On these scales, “1” represented “low” and “7” “high”.

5.10.5 Laboratory analyses

Samples were separated by centrifuge and the plasma immediately frozen at −20°C pending analysis. The glucose concentration of each was determined by the glucose oxidase method (237). Double antibody radioimmunoassay determined insulin concentrations (238). Banting and Best Diabetes Centre Core Laboratory, Toronto, Canada performed the analyses.

5.10.6 Statistical analyses

Plasma glucose and insulin curves were plotted. Intra-subject variation for insulin and glucose was calculated as the CV at each time point (0, 15, 30, 45, 60, 90, 120 minutes). This measure expresses the SD of the three repeats for each volume (300ml, 600ml, and 900ml) as a percentage of their mean (CV = SD/mean × 100%). Statistical analyses were then performed using the Number Cruncher Statistical System (NCSS statistical software, Kaysville, Utah). One-way ANOVA adjusted for multiple pair-wise comparisons with the Newman Keuls procedure assessed differences between the three groups in demographic and anthropometric characteristics. The repeated measures version of this same statistic assessed differences in the replicate mean values for plasma glucose and insulin concentrations between the 300ml-, 600ml-, and 900ml-OGTTs within each group. It also assessed differences in CVs for plasma glucose and insulin within each group and differences in the replicate mean ratings of palatability, acceptability, satiety, dizziness, and nausea for the entire data set between the three volume doses. All results were expressed as mean±SEM and considered statistically significant if p<0.05.

5.11 Results

All 35 subjects were able to follow the study protocol without difficulty. The test meals were consumed in approximately the 5 minutes allotted: mean consumption times for the 300ml-,
600ml-, and 900ml-OGTT were 4:59±0:04, 5:19±0:08, and 5:54±0:15 minutes respectively. In addition, only three tests needed to be repeated because of violation of the study protocol. Subject #2 had to repeat a 900ml-meal due to poor venous blood collection, subject #28 had to had to repeat a 900ml-meal due to vomiting during the test and subject #29 had to repeat a 600ml-OGTT for consuming a post-test snack prior to collection of the last blood sample.

5.11.1 Group characteristics

Table 5.1 shows the demographic and anthropometric data of the three groups studied. The obese group differed significantly from the lean and normal groups in BMI, WC, and WHR measurements. Both the normal and obese groups differed significantly from the lean group in ratio of males to females (p<0.05). All three groups differed significantly from one another in body fat and truncal and peripheral skinfold thickness (p<0.00001).

Table 5.1 – Demographic and anthropometric characteristics of the groups

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=11)</th>
<th>Normal (n=12)</th>
<th>Obese (n=12)</th>
<th>All (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male:female)</td>
<td>9:2*</td>
<td>5:7</td>
<td>4:8</td>
<td>18:17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±3.2</td>
<td>33±2.9</td>
<td>41±4.5</td>
<td>36±2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.8±4.1</td>
<td>67.0±3.1</td>
<td>96.3±3.1*</td>
<td>79.8±3.2</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.1±0.8</td>
<td>23.9±0.7</td>
<td>34.5±1.7*</td>
<td>27.6±1.1</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>79.8±2.7</td>
<td>78.7±3.0</td>
<td>106.2±4.4*</td>
<td>88.5±2.9</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>97.5±2.3</td>
<td>98.4±2.2</td>
<td>118.4±1.9*</td>
<td>104.9±2.2</td>
</tr>
<tr>
<td>WHR (cm:cm)</td>
<td>0.82±0.02</td>
<td>0.80±0.02</td>
<td>0.89±0.03*</td>
<td>0.84±0.01</td>
</tr>
<tr>
<td>BF (%)</td>
<td>11.5±1.5*</td>
<td>24.3±1.3*</td>
<td>34.2±1.5*</td>
<td>23.6±1.8</td>
</tr>
<tr>
<td>Skinfold Thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truncal</td>
<td>34.8±4.0*</td>
<td>59.4±6.0*</td>
<td>108.9±1.6*</td>
<td>69.6±6.0</td>
</tr>
<tr>
<td>Peripheral</td>
<td>21.7±2.7*</td>
<td>47.6±3.0*</td>
<td>72.9±3.1*</td>
<td>48.9±4.1</td>
</tr>
</tbody>
</table>

*Indicates significance from other groups (between columns) within the same row, not including the “all” category (p<0.05, one-way ANOVA adjusted for multiple pairwise comparisons with the Newman Keuls procedure). WC, HC, WHR, and BF denote waist circumference, hip circumference, waist-to-hip ratio, and body fat respectively. Truncal and peripheral skinfold thickness measurements are for n=10 in the lean group and n=34 in “all” subjects.
5.11.2 Plasma glucose and insulin

Figure 5.1 shows the mean plasma glucose and insulin responses following three repetitions of a 75g-OGTT at 300ml, 600ml, and 900ml in the lean (n=11), normal (n=12), and obese (n=12) groups. There was a significant affect of dilution on postprandial plasma glucose at 30 (p=0.043), 45 (p=0.017), 60 (p=0.0082), and 90 (p=0.023) minutes in the lean group. Pairwise comparisons demonstrated that in each case, plasma glucose concentrations were significantly higher for the 300ml-meal than the 900ml-meal (9.1±0.3 vs. 8.4±0.5mmol/L at 30 minutes, 8.6±0.5 vs. 7.3±0.6mmol/L at 45 minutes, 7.7±0.5 vs. 6.1±0.4mmol/L at 60 minutes, and 6.0±0.4 vs. 5.1±0.3mmol/L at 90 minutes, p<0.05). There was also a significant affect of dilution on plasma insulin in this same group at 60 minutes (p=0.0099). Pairwise comparisons showed that plasma insulin was significantly higher for the 300ml-OGTT (180.5±28.9pmol/L) than the 600ml-OGTT (137.5±21.7pmol/L) (p<0.05).

Similar results were seen in the normal group. Dilution significantly affected plasma glucose at 45 minutes (p=0.025). Pairwise comparisons showed that glycemia was significantly higher after the 300ml-OGTT (8.6±0.5mmol/L) than the 600ml-OGTT (7.6±0.5mmol/L) (p<0.05). A significant affect of volume was also noticed on plasma insulin at 30 (P=0.00055) and 60 (P=0.017) minutes. Pairwise comparisons showed that insulin levels were significantly higher for the 900ml-meal (319.4±35.6pmol/L) than both the 600ml-OGTT (245.1±22.3pmol/L) and the 300ml-OGTT (235.7±26.4pmol/L) at 30 minutes and that insulin levels were significantly higher for the 300ml-meal (277.8±47.8pmol/L) than the 600ml-OGTT (204.2±35.3pmol/L) at 60 minutes (p<0.05).

No effect of dilution was noticed on either plasma glucose or insulin at any time points in the obese group.
Figure 5.1 - Comparison of mean plasma glucose and insulin responses after three repetitions of a 75g oral glucose tolerance test at 300ml (O), 600ml (□), or 900ml (△) in 11 lean, 12 normal-weight, and 12 obese individuals. Points at the same time interval with different letters are significantly different (p<0.05, repeat measures one-way ANOVA with the Newman-Keuls procedure). All values are mean±SEM.

5.11.3 CV for plasma glucose and insulin

Figure 5.2 shows the intra-subject variation for plasma glucose and insulin after three repeated 75g-OGTT meals at 300ml, 600ml, and 900ml in the lean (n=11), normal (n=12), and obese groups (n=12). Dilution was found to affect CV for plasma glucose at 45 minutes in the lean group (p=0.024). Pairwise comparisons showed that the CV of plasma glucose was significantly lower after the 300ml-OGTT (10.0±2.1%) than both the
600ml-(17.9±2.1%) and 900ml-(19.9±4.4%) OGTTs (p<0.05). Dilution was also noticed to affect CV of plasma insulin at 60 minutes in the obese group (P=0.0066). Pairwise comparisons showed that plasma insulin was significantly lower for both the 300ml-(30.6±4.5%) and 600ml-(30.6±4.1%) OGTTs than the 900ml-OGTT (53.7±7.9%) (p<0.05) in this group. No significant differences between CVs of glucose or insulin however were detected in the normal group.

Figure 5.2 - Comparison of coefficients of variation (CV) for plasma glucose and insulin after three repetitions of a 75g oral glucose tolerance test at 300ml (O), 600ml (□), or 900ml (△) in 11 lean, 12 normal-weight, and 12 obese individuals. Points at the same time interval with different letters are significantly different (p<0.05, repeat measures one-way ANOVA with the Newman-Keuls procedure). All values are mean±SEM.
5.11.4 Affective ratings

Figure 5.3 shows the mean 7-point analogue scale ratings for palatability, acceptability, satiety, dizziness, and nausea following three repetitions of a 75g-OGTT meals at 300ml, 600ml, and 900ml in the entire study sample (n=35). Dilution was observed to have a significant effect on palatability (p=0.0015), acceptability (p=0.0014), satiety (p<0.0001), and nausea (p=0.011). Pairwise comparisons showed: both the
900ml- (4.6±0.2) and 600ml- (4.9±0.2) OGTTs received significantly higher ratings than the 300ml-OGTT (4.1±0.3) for palatability; the 600ml-OGTT (5.0±0.2) received a significantly higher rating than both the 900ml- (4.5±0.2) and 300ml- (4.1±0.3) OGTTs for acceptability; the 900ml-OGTT received the highest (5.2±0.2), 600ml-OGTT the second highest (4.4±0.2), and 300ml-OGTT the lowest (3.7±0.2) ratings for satiety; and both the 300ml- (1.9±0.2) and 600ml- (1.9±0.2) OGTTs received significantly lower ratings than the 900ml-OGTT (2.3±0.2) for nausea (P<0.05). No other effects of dilution were observed. This included an effect on dizziness, although differences in this rating between the 600ml-, 300ml-, and 900ml-OGTTs were approaching significance (1.7±0.2 vs 1.9±0.2 vs. 2.0±0.2 respectively, p=0.093).

5.12 Discussion

Our findings indicate that dilution of the 75g-OGTT has only a minor impact on the intra-subject variation of plasma glucose and insulin responses with no effect on the diagnostically relevant 2h-endpoint irrespective of body composition. Dilution significantly increased the CV of plasma glucose and insulin at just one intermediate time point in lean and obese individuals respectively. No points of significance were detected in the normal group. This lack of effect was noticed despite finding more meaningful changes in affective ratings and absolute plasma glucose and insulin values. We observed that the 600ml-OGTT received the highest ratings for palatability and acceptability. It was also observed that dilution reduced the mean absolute glycemic and insulinemic responses at various intermediate time points in the lean and normal groups but not in the obese group. There was one exception among the effected time points (30 minutes in the normal group) at which insulin was most pronounced for the 900ml-OGTT.

The direction of these last findings for glucose and insulin is surprising. As indicated earlier, an opposite effect of dilution was observed in previous studies in which liquid test meals
were used. Increasing the volume of oral glucose solutions of varying carbohydrate loads was noticed to increase glycemia significantly (59,232,233). Others using mixed solid test meals in people with diabetes however have also reported findings that were at variance with a glucose raising effect of dilution. Gregersen and co-workers (62) found that the glycemic response to a solid meal consumed with 90ml of water was no different from that to the same meal taken with 600ml of water, in type 2 diabetic subjects. Similarly, Torsdottir and Anderson (61) noticed that the addition of 300ml of water to a solid mixed meal had no significant effect, in poorly controlled type 2 diabetic subjects, although the same intervention was noticed to increase glycemia in nondiabetic and well-controlled type 2 diabetic subjects.

Reasons for the contradiction are not clear. Selectively administering one of the test volumes over another at different phases of gastrointestinal tract activity may have been a contributing factor. It was previously noticed that administering a 75g-OGTT during periods of gastric motility increased emptying of the stomach, compared to administering the test during quiescence. The effect was to increase glycemia (215). Conditioning of the cephalic phase of digestion by repeated testing may offer another explanation. It has been suggested that insulin levels are sensitive to cephalic cues, including meal size (239). Perhaps over repeated tests the participants responded to the high volume 900ml-OGTT by increasing insulin secretion, thereby decreasing glycemia. There was a slight tendency for higher initial insulin secretion as evidenced by higher plasma insulin for the 900ml- and 600ml-OGTTs at 30 minutes in all three groups that may support this hypothesis. This trend was not confirmed by the statistics in either the lean or obese groups. Alternatively, it is possible that the conflicting results are simply a function of the high intra-subject variation of this test.

Despite not finding a glucose raising effect of dilution, the gastric emptying mechanism that has been hypothesized to explain such an effect (59,61,232,233) may still be operating. Schwartz and colleagues (15) suggested that the acute adverse symptoms of nausea and dizziness
that are associated with the OGTT might be related to irregular patterns of gastric emptying, resulting from the test's high osmolarity. They found that symptoms to a 50g-OGTT attenuated by increasing the volume from 150ml to 450ml. It was concluded that the 450ml-meal might have been more physiological. Because we noticed the 600ml-OGTT received the highest ratings for palatability and acceptability and lower though not significant ratings for dizziness, it is possible that it had a more agreeable pattern of gastric emptying. Any regularity in gastric emptying that these findings suggest may have been gained by modulating volume did not however translate into a meaningful improvement in reproducibility.

Our findings support the notion that the 75g-OGTT irrespective of volume has high diagnostic intra-subject variability. Compared to FPG, the diagnostic 2hPG endpoint had approximately double the intra-subject variability whether given at 300ml, 600ml, or 900ml in lean (15.7±3.3, 15.3±2.9, and 13.5±2.5% respectively vs. 6.2±0.6% for FPG, p<0.05) normal (14.5±2.2, 13.8±2.8, and 10.9±1.7% respectively vs. 6.2±0.6% for FPG, p<0.05) and obese (12.1±3.8, 13.8±1.3, and 13.6±1.9% respectively vs. 5.9±0.6% for FPG, p<0.05) subjects. This is consistent with the findings of several other studies that have made this comparison. Cummings and Fraser (72) in 14 healthy subjects found the CV of 2hPG was 11.0% after a 75g-OGTT compared to 4.7% for FPG. Similarly, both Feskens et al (73) and Mooy et al (113) in large samples of subjects with varying glucose tolerance found the CV of 2hPG was 17.7% and 16.7% compared to 12.0% and 6.4% for FPG respectively. Other studies in which the OGTT was compared to a more physiological substitute test meal have shown similar results. In 10 healthy subjects (114) and then later in 36 mixed subjects (112) we found that the intra-individual CV for 2hPG after a 75g-OGTT was significantly greater than that after a diabetes screening test bar (12.9% versus 4.7% and 12.7% versus 10.5% respectively). Together these findings and our own bring into question the reliability of this test and support the position of the ADA that the OGTT is poorly reproducible (70).
In conclusion, dilution of the 75g-OGTT due either to the use of protocols that are less restrictive (71,75) or allowing additional water at the request of subjects may improve the overall acceptability of the test but will likely not affect diagnostic reproducibility. No one volume presented as superior in the lean, normal, or obese groups. Practitioners may therefore choose to give the best-tolerated dilution. The increased flexibility allowed by this situation may help to mitigate some of the negative feelings practitioners and subjects have toward the test.

5.13 Acknowledgements

We would like to thank MuscleTech Research and Development Incorporated for their generous financial sponsorship of this study; Technilab for donating the 75g Glucodex® test meals used in this study; Denise Lamure for excellent technical assistance in the managing and processing of samples; and Jeremy Kwan at the Banting and Best Diabetes Centre Core Laboratory for prompt expert analyses.
CHAPTER 6.

GENERAL DISCUSSION AND CONCLUSIONS
6. GENERAL DISCUSSION AND CONCLUSIONS

6.1 Summary

Three studies were undertaken to assess the effect of dilution on the glycemic response to oral sugar meals. A significant effect of dilution on postprandial glycemia was demonstrated when the dilutions studied were not repeated. The first and second study showed that a 3-fold dilution either of 25g oral sucrose, glucose, or fructose tolerances tests or a 75g-OGTT increased AUC and glycemic concentrations at various intermediate time points. Within subject repetition of the tests however changed the outcome in the third study. When the same levels of dilution of the 75g-OGTT were repeated three times each, this glycemia raising effect of dilution either disappeared or was reversed. In addition the hypothesized affect of dilution on reproducibility was not observed. Reasons for these discrepancies were not clear. It was speculated that the high variability of results inherent in oral glucose tolerance testing (72,73) might have overridden any effect of dilution on glycemia. Conditioning of the cephalic insulin release by repeated testing might also have played a role, such that with subsequent repeated tests the participants responded to the higher volumes by increasing insulin secretion, thereby decreasing glycemia (239). Support for this hypothesis was provided by higher plasma insulin at 30 minutes for the 900ml-OGTT in the normal group. On the other hand, selective administration of one of the test volumes over another at different phases of gastrointestinal tract activity may have compensated for any effect of dilution either on absolute glycemia or its reproducibility (215). Taken together the findings from these three studies were inconclusive, but do not preclude the possibility that the addition of water may alter postprandial glycemia.

6.2 Mechanism of Action

The present findings also do not preclude the possibility that the effects observed might have been attributable to the gastric emptying mechanism that was hypothesized earlier (59,61,232,233). Because the higher volume meals in the first two studies amplified postprandial
glycemia at intermediate time points including "peak" glycemia, the suggestion was that dilution had its effect during the phase of the glycemic curve that marks the "absorptive" phase of carbohydrate metabolism as opposed to the "disposal" phase. It was concluded therefore that our findings were consistent with accelerated gastric emptying of the high volume meals, due to their higher volume and lower osmolality (208-211). It was also suggested that this mechanism might have remained operating in the third study, although repetition of the dilutions did not produce the effect observed in the first two studies. Because we noticed the 600ml-OGTT received the highest ratings for palatability and acceptability and lower though not significant (p<0.1) ratings for dizziness, we might conclude, as did Schwartz and coworkers (59), that it had a quicker, more agreeable pattern of gastric emptying. That is, the 600ml-OGTT meal was more physiological.

6.3 Therapeutic Implications

Whether or not gastric emptying mediates an effect of dilution on postprandial glycemia, a public health message for the use of either low or high volume meals is not supported by the data. While the reductions in postprandial glycemia that were observed when volume was decreased in the first two studies were similar to the reductions in the glycemic load of the diet associated with a decrease in the development of diabetes (84,85) and improvement in glycemic control in people with diabetes (146,147), it is difficult to extrapolate the therapeutic value of manipulating dilution. Several reasons can be cited for this conclusion. First, the inconclusive nature of our findings after repeated testing of the OGTTs at different dilutions makes it unclear as to whether a blood glucose raising effect of dilution would persist from day to day. Second, concerns were raised regarding the safety and practicality of limiting water consumption during meals in order to control mealtime hyperglycemia. Third, reported findings for an effect of dilution on postprandial glycemia are inconclusive in people with diabetes (61,62), the group that would arguably benefit the most from a maneuver for controlling mealtime hyperglycemia.
Finally, dilution may have the opposite effect on hunger (221-223) and energy intake (222,223). Our data, instead of supporting a reduction of water volume with meals, therefore support further investigation of dilutions possible longterm effects.

6.4 Methodological Implications

This variable effect of dilution we observed on postprandial glycemia might nevertheless have direct implications for glycemic testing methodology. As indicated the volume of test meals is not rigorously controlled in glycemic testing. Since glycemia at intermediate time points and AUC, both of which were affected, are used as endpoints in this testing, it was concluded that alterations in dilution might contribute to variability, compromising both reliability and validity of results. In other words, differences in "test-retest" results between the same treatments might occur because of dilution differences and differences between different treatments might be attributable to dilution or its interaction with the "main-effect" being studied as opposed to the "main-effect" alone. The result of this situation may be to confound comparisons within and between the findings of different studies.

Although discrepancies between protocols and the addition of extra water to compensate for poor tolerability might also mean that dilution is not rigorously controlled in oral glucose tolerance testing, our findings do not likely have implications for diabetes screening methodology. Because 2hPG was insensitive to alterations in volume and no effect on reproducibility was observed, dilution seems an unlikely candidate to affect diagnosis using 75g-OGTT criteria that is based solely on this parameter. That being said, the interpretation of older criteria, namely NDDG criteria (75), that rely on intermediate time points may be affected by dilution. Specifically, false diagnoses might result, as might false comparisons of prevalence/incidence data derived from an OGTT at one volume with an OGTT at another volume. Our data must therefore be viewed as supporting the move of the ADA (70) and CDA (71) in recommending the sole use of 2hPG for diagnosis following a 75g-OGTT. Abandonment of
intermediate time points in diagnosis has likely minimized the confounding effect of dilution, thereby improving reproducibility.

The overall message from our data is that investigators conducting postprandial carbohydrate metabolism studies may wish to control for an effect of dilution in their study design when glycemic concentrations at intermediate time points and AUC are outcome measures. This precaution is already in place in GI testing. In oral glucose tolerance testing in which 2hPG is used as the sole basis for diagnosis, investigators and practitioners may however consider administering the best-tolerated dilution. In this last regard, because the 600ml-OGTT had the best tolerability profile, a more diluted test may be desirable.

6.5 Future Research

Future studies are warranted. As suggested, these should include efforts to explore dilutions longterm effects. Also included should be studies of the effect of dilution on glycemia and OGTT reproducibility in IGT and type 2 diabetic subjects. This avenue of research would be important on two-levels. First, they are the two groups most likely to be diagnosed, so the data provided by such studies would be more directly applicable. Second, glucose tolerance may interfere with an effect of dilution, remembering that there was a discrepancy in the effect of dilution between both people without diabetes and well-controlled diabetes and those with poorly controlled diabetes (61,62). Other investigative efforts should explore the proposed gastric emptying hypothesis. This sort of study would go to clarifying the effect of dilution on postprandial glycemia.
CHAPTER 7.

REFERENCES
7. REFERENCES


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8.1 APPENDIX 1: “STUDY 1” CONSENT FORM

TITLE OF RESEARCH STUDY: The Effect Of Volume in the glycemic testing of oral sugar meals

PRINCIPAL INVESTIGATOR: Vladimir Vuksan, PhD

After the consumption of a food, a person will experience a change in his/her blood sugar level. This change is termed the blood glucose response to that food. Dietary factors, which influence the blood glucose response, include type of carbohydrate, amount of carbohydrate, food form, physical preparation, ripeness, and fibre, fat, protein, and antinutrient content. Evidence suggests that water volume may also be a factor. The purpose of this study is to confirm this latter relationship by assessing whether a two-fold increase in the volume of 25g oral sucrose, glucose or fructose test meals will affect the blood glucose response.

I agree to participate in a research study with this expressed purpose being conducted by the Nutritional Sciences and Risk factor Modification Centre, St. Michael’s Hospital and Faculty of Medicine, Department of Nutritional Sciences University of Toronto.

As part of the conditions of my participation, there is a protocol I agree to follow. I will come to the Risk Factor Modification Centre at St. Michael’s Hospital, 61 Queen Street East on six designated mornings between 09:00 and 10:30 am. The evening before each test, I will fast overnight for 10-16 hours and maintain my usual dietary and exercise patterns. Also on test mornings, I will avoid any exercise. As part of the test, I understand I will consume a GLUCODEX® OGTT meal consisting of 25g of sucrose, glucose, or fructose dissolved in 200ml or 600ml of water. Each of these meals will be given in random order. I will consume each over exactly five minutes ensuring to drink it steadily and evenly over this period. Should I not be able to consume the test meal in the allotted 5 minutes, I understand that I will be given an additional 5 minutes to drink as much of it as I possibly can. I also understand that I will give seven finger prick blood samples of about 2 to 3 drops each using an automatic Monoejector lancet device. These samples will be obtained from me before the start of the meal and at 15, 30, 45, 60, 90, and 120 minutes after the start of the test meal. During this time, I will be expected to remain seated and not smoke, eat, or drink. The entire time spent in the clinic will be approximately two hours and fifteen minutes.

There are several precautions I agree to observe. First, to prevent the transmission of disease, I will not share the Monoejector lancet device I have been assigned with anyone else and will personally change the device’s needle after every test. Second, to become as comfortable as possible with the use of the Monoejector lancet device, I will practice giving blood under the direction and supervision of the study investigators before starting the study. This will be done until I am sufficiently familiar with the procedure involved. Third, to minimize the pain and discomfort, and possible bruising that may accompany a finger prick, I will consider pricking the sides of my fingers as opposed to the tips. The tips tend to be more sensitive than the sides.

I understand that I will not receive any direct benefit from participating in this study. Others, however, may benefit from the knowledge gained through my participation. I also understand that there are some possible risks that may result from my involvement. I may experience discomfort, bruising, and rarely infection from the blood collection. Additionally, there is a risk of disease transmission if I do not observe the precautions outlined above for the proper use of the Monoejector lancet device.

I understand that I shall be informed of the results of any tests I undertake and that these results will be confidential and shown to no one with out my expressed consent. If I desire, then my results may be forwarded to my family doctor.

My involvement in this study is strictly voluntary, and, as such, I understand that I am free to withdraw from this study at any time. I also understand that if I choose to participate, then I will not be identified by name in publications of the results of this study.

Toronto’s Urban Angel
I certify that I have read the preceding and understand its content. I have discussed any questions pertaining to this research with either Dr. Vladimir Vuksan or Mr. John Sievenpiper and I understand that if I have any further questions about the study, then I may reach them at 416-867-7450. In addition, if I have any questions as a research subject, then I may contact Dr. Andrew Baker, chairman of research ethics, at (416) 864-5720.

Name of participant:________________________________________

Signature of participant:____________________________________ Date:________

Signature of Investigator:____________________________________ Date:________

Signature of witness:________________________________________ Date:________
8.2 APPENDIX II: “STUDY 2” CONSENT FORM

TITLE OF RESEARCH STUDY: The Effect Of Volume In Oral Glucose Tolerance Testing

PRINCIPAL INVESTIGATOR: Vladimir Vuksan, PhD

After the consumption of a food, a person will experience a change in his/her blood sugar level. This change is termed the blood glucose response to that food. Dietary factors, which influence the blood glucose response, include type of carbohydrate, amount of carbohydrate, food form, physical preparation, ripeness, and fibre, fat, protein, and antinutrient content. Evidence suggests that water volume may also be a factor. The purpose of this study is to confirm this latter relationship by assessing whether a two or three fold increase in the volume of an oral glucose tolerance test (OGTT) meal, used in the diagnosis of diabetes, will affect the blood glucose response.

I agree to participate in a research study with this expressed purpose being conducted by the Nutritional Sciences and Risk factor Modification Centre, St. Michael’s Hospital and Faculty of Medicine, Department of Nutritional Sciences University of Toronto.

As part of the conditions of my participation, there is a protocol I agree to follow. I will come to the Risk Factor Modification Centre at St. Michael’s Hospital, 61 Queen Street East on three designated mornings between 09:00 and 10:30 am. The evening before each test, I will fast overnight for 10-16 hours and maintain my usual dietary and exercise patterns. Also on test mornings, I will avoid any exercise. As part of the test, I understand I will consume a GLUCODEX® OGTT meal consisting of 75 g of glucose dissolved in 300ml (approx. 1 glass), 600 ml (approx. 2 glasses) 900ml (approx. 3 glasses) of water. Each of these meals will be given in random order. I will consume each over exactly five minutes ensuring to drink it steadily and evenly over this period. Should I not be able to consume the test meal in the allotted 5 minutes, I understand that I will be given an additional 5 minutes to drink as much of it as I possibly can. I also understand that I will give seven finger prick blood samples of about 2 to 3 drops each using an automatic Monojector lancet device. These samples will be obtained from me before the start of the meal and at 15, 30, 45, 60, 90, and 120 minutes after the start of the test meal. During this time, I will be expected to remain seated and not smoke, eat, or drink. The entire time spent in the clinic will be approximately two hours and fifteen minutes.

There are several precautions I agree to observe. First, to prevent the transmission of disease, I will not share the Monojector lancet device I have been assigned with anyone else and will personally change the device’s needle after every test. Second, to become as comfortable as possible with the use of the Monojector lancet device, I will practice giving blood under the direction and supervision of the study investigators before starting the study. This will be done until I am sufficiently familiar with the procedure involved. Third, to minimize the pain and discomfort, and possible bruising that may accompany a finger prick, I will consider pricking the sides of my fingers as opposed to the tips. The tips tend to be more sensitive than the sides.

I understand that I will not receive any direct benefit from participating in this study. Others, however, may benefit from the knowledge gained through my participation. I also understand that there are some possible risks that may result from my involvement. I may experience discomfort, bruising, and rarely infection from the blood collection. Additionally, there is a risk of disease transmission if I do not observe the precautions outlined above for the proper use of the Monojector lancet device.

I understand that I shall be informed of the results of any tests I undertake and that these results will be confidential and shown to no one with out my expressed consent. If I desire, then my results may be forwarded to my family doctor.

My involvement in this study is strictly voluntary, and, as such, I understand that I am free to withdraw from this study at any time. I also understand that if I choose to participate, then I will not be identified by name in publications of the results of this study.

Toronto’s Urban Angel
I certify that I have read the preceding and understand its content. I have discussed any questions pertaining to this research with either Dr. Vladimir Vuksan or Mr. John Sievenpiper and I understand that if I have any further questions about the study, then I may reach them at 416-867-7450. In addition, if I have any questions as a research subject, then I may contact Dr. Andrew Baker, chairman of research ethics, at (416) 864-5720.

Name of participant: __________________________

Signature of participant: __________________________ Date: _______

Signature of Investigator: __________________________ Date: _______

Signature of witness: __________________________ Date: _______
8.3 APPENDIX III: “STUDY 3” CONSENT FORM

TITLE OF RESEARCH STUDY: The Effect Of Volume In Oral Glucose Tolerance Testing For Diabetes

INVESTIGATORS:
Dr. Vladimir Vuksan, PhD
Assistant Professor,
Department of Nutritional Sciences
Faculty of Medicine, University of Toronto
and Associate Director of Clinical Trials
Risk Factor Modification Centre,
St. Michael’s hospital
tel: 967-7450

-and-
Mr. John L Sievenpiper, BASc, MSc student
Supervisor: Dr. Vladimir Vuksan
Department of Nutritional Sciences.
Faculty of Medicine, University of Toronto
and Research Assistant,
Risk Factor Modification Centre
St. Michael’s hospital
tel: 867-7460, ext. 8059

STUDY SPONSOR:
MuscleTech

PURPOSE OF THE RESEARCH:
After the consumption of a food, a person will experience a change in his/her blood sugar level. This change is termed the blood glucose response to that food. Dietary factors which influence the blood glucose response include type of carbohydrate, amount of carbohydrate, food form, physical preparation, ripeness, and fibre, fat, protein, and antinutrient content. Evidence suggests that water volume may also be a factor. The purpose of this study is to confirm this latter relationship by assessing whether a two, three, or four fold increase in the volume of an oral glucose tolerance test (OGTT) meal, used in the diagnosis of diabetes, will affect blood glucose. Establishing a connection may help to explain some of the high variability of blood glucose based test results, a major criticism of this tool. Future application of the results may thereby have the effect of reducing the chances of misdiagnoses.

DESCRIPTION OF THE RESEARCH:
As part of the conditions of my participation, there is a protocol I agree to follow. Before beginning the study I will submit to measurements of my weight using a beamscale, height using a measuring tape, and blood pressure using a cuff attached to a standard blood pressure measuring device. I will also submit to measurement of my percentage body fat using a FUTREX® machine which will direct an infrared beam of light into my bicep and using calipers which will measure my tricep, bicep, and subscapula (shoulder blade) skin-fold thickness. Upon commencement of the study, I will come to the Risk Factor Modification Centre at St. Michael’s Hospital, 12 Queen Street East on twelve designated mornings between 08:00 and 10:00 am. For the three days prior to each visit, I will consume a minimum of 150g of carbohydrate each day. This represents the equivalent to three servings of any of the following alone or in combination: 2 slices of bread, 1 cup of cooked rice or pasta, 1 medium potato, 1 bowl of cereal with milk, 1 glass of juice/soft-drink, 3 oranges/apples, or 1 bowl of ice cream. The evening before each test, I will fast overnight for 10-16 hours and maintain my usual dietary and exercise patterns. Additionally, on test mornings, I will avoid any exercise. As part of the test, I understand I will consume a GLUCODEX® OGTT meal consisting of 75 g of glucose dissolved in either 300ml (approx. 1 glass), 600 ml (approx. 2 glasses) 900ml (approx. 3 glasses), or 1200ml (approx. 4 glasses) of water. Each of these meals will be repeated three times for a total of twelve tests and given in random order. I will consume each over exactly five minutes ensuring to drink it steadily and evenly over this period. Should I not be able to consume the test meal in the allotted 5 minutes, I understand that I will be given an additional 5 minutes to drink as much of it as I possibly can. I also understand that I will give seven venous blood samples of about 5ml each. These samples will be obtained by a registered nurse from an indwelling cannula (small needle) inserted in a forearm vein. This device will be inserted at the beginning of test, held in place for its two hour duration by medical tape, and kept open with the injection of a small amount of sterile saline (salt solution) after each blood collection. The timing of blood collections will be once before the start of the test meal and then at 15, 30, 45, 60, 90, and 120 minutes after its start. During this time, I will be expected to remain seated and not smoke, eat, or drink. The entire time spent in the clinic will be approximately two hours and fifteen minutes.

POTENTIAL HARMs (INJURY, DISCOMFORTS, OR INCONVENIENCE):
I understand that there are some possible risks that may result from my involvement. I may experience discomfort and bruising from the blood collection. Additionally, there is a risk of further bruising and possible disease.
transmission if I attempt to alter, move or bump the cannula during the test. Any adjustments to the device I agree to leave to the registered nurse.

**POTENTIAL BENEFITS:**
I will not benefit directly from participating in this study. Others, however, may benefit from the knowledge gained through my participation.

**COMPENSATION AND REIMBURSEMENT:**
I understand that there is financial compensation for my involvement. I will receive $25.00 per session in the form of a cheque, payable in one installment of $300.00 upon successful completion of all 12 tests. I will also be reimbursed $5.00 in cash per session for transportation costs ($60.00 in total).

**CONFIDENTIALITY AND PRIVACY:**
I understand that I shall be informed of the results of any tests I undertake and that these results will be confidential and shown to no one with out my expressed consent unless required by law. If I desire, then my results may be forwarded to my family doctor.

**PUBLICATION OF RESULTS:**
The results from this study may be presented at public forums such as conferences or seminars. Publication of these results may also occur in scientific, lay, or promotional media. In either case I understand that I will not be identified by name. Instead only data for the group as a whole will be reported or where it is necessary to report individual data, non-traceable identification numbers will be used.

**PARTICIPATION AND WITHDRAWAL:**
Participation in research is voluntary. I understand that should I choose to participate, I am free to withdraw from this study at any time without prejudice, loss of compensation, or any affect on the care I will receive at St. Michael’s Hospital. Alternatively, if I choose not to participate, then I understand that I will continue to have access to customary care at St. Michael’s Hospital.

**RESEARCH ETHICS BOARD CONTACT:**
I understand if I have any questions as a research subject, then I may contact Dr. Andrew Baker, cochair of research ethics, at (416) 864-5720.

**CONSENT:**
I acknowledge that I have been given sufficient time to read and understand the preceding, the research study described here-in has been adequately explained, and any questions that I had have been answered to my satisfaction. I certify that I have been informed of (1) the procedures I will follow; (2) the potential risks, harms, and discomforts that may result from these; (3) compensation I will receive, should I choose to participate; (4) assurances that records relating to my involvement will be kept confidential and information will not be released with out my permission unless required by law; (5) the possibility of publication or presentation of the results of this study and the means that will be taken to ensure confidentiality; and (6) alternatives to participation in this study, including the right not to participate and withdraw without compromising the quality of medical care at St. Michael’s Hospital for me or for other members of my family. If I have any further questions regarding these matters, then I know that I may ask them now or in the future.

By agreeing to participate, I understand that I have not waived my legal rights nor released the investigators sponsors or involved institutions from their legal and professional duties.

I hereby consent to participate and have been given a copy of this consent form.

Participant name: ___________________________ Participant signature: ___________________________ Date: __________

Investigator name: ___________________________ Investigator signature: ___________________________ Date: __________

Witness name: ___________________________ Witness signature: ___________________________ Date: __________
8.4 APPENDIX IV: GLYCEMIC TESTING QUESTIONNAIRE

Name: ___________________ Date: __________

Weight: ______ kg Height: ______ cm

Test meal eaten (please circle one): A200 B200 C200

A600 B600 C600

Start Time: ___________ Time required to finish Meal: ___________ 

Pretest information:

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In the adjacent column, please describe the dinner and any snacks you consumed last night prior to beginning your fast.

Did you consume at least 150g (6oz.) of carbohydrate on each of the three days previous to this test? This amount is equivalent to three servings of any of the following alone or in combination: 2 slices of bread, 1 cup of cooked rice or pasta, 1 medium potato, 1 bowl of cereal with milk, 1 glass of juice/soft-drink, 3 oranges/apples, or 1 bowl of ice cream.

☐ Yes  ☐ No

Did you do anything last night that is not in part of your regular routine? This may include exercise or use of alcohol, medications, or supplements. If yes, then please describe.

☐ Yes  ☐ No

How many hours of sleep did you have last night?

Does this represent a typical amount?

☐ Yes  ☐ No

--------- hours

Did you do anything before the test this morning that is not in compliance with the study protocol or part of your regular routine? If yes, then please describe.

☐ Yes  ☐ No

What was your mode of transportation to the clinic this morning?

How would you rate your current level of health/well-being. Please comment on anything unusual.

☐ Excellent  ☐ Good  ☐ Fair  ☐ Poor
### 8.5 APPENDIX V: OGTT QUESTIONNAIRE

Name: ____________________ Date: __________

Weight: ______kg  Height: ______cm

Test meal eaten (please circle one):  300  600  900

Start Time: __________  Time required to finish Meal: __________

**Pretest information:**

In the adjacent column, please describe the dinner and any snacks you consumed last night prior to beginning your fast.

Did you consume at least 150g (6oz.) of carbohydrate on each of the three days previous to this test? This amount is equivalent to three servings of any of the following alone or in combination: 2 slices of bread, 1 cup of cooked rice or pasta, 1 medium potato, 1 bowl of cereal with milk, 1 glass of juice/soft-drink, 3 oranges/apples, or 1 bowl of ice cream.

- [ ] Yes  - [ ] No

Did you do anything last night that is not in part of your regular routine? This may include exercise or use of alcohol, medications, or supplements. If yes, then please describe.

- [ ] Yes  - [ ] No

How many hours of sleep did you have last night?
Does this represent a typical amount?

- [ ] Yes  - [ ] No

Did you do anything before the test this morning that is not in compliance with the study protocol or part of your regular routine? If yes, then please describe.

- [ ] Yes  - [ ] No

What was your mode of transportation to the clinic this morning?

How would you rate your current level of health/well-being. Please comment on anything unusual.

- [ ] Excellent  - [ ] Good  - [ ] Fair  - [ ] Poor

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_______ hours
### 8.6 APPENDIX VI: OGTT QUESTIONNAIRE WITH SCALES

Name: ___________________________ Date: ____________

Weight: _______ kg  Height: _______ cm

Test meal eaten (please circle one): 300  600  900

Start Time: ____________  Time required to finish Meal: ____________

**Pretest information:**

In the adjacent column, please describe the dinner and any snacks you consumed last night prior to beginning your fast.

Did you consume at least **150g** (6oz.) of carbohydrate on **each** of the three days previous to this test? This amount is equivalent to **three** servings of any of the following alone or in combination: 2 slices of bread, 1 cup of cooked rice or pasta, 1 medium potato, 1 bowl of cereal with milk, 1 glass of juice/soft-drink, 3 oranges/apples, or 1 bowl of ice cream.

- □ Yes  □ No

Did you do anything last night that is not in part of your regular routine? This may include exercise or use of alcohol, medications, or supplements. If yes, then please describe.

- □ Yes  □ No

How many hours of sleep did you have last night? Does this represent a typical amount?

- □ Yes  □ No

<table>
<thead>
<tr>
<th>Time</th>
<th>Food item</th>
<th>Quantity</th>
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Did you do anything before the test this morning that is not in compliance with the study protocol or part of your regular routine? If yes, then please describe.

- □ Yes  □ No

What was your mode of transportation to the clinic this morning?

How would you rate your current level of health/well-being. Please comment on anything unusual.

- □ Excellent  □ Good  □ Fair  □ Poor

---

**Post-meal Palatability, Acceptability, Satiety, Dizziness, and Nausea Scales:** Place an "X" on the lines

<table>
<thead>
<tr>
<th>Low palatability</th>
<th>Low acceptability</th>
<th>Low satiety/fullness</th>
<th>Low dizziness</th>
<th>Low Nausea</th>
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<td>1——2——3——4——5——6——7</td>
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</table>

High Palatability  
High acceptability  
High satiety/fullness  
High dizziness  
High Nausea
September 4, 1998

Dr Vladimir Vuksan
Risk Factor Modification Centre
St Michael's Hospital

Dear Dr Vuksan

Re: REB 97-016: Effect of volume in oral glucose tolerance testing for diabetes

Thank you for your communications of August 21, 1998 regarding the above named study. As I understand, this project represents a continuation of the previously approved study entitled “Effect of the water content of food on the postprandial glycemic response: Implications for glycemic testing”.

In the continuation protocol, there are only three main differences between the protocol for this project and the last. First, you will be drawing venous blood instead of finger prick capillary samples. Second, in addition to 300 ml and 600 ml meals, you will also be looking at those of 900 ml and 1200 ml. And third, subjects will be financially compensated.

Consent form has been modified to reflect these changes.

I am happy to issue approval for this study for a period of 12 months from the date of this letter. Continuation beyond that date will require further annual review of the REB approval.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the Research Ethics Board.

Good luck with your investigation.

With best wishes

Andrew Baker MD
Co-Chair
Research Ethics Board

AB/dp

Toronto's Urban Angel
16 September 1999

Vladimir Vuksan, PhD
Associate Director
Clinical Nutrition and Risk Factor
Modification Centre
30 Bond Street
Toronto ON M5B 1W8

Dear Dr. Vuksan:

Re: Dilution of the 75g oral glucose tolerance test increases postprandial glycosmia: implications for diagnostic criteria (899-0886)

I am pleased to inform you that subject to minor revisions an edited copy of your manuscript will be suitable for publication in CMAJ. It will also appear in Ovid Online, a computer-assisted data retrieval service of Ovid Technologies, New York. Your article may also appear on CMA Online, the Canadian Medical Association's Internet service (www.cma.ca).

Your manuscript will now be sent for copy-editing. The copy editor will make changes to adhere to CMAJ style and will send you a proof for your approval.

In order to proceed with publication of your manuscript, we require that the following information be sent to us in the next two weeks:

1. Summary of paper: Provide a 75-word summary of your paper. The summary should describe the reason for doing the research (why your study is important), the main result, and your main message (what you want readers to remember about your study). This may be published on a special page in the journal devoted to summaries of manuscripts.

2. Press release: Provide a 75-word paragraph suitable for us to use in a press release. It should describe the work you did, give the main results and explain their meaning and, lastly, describe why this study is important. Use language that can be easily understood by a non-medical reader. CMAJ issues a press release for each issue, highlighting important topics and explaining the significance of scientific research.
3. **Key messages:** List up to 6 points that you want readers to remember about your study. These points should include a reminder of what the problem is, your main findings, and what you think are its implications for the practice of medicine or for the health of patients/population. This may be published with the article as a quick reference for readers.

Please refer to the above file number when corresponding with us. Thank you for your contribution.

Sincerely yours,

[Signature]

John Hoey, MD
Editor-in-Chief
*Canadian Medical Association Journal*