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Intra- and Inter-Person Variability in Glycemic Responsiveness

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INTRA- AND INTER-PERSON VARIABILITY IN GLYCEMIC RESPONSIVENESS

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List of Abbreviations

AA = African American

ANOVA = Analysis Of Variance

ANS = Autonomic Nervous System

AUC = Area Under the Curve

BG = Blood Glucose

BMI = Body Mass Index

CAD = Coronary Artery Disease

CHD = Coronary Heart Disease

CNS = Central Nervous System

CPG = Casual Plasma Glucose

CV = Coefficient Of Variation

CVD = Cardiovascular Disease

dL = Deciliter

DM = Diabetes Mellitus

EA = European-American

FBG = Fasting Blood Glucose

FPG = Fasting Plasma Glucose

g = Gram

GDM = Gestational Diabetes Mellitus

GI = Glycemic Index

Hb = Hemoglobin

HDL = High-density Lipoproteins

HFCS = High-Fructose Corn Syrup

IDDM = Insulin Dependent Diabetes Mellitus

IGT = Impaired Glucose Tolerance

kg = Kilogram

LDL = Low-density Lipoprotein

LSU = Louisiana State University & Agricultural and Mechanical College

m = Meter

m^2 = Meter Squared

mg = Milligram

mM = Millimole

NHANES III = Third National Health and Nutrition Examination Survey

NIDDM = Non-Insulin Dependent Diabetes Mellitus

OGTT = Oral Glucose Tolerance Test

SD = Standard Deviation

SMBG = Self-monitoring of Blood Glucose

TG = Triglycerides

< = less than

> = greater than

$\leq$ = less that or equal to

$\geq$ = greater than or equal to

DEFINITIONS

Autoimmune disease = a disease resulting from a disordered immune reaction in which antibodies are produced that damage components of the body.

Chronic disease = disease having long duration

Diabetes Mellitus = a chronic metabolic disorder characterized by high blood glucose and either insufficient or ineffective insulin.

Fasting = a period of no energy intake, usually 10-12 hours overnight

Fed state = 0 to 3 hours after consumption of food.

First Degree Relative = mother, father, sister, brother, child

Gluconeogenesis = generation of glucose from non-carbohydrate precursors.

Glycemic index = a measure of the effect of carbohydrate-containing foods on blood glucose.

Ketone = certain substances (acetone, acetoacetic acid, and B-hydroxybutyric acid) produced by the incomplete oxidation of fats, especially when fats are being rapidly catabolized.

Normal Range of Blood Glucose Level = a fasting blood glucose level of 70-110 mg/dL.

Postabsorptive state = 3 to 16 hours after consumption of food

Postprandial = after consumption of food

Prolonged fasting = fasting more than 12 hours

Resistant Starch = the sum of starch and starch degradation products not absorbed in the small intestine of healthy adult

Syndrome X = a cluster of atherogenic risk factors including hyperinsulinemia, obesity with an abdominal pattern of distribution, some degree of carbohydrate intolerence, hypertension, and an abnormal blood lipid pattern of increased triglyceride and decreased high-density lipoprotein subfractions.

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ABSTRACT

Nearly one half of the adults in the United States are overweight or obese. Increased intake of high glycemic index (GI) carbohydrates (CHO) has paralleled the increase in obesity. Clinical implications of the GI and eating low GI foods include: improved glycemic control for diabetics and weight loss. Many studies on the GI have failed to address basic issues such as intra- and inter-variability in individuals. The purpose of this study was to evaluate the intra- and inter-variability in glycemic responsiveness to a white bread standard.

Study participants (n=10) were without diagnosed glucose metabolism impairment. Coefficient of variation (CV) was determined for the assay (2 subjects; 10 replicates) and for fasting blood glucose (BG) levels (10 subjects). Using a standard finger stick method, BG levels were determined at baseline and 15, 30, 45, 60, 90, and 120 minutes after ingesting pre-weighed portions of white bread. Four levels of carbohydrate (CHO) were tested in triplicate: 25 grams (g) CHO (54g white bread) (n=10), 50 g CHO (108g white bread) (n=10), 75g CHO (154g white bread) (n=10), and 100g CHO (216g white bread) (n=3). In addition to the standard seven finger sticks, samples were also taken at 150, 180, and 210 minutes for the 100g treatment.

CV for the assay was small: 2.22, 4.48. There was no significant difference among subjects for fasting BG. There was a significant difference among subjects for all levels of CHO tested except 100g CHO: 25g CHO ($p<0.004$), 50g CHO ($p<0.008$), and 75g CHO ($p<0.009$). Three subjects had a dose response to increasing levels of CHO: ($r^2=.0.736$; $p<0.001$), ($r^2=0.352$; $p<0.042$), and ($r^2=0.565$; $p<0.020$).

The variability observed in this study is inconsistent with results in the literature and suggests that when using the GI for therapeutic diets, individual responsiveness must be considered.

CHAPTER 1

INTRODUCTION

The mean percent of total energy from total fat and saturated fat has significantly decreased among the United States (US) population since the 1960's[1,2]; however, the prevalence of diet-related diseases such as type 2 diabetes mellitus (DM)[3] and obesity[4] continues to rise. This suggests that dietary fat is not the only dietary component responsible for increasing disease risk. While fat intakes have fallen, dietary carbohydrate (CHO) intakes have increased.[5] Type and amount of dietary CHO may be a determining factor in the development of obesity and type 2 DM. In the 1980's, the glycemic index (GI) was developed as a system to rank foods according to the blood glucose response produced after eating them.[6] Since its development, the GI has been studied and positively associated with risk for diabetes[7,8] and obesity.[9]

Glycemic index is defined as the area under the blood glucose response curve above the fasting level for 50 grams (g) of a test food divided by the response to 50g of a reference food.[10] The GI for a particular food reflects the mean response of a group of people[10]; because of this, the GI does not allow for individual variations in responsiveness. Studies have indicated a significant difference between individual responses[11,12]; yet, they have failed to address the implications of intra- or inter-person variability.

The current study was conducted to determine if glycemic responsiveness differs among or within individuals.

Objectives

There were two objectives: 1) to determine if glycemic responsiveness varies within an individual; and, 2) to determine if glycemic responsiveness varied among individuals on the basis of age, race, gender, body mass index (BMI), or percent body fat.

Hypotheses

- An individual experiences similar glycemic responses to a standard food.
- An individual's glycemic response varies according to the amount of CHO ingested.
- Glycemic responsiveness differs among individuals according to differences in their:
  - Age
  - Percent body fat
  - BMI
  - Gender
  - Race

Assumptions

Assumptions that were made in the design and implementation of this study were:

1. The results of this study are applicable to the general public;
2. The instruments used in determining blood glucose levels, weight, and percent body fat were accurate and reliable tools;
3. The measurement of glucose in capillary blood is a good indicator of glycemic response; and
4. The subjects included in the sample complied with instructions regarding fasting before testing glycemic responses.

Limitations

Limitations of this study are:

1. The sample was not selected randomly. Participants were volunteers who were informed of the study through flyers distributed within Louisiana State University Agricultural and Mechanical College-School of Human Ecology or by announcements made in dietetics courses.
2. The sample population was too small to include adequate representation of all groups—*e.g.* age, percent body fat, BMI, gender, gender,
3. The small sample size (n=10) may limit applicability of data to the general public.
4. Participants were free-living individuals. There was no method of determining if subjects had indeed fasted for the specified time before the glycemic response tests.
5. Participants were not asked to report a family history of diabetes.
6. Participants were not asked to report any use of medications.
7. Parallel insulin responses were not measured.

Justification

A significant difference in glycemic responsiveness among subjects has been demonstrated in non-diabetic and diabetic populations[11,12]; however, reasons for varying responsiveness of individuals has not been well studied.

As the prevalence of diabetes increases[3], it is important to develop a diet to manage and prevent long-term complications due to prolonged hyperglycemia. A low GI diet has been indicated to improve glycemic control[13,14,15,16] , as well as increase satiety[9]; thereby decreasing subsequent food intake. How does individual variation impact a

person's glycemic status? Brand-Miller, a major proponent of the GI, argues that diabetic diets should be determined according to GI principles.[7] If responses are significantly different either within or among people, the GI cannot predict accurately the response a person will experience at a particular time. This would greatly reduce the utility of the GI as a means for developing therapeutic diets. Information from this study can be used to design future studies to explore the clinical utility of the GI.

CHAPTER 2

REVIEW OF THE LITERATURE

Carbohydrates

Carbohydrates are a class of organic compounds composed of carbon, hydrogen, and oxygen. Carbohydrates are classified as (1) simple and (2) complex, according to the number of saccharides, or sugar, units they possess. Simple carbohydrates, the sugars, consist of either one saccharide or several saccharides linked together. Complex carbohydrates, starch and fiber, are composed of saccharide linkages to form very large molecules.[18] These are discussed below.

Simple Carbohydrates

Simple carbohydrates are categorized further into the monosaccharides, disaccharides, and oligosaccharides.

Monosaccharides

Monosaccharides contain one saccharide unit. These three mononsaccharides of nutritional significance are glucose, fructose, and galactose. The monosaccharides are isomers of each other; that is, each possesses the same molecular formula $C_6H_{12}O_6$, but different structural formulas.[19]

Glucose (Figure 1) is the most abundant monosaccharide; however, it seldom exists naturally in its monosaccharide form. It is the major building block of di- and polysaccharides. Glucose is present in small amounts in all fruits and vegetables and is the preferred source of energy for cells.[20]

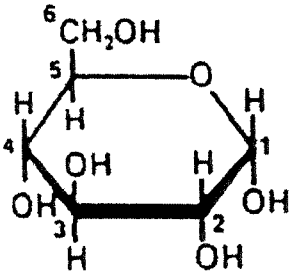


Figure 1. Structure of Glucose.

Fructose is the sweetest simple sugar and is largely responsible for the sweetness of honey and fruit. Due to its intense sweetness, fructose is used, usually as high-fructose corn syrup (HFCS), by the food industry to sweeten processed foods.[18] Galactose, one of the two saccharides linked to form the disaccharide lactose, is generally not found free in nature.[18,118]

Disaccharides

Disaccharides are pairs of monosaccharides linked through condensation reactions. The three disaccharides digestible by humans are sucrose, lactose, and maltose. Glucose occurs in all three. Sucrose is comprised of glucose + fructose. Sucrose, commonly referred to as table sugar, is extracted from sugar cane or sugar beets, then granulated and processed into commercial products such as brown, white, or powdered sugar. Lactose, or "milk sugar," occurs naturally only in milk and milk products. Lactose is comprised of galactose + glucose. Maltose, glucose + glucose, generally does not exist as a free molecule in nature. Maltose is a breakdown product of starch and is present in malted or germinated grains and corn syrup.[20]

Oligosaccharides

Oligosaccharides consist of 3-10 saccharide units. Humans cannot digest oligosaccharides found in nature. They can, however, be digested by bacteria in the human intestinal tract, which results in gas formation. Oligosaccharides are present in legumes and squash.[19]

Complex Carbohydrates

Complex carbohydrates, polysaccharides, are comprised of long chains of monosaccharides. The number of saccharide units in a polysaccharide range from 11 to literally thousands. Three polysaccharides of importance in the human diet are starch, glycogen, and fiber.[20]

Starch

Starch is the storage form of energy in plants. The plant uses these stores during times when photosynthesis is not possible. A molecule, or granule, of starch is a long chain of linked glucose units in an organized crystalline pattern. Three crystalline forms (A, B, and C) of starch have been identified. Type A is common in cereal starches (wheat, rice, corn) and is characterized by the presence of water between molecules.[21] Type B is found in potatoes and bananas and contains densely packed starch molecules.[22] Type C is present in legumes. Its formation is a mixture of A and B patterns.

Two components of starch are amylose (Figure 2) and amylopectin (Figure 3). Amylose is a long, linear chain of glucose units linked by alpha-1,4 glycosidic bonds. The linear linkage of glucose units creates a hydrophobic molecule, which is insoluble in water.[23] When foods high in amylose are heated in water, the starch granule swells irreversibly and leaks amylose into the water. This is the process of gelatinization and

results in thickening the solution. The amylose forms a three-dimensional network, or sol. Pasting occurs with the continued heating of gelatinized starch. A gel forms, upon cooling of a gelatinized starch paste.[18] Aging of the gel results in retrogradation to a B-type crystalline structure.[24]

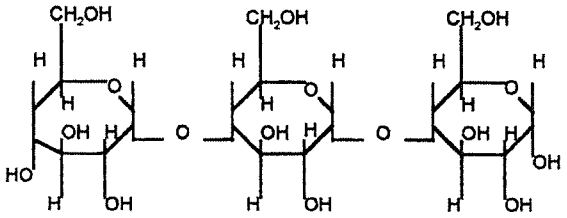


Figure 2. Structure of Amylose.

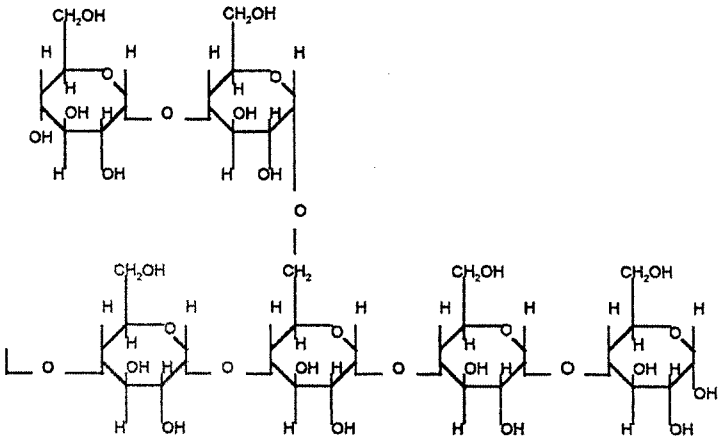


Figure 3. Structure of Amylopectin.

Amylopectin is a branched-chain molecule.[23] It resembles amylose, with its glucose backbone of alpha-1,4 glycosidic bonds. Chains of glucose units branch off the backbone every 20-25 glucose units. These chains are attached to the backbone by alpha-1,6 glycosidic bonds. The branching leads to the formation of a globular molecule, which makes it hydrophilic and soluble in water. Because amylopectin is a branched molecule, there are many sites vulnerable to enzymatic attack. Therefore, amylopectin is

more rapidly degraded than is straight-chain amylose. Cooked starches high in amylopectin, with little or no amylose, will thicken but not gel upon cooling.[18]

All starchy foods are of plant origin—grains, tubers, and legumes. Sources of starch in the human diet include potatoes, beans, peas, pasta, rice, corn, grains, and grain products such as cereal, bread, crackers, and baked goods.[25]

Glycogen

Glycogen is the energy storage polysaccharide found in animal cells. In humans, cells of the liver, muscles, kidney, uterus, and vagina produce glycogen. Glycogen resembles starch in that it is composed of the linkages of glucose units. Like amylopectin, glycogen is a branched molecule; however, glycogen is branched every 8-12 glucose units. This makes glycogen much more soluble in water than is starch. When the concentration of glucose in the blood is higher than the amount needed to provide immediate energy to the body's cells, the liver, and the other tissues that make glycogen, links glucose units to form glycogen molecules. Glycogen molecules are eventually broken into individual glucose units to be used for energy when there is no food intake. The molecular arrangement of glycogen allows for rapid hydrolysis. Because stores of glycogen in the body are limited, they provides only a temporary supply of energy.[20]

Fiber

Dietary fiber refers to the remnants of plant cells that are resistant to hydrolysis or breakdown by human digestive enzymes. Sources of dietary fiber in the human diet include whole grains, fruits, vegetables, seeds, nuts, and skins of plant products.[25] Fiber is a complex mixture of polysaccharides including cellulose, hemicelluloses, beta-glucans, pectins, gums, and lignin.[118]

Fiber is classified according to its chemical properties, solubility, or physical properties. Physical properties of fiber include water-holding capacity, viscosity, cation-exchange capacity, bile-binding capacity, and fermentability by the bacteria in the human gastrointestinal tract. The water-holding capacity of fiber contributes to its ability to produce larger, softer stools, and to increase the frequency of defecation. Insoluble fibers include cellulose, hemicellulose, and lignin. Soluble fibers include gums, mucilages, and pectins. Soluble fiber swells with water in the digestive tract and helps move materials that might otherwise stagnate, ferment, and generate toxins in the intestine.[23] Soluble fiber has the beneficial effect of delaying monosaccharide absorption in the small intestine. Both soluble and insoluble fibers contribute to a feeling of fullness and satiety when eaten.[19]

Cellulose is composed of thousands of glucose units linked by beta-1,4 glycosidic bonds. It is a structural polysaccharide providing strength in the cell walls of plants[19]; and it is the main component of wood, cotton, and paper. Cellulose holds water, reduces elevated pressure in the colon, and binds zinc. Cellulose may be chemically modified to thicken, stabilize, gel, and provide bulk in processed foods.[118] It is indigestible by humans.[19]

Hemicelluloses are also found in plant cell walls; they contain a variety of different monosaccharide building blocks. A molecule of hemicellulose may have branching side chains.[118] Hemicellulose holds water, increases stool bulk, reduces elevated colon pressure, and binds bile acids.[25]

Beta-glucans are polysaccharides made of glucose molecules. The glucose molecules are linked together in an order different from that of cellulose. Beta-glucan

molecules are more soluble in water than cellulose. Good sources of beta-glucans are oats and barley.[118]

Pectins are polysaccharides found in the spaces between plant cells and in the cell walls. Pectin aids in holding plant cells together. A derivative of galactose, galacturonic acid, is the building block of pectic substances. Protopectin is the largest pectic molecule. It is found in unripe fruit and is hydrolyzed to pectin as the fruit ripens. Pectin is responsible for forming gels in jams, jellies, and preserves. It is used as a thickener and stabilizer in the food industry. Pectins form gels in water and occur naturally in many fruits, especially in apples, citrus fruits, and strawberries.[118]

Gums and mucilages are long-chain polymers of monosaccharides, usually galactose, and are similar in structure. They are added to foods as thickeners and stabilizers. Gums help to retain water and reduce evaporation rates.[118]

Lignin is a noncarbohydrate molecule that may also be part of the fiber complex.[118] Lignin is found in the woody part of plants and is insoluble in water. Lignin acts as an antioxidant and binds bile acids, cholesterol, and metals.[25]

Digestion and Absorption of Carbohydrates

The body uses carbohydrates in the form of monosaccharides. Carbohydrates are rarely consumed in the monosaccharide form, therefore they must by hydrolyzed to monosaccharide units. The hydrolytic enzymes required for carbohydrate digestion are collectively called glycosidases or carbohydrases.[26] The glycosidases include: salivary alpha-amylase, pancreatic alpha-amylase, isomaltase, maltase, sucrase, and lactase. Starches are primarily hydrolyzed by alpha-amylase, which hydrolyzes the alpha-1,4

glycosidic bonds; however, beta-1,4 bonds of cellulose and alpha-1,6 bonds of amylopectin are resistant to alpha-amylase.[20,26]

Digestion of polysaccharides begins in the mouth. Chewing food stimulates the secretion of saliva that mixes with the food to form a bolus. Saliva contains salivary alpha-amylase, which hydrolyzes starch into shorter polysaccharide chains. Very little digestion actually occurs at this point.[20]

The bolus travels down the esophagus to the stomach where starch is hydrolyzed to dextrins, short chain polysaccharides, and maltose; however, virtually no digestion of di- or monosaccharides occurs.[26] In the stomach, the bolus is penetrated by hydrochloric acid which inactivates salivary alpha-amylase by lowering the pH to a point inconducive to enzymatic activity. Digestion of starch is delayed at this point. The presence of fiber in the bolus delays gastric emptying. This property of fiber leads to a feeling of fullness and satiety.[20]

From the stomach, dextrins, disaccharides, and monosaccharides move into the small intestine, where the majority of digestion takes place. Pancreatic alpha-amylase enters the small intestine through the pancreatic duct. Here, pancreatic bicarbonate and bile raise the pH to a level conducive to enzymatic activity. The action of pancreatic alpha-amylase on starch yields maltose, glucose, and isomaltose, with alpha-1,6 bonds. Digestible carbohydrate is now in the form of mono- and disaccharides. Brush-border enzymes on the microvilli lining of the small intestine cleave the glycosidic bonds of disaccharides. These enzymes include maltase, sucrase, lactase, and isomaltase that hydrolyze maltose, sucrose, lactose, and isomaltose, respectively.

The resulting monosaccharides proceed through the mucosal cells lining the small intestine through the capillary of the villus, pass into the bloodstream, and are transported to the liver by the portal vein. Glucose and galactose are absorbed quickly by active transport. The absorption of fructose occurs much more slowly and is less understood than the absorption of either glucose or galactose.[26]

Shi and colleagues suggest that fructose is absorbed through both facilitated diffusion and a paracellular pathway.[27] A review of four studies concerning fructose absorption and concluded that healthy subjects absorb much less fructose than glucose or sucrose.[28] Malabsorption of fructose is a common occurrence. Of the four studies reviewed, 58-80% of subjects participating in each study were unable to absorb completely doses of fructose in the range of 20-50 grams (g) (a level easily found when consuming products sweetened with HFCS).[28] The delay and malabsorption of fructose results in a much lower blood glucose response after ingesting fructose.[29] Fructose absorption is enhanced significantly in the presence of glucose, with the highest efficiency occurring with equal amounts of fructose and glucose.[28]

Once the monosaccharides have been transported to the liver, glucose is transported to the tissues. Fructose and galactose are converted to glucose derivatives, and delivered to the tissues. The use of glucose is dependent on the body's energy demands.[19,20,26]

Carbohydrates that reach the large intestine are resistant starches and indigestible fibers. Resistant starch is defined as the sum of starch and starch degradation products not absorbed in the small intestine of healthy adult.[30] Some resistant starch may be

hydrolyzed in the stomach, but most escape digestion in the upper intestinal tract. Both physical and extrinsic factors affect the digestibility of starch.

Physical characteristics of resistant starch include: (1) intact granules, partly milled, whole grains or seeds that are inaccessible to digestive enzymes; (2) B-type starch granules present in uncooked potatoes and green bananas; and (3) retrograded amylose occurring in processed foods.[31] Other factors contributing to resistance may include the formation of amylose-lipid complexes, possible retrogradation of amylopectin, and creation of enzyme resistant glycosidic bonds by dry heating at high temperatures.[30] Extrinsic factors contributing to resistance include the extent of chewing, mouth to terminal ileum transit time, amylase concentration in the gut, amount of starch present, and food components that might retard starch hydrolysis.[30]

Resistant starch and fiber provide substrates for bacterial fermentation in the colon. Fermentation results in gas and short-chain fatty acid formation. The short-chain fatty acids provide an energy source and may be a protective factor against colorectal cancer.[31] Short-chain fatty acids are also transported to the liver, where they inhibit hydroxymethyl glutaryl-coenzyme A reductase. This results in decreased serum cholesterol levels and, subsequently, a decreased risk for coronary heart disease.

Glucose in the Body

The primary function of glucose is to provide energy for cells. Glucose enters cells by active transport through a process that is dependent on the hormone insulin. Glucose is then oxidized through biochemical pathways to produce energy.[20]

If the body's supply of glucose exceeds its energy needs, the liver, and other tissues that are capable, reassemble glucose units into glycogen or fat for energy storage

through processes called glycogenesis and lipogenesis, respectively. Through the process of glycogenolysis, glycogen is eventually broken into glucose units to provide energy when needed. Because glycogen is a bulky molecule, the body stores are limited. Glycogen can provide the body with energy for a few hours, long enough to sustain exercise and normal daily activities until more carbohydrates are eaten.[20]

When glycogen stores are full and glucose is in excess, the liver breaks glucose into smaller fragments and puts them together as fatty acids through lipogenesis. The fatty acids are transported to adipose tissue, which has unlimited storage capacity, and put into long-term energy storage. This biochemical process is metabolically expensive since it requires more energy than it produces. Dietary fat is more efficiently converted to adipose tissue.[20]

The brain and red blood cells are almost exclusively reliant upon glucose for energy, so a constant supply of glucose must be available for these tissues. When glycogen stores are depleted and not replenished by eating carbohydrates, the liver breaks down body protein to make glucose through the process of gluconeogenesis, or generation of glucose from non-carbohydrate precursors. Gluconeogenesis from protein sources converts amino acids to glucose.[26]

In between meals, the postabsorptive state, the liver, muscle, and adipose tissue will use stored fat as an energy source. In the process of lipolysis, triglycerides (TG) in adipose tissue are converted to glycerol and fatty acids. Acetic acid molecules are successively split from the end of fatty acids in beta-oxidation.[32] Some carbohydrate must be present for the complete oxidation of fatty acids. If insufficient glucose is present, the acetic acid molecules combine in pairs to form ketone bodies. Ketones travel

in the blood and can be used for energy by cells, such as those in the heart, muscles, and kidneys.[32] Fats are unable to pass the blood-brain barrier, but lipolysis releases glycerol, which is allowed passage to the brain where it can be used for energy.

Ketones are produced whenever fat mobilization occurs; during moderate exercise, an overnight fast, and during periods of low food intake.[32] Ketones not used for fuel can build up in the blood, causing ketosis. If fluid intake is too low to produce enough urine to excrete ketones or if ketone production is high, ketosis will result.[33] Circulating ketones in the "fed state" are approximately 0.1 millimoles (mM). Ketone levels do not normally rise above 6 to 8 mM.[34] In severe diabetes, however, ketone levels may rise to 12 to 14 mM, due to the insufficient use of insulin.[34] The blood ketone body concentration of an individual on a high-protein, carbohydrate-free diet is typically 2 to 3 mM.[34]

Ketosis increases the acidity of the blood and can result in coma and death[33]; this is usually observed in individuals with diabetes. Symptoms associated with mild ketosis include lethargy, headache, and loss of appetite.[32] Fifty to 100 grams of carbohydrate must be consumed daily to spare body protein and prevent ketosis.[20,26]

The ketogenic state is protein-sparing and for that reason is crucial for surviving periods of starvation. During prolonged fasting, the body protein is initially metabolized to provide glucose for the brain and nervous system. After a number of days, some of the brain and nervous system adapt to the use of ketone bodies for energy; and thus, survival is possible.[32]

Blood Glucose Homeostasis

In the postabsorptive state, the plasma glucose appearance rate is about 8 to 10 g per hour.[34] The free glucose pool of the body, about 16 g, is replaced every 2 hours. Hormonal control maintains blood glucose (BG) homeostasis. Normal fasting levels of blood glucose in non-pregnant adults range from 70 to 110 milligrams per deciliter (mg/dL) and should not exceed 140 mg/dL two hours postprandial.[35] Derangement of BG adversely affects normal bodily function. Hypoglycemia can result in coma and death. Conditions associated with uncontrolled BG levels are described in the section "Conditions of Impaired Glucose Metabolism" below.

As carbohydrates are digested and absorbed, BG levels rise. This triggers the pancreas to produce the endocrine hormone insulin from the beta cells of the islets of Langerhans. Insulin is secreted in direct proportion to the BG level. Insulin binds to its corresponding receptor on the cell, where it facilitates the entry of glucose into the cell. Insulin increases the rate of glucose utilization for oxidation, glycogenesis, and lipogenesis. As glucose is used for these metabolic activities, blood glucose levels drop. Insulin secretion ceases when blood glucose levels reach a mean value of 83 mg/dL.[36]

Approximately 3-4 hours after a meal, catabolic processes are used to maintain BG levels within normal limits. Glucagon is the principal catabolic hormone that antagonizes insulin. Glucagon is produced and secreted from the alpha cells of the islets of Langerhans in the pancreas when blood glucose levels are approximately 68 mg/dL.[36] Glucagon acts to increase BG levels by increasing glycogenolysis and gluconeogenesis. Glucagon is the primary hormone that protects against hypoglycemia.

Other catabolic hormones that raise BG levels are epinephrine, glucocorticoids, thyroxine, and growth hormone. These are not the principal hormones responsible for BG homeostasis. They hormones are secreted during periods of metabolic stress, when energy requirements are increased. Epinephrine, the "stress hormone," is produced by the adrenal medulla and is released in response to fear, anger, or anxiety. Epinephrine is secreted when glucose levels fall to approximately 69 mg/dL.[36] This triggers glycogenolysis, thereby raising BG levels to provide extra energy in times of stress.[37]

Glucocorticoids, steroid hormones, are produced by the adrenal cortex and stimulate gluconeogenesis to make more glucose. Their secretion inhibits oxidation of glucose in certain tissues. Cortisol (hydrocortisone) is a glucocorticoid secreted in response to tissue damage. Cortisol is released when BG levels are approximately 58 mg/dL.[36]

Thyroxine, a thyroid hormone, stimulates carbohydrate metabolism. Thyroxine enhances gastrointestinal absorption of glucose, cellular uptake of glucose, glycolysis, and gluconeogenesis.[38] Thyroxine secretion is stimulated by cold weather. The metabolism of carbohydrates releases heat, thereby lowering BG levels.[23] Thyroxine promotes normal metabolism of carbohydrates. Hyperthyroidism over-stimulates glycogenolysis and gluconeogenesis and results in hyperglycemia. Severe hyperthyroidism impairs glucose tolerance.[39]

Growth hormone, or somatotropin, is secreted by the anterior pituitary and promotes tissue and organ growth. Growth hormone is secreted when BG levels drop to 66 mg/dL.[36] It acts to increase blood glucose levels. Upon its secretion, protein synthesis

is increased in relation to breakdown; and adipose tissue is mobilized for energy use, thereby inhibiting the cellular oxidation of glucose.[23]

Conditions of Impaired Glucose Metabolism

Hypoglycemia

Hypoglycemia is a clinical syndrome, in which low levels of plasma glucose eventually lead to neuroglycopenia.[40] Symptoms of hypoglycemia can occur when BG levels drop to approximately 60 mg/dL. Impaired brain function can occur at 50 mg/dL; however, major central nervous system (CNS) dysfunction may not occur until BG concentrations fall to 20 mg/dL. Hypoglycemic symptoms differ among individuals but are usually constant within the same person.[40]

Symptoms of hypoglycemia, discussed below, are classified into two groups: those related to the autonomic nervous system (ANS) and those that are related to CNS function.[40] Symptoms related to the ANS are sweating, tremors, tachycardia, anxiety, and hunger. Central nervous system symptoms include dizziness, headache, blurred vision, disorientation, loss of fine motor skill, confusion, abnormal behavior, convulsions, loss of consciousness, seizure, coma, and death.[37]

Disturbance in CNS function is due to an insufficient supply of glucose to the brain. As stated previously, the brain can use glycerol produced from fatty acid breakdown for energy. However, ketosis is not well established for up to 16 hours after glycogen stores have been depleted. Therefore, ketogenesis is not protective against acute hypoglycemia.[41]

The major causes of hypoglycemia are the underproduction or overutilization of glucose. Hormone deficiencies (*e.g.* glucagon deficiency); enzyme defects (in glycolytic

pathway); substrate deficiency; acquired liver disease; and drugs, such as injected insulin, sulfonylureas, alcohol, propranolol, and salicylates, can cause the underproduction of glucose. The overutilization of glucose occurs primarily through the over-treatment of hyperglycemia.[41] Either insulin or oral hypoglycemic agents, intended to lower BG levels quickly to within normal limits, are administered in excessive amounts. The insulin rapidly delivers glucose to the cells, which produces acute reactive hypoglycemia.[41]

Diagnosis of hypoglycemia is best confirmed with a documented low BG level, the presence of symptoms, and relief of symptoms after the rise of BG. If it is not practical to determine a BG level at the time when symptoms occur, it is appropriate to treat on the basis of symptoms alone. The risk of untreated hypoglycemia outweighs the risk of transient hyperglycemia. Eating carbohydrates, especially in the form of a glucose tablet, is usually an effective treatment for hypoglycemia. A 20 g dose of glucose from CHO-containing juices, soft drinks, candy, cracker, or a meal is recommended.[37] However, if there is a loss of consciousness, intravenous glucose, glucagon, or epinephrine is required.[37]

Hyperglycemia

Hyperglycemia is characterized by a fasting BG level of >110 mg/dL or BG of >140 mg/dL 2 hours postprandial.[35] Chronic hyperglycemia leads to excessive glycosylation, or binding of glucose to proteins, causing structural and functional changes of body proteins affecting micro- and macrovascular systems. Microvascular complications include retinopathy, nephropathy, and neuropathy.[42] Macrovascular complications include coronary artery disease (CAD), cerebrovascular disease (CVD),

and peripheral vascular disease.[43] Hyperglycemia may be the result of insufficient or ineffective insulin, or an increased hepatic production of glucose.

Clinical syndromes associated with glucose intolerance are type 1 diabetes mellitus (DM), type 2 DM, impaired glucose tolerance (IGT), gestational diabetes mellitus (GDM), and syndrome X. Diagnosis of these conditions is determined by assessment of BG levels. Three diagnostic tests can be performed: a fasting plasma glucose test, casual plasma glucose test, and an oral glucose tolerance test. Fasting plasma glucose (FPG) is determined on a blood sample taken after an overnight (10-12 hour) fast. A casual plasma glucose (CPG) value is obtained from a blood sample taken without regard to timing of meals. In an oral glucose tolerance test (OGTT), plasma glucose level is measured 2 hours after administration of 75 grams (g) of glucose. An OGTT for pregnant women involves measuring plasma glucose level 3 hours after administration of 100g of glucose. Table 1 gives the diagnostic criteria for the corresponding condition.[35]

Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose and either insufficient or ineffective insulin. There are 2 principal types of DM, type 1 and type 2. Approximately 16 million Americans, 6% of the population, have DM; there are 10.3 diagnosed and 5.4 undiagnosed cases.[44] Diabetes is increasingly prevalent in minorities, elderly, and women.[45]

The prevalence of DM also increases with age. In the elderly population, age 65 years or older, 6.3 million people (18.4%) have DM. Of all people 20-65 years old, 15.6

million people, (8.2%) have DM. Only 0.16%, 123,000 people, of people under the age of 20 have DM.[44]

Table 1. Diagnosis of Impaired Glucose Metabolism

Condition	Criteria
Diabetes Mellitus	- FPG $\geq$ 126 mg/dL on 2 occasions - CPG $\geq$ 200 mg/dL - FPG $\geq$ 126 mg/dL and 2 OGTTs including 2-hour PG $\geq$ 200 mg/dL and 1 intervening value $\geq$ 200 mg/dL
Impaired Glucose Tolerance	- FPG > 110 mg/dL, but < 126 mg/dL and OGTT including 2-hour PG $\geq$ 140 mg/dL with 1 intervening value > 200 mg/dL
Gestational Diabetes Mellitus	-If 2 of the following values occur: - FPG $\geq$ 105 mg/dL - 1-hour PG after OGTT $\geq$ 190 mg/dL - 2-hour PG after OGTT $\geq$ 165 mg/dL - 3-hour PG after OGTT $\geq$ 145 mg/dL
Normal	- FPG 70-110 mg/dL - 2-hour PG after OGTT < 140 mg/dL with intervening PG < 200 mg/dL

Adapted from *Krause's Food, Nutrition, & Diet Thereapy 10th ed.*[19]

In the United States, diabetes is more prevalent among minorities than among the majority European-American population. Among European-Americans, 11.3 million people (7.8%) have DM. Of African-Americans, 2.3 million people (10.8%) have DM. Among Mexican-Americans, 1.2 million people (10.6%) have DM. Of all Native-Americans and Alaska Natives, 9% have DM.[44]

Type 1 Diabetes Mellitus

Type 1 DM, also referred to as juvenile-onset diabetes, insulin dependent diabetes (IDDM), or ketosis-prone diabetes accounts for 5-10% of all cases.[44] It is more common in youth, but it can occur at any age. Peak onset in the U.S. occurs in adolescence.[46] The

condition is the result of an autoimmune disease, which targets the beta cells of the islets of Langerhans in the pancreas. Beta cells are damaged and can no longer produce insulin.[47] The lack of insulin leads to hyperglycemia, and susceptibility to ketosis. In fact, at the point of diagnosis, many individuals with type 1 DM are in diabetic ketoacidosis.[46]

Type 1 DM must be treated with insulin through injections or an insulin pump that delivers insulin to the body in a manner similar to the pancreas. Insulin must be injected since as a protein it would be digested if taken by mouth and therefore not delivered to cells to perform its intended function. Cells respond normally to injected insulin. Type 1 DM is also treated through diet and exercise. Tight control of BG within normal limits, by intensive insulin therapy, can delay the onset and slow the progression of complications in type 1 DM.[48]

Type 2 Diabetes Mellitus

Type 2 is the more common form of DM, accounting for 90-95% of all cases.[44] It can occur at any age, but onset is more common in adults over 40 years of age. However, the incidence of type 2 DM is increasing in adolescents—maturity onset-type diabetes of youth.[49] This increase corresponds with the increase in childhood obesity.[49] Exact etiology of type 2 DM is unknown, but it involves both genetic and environmental factors.[35]

Type 2 DM is characterized by ineffective use of glucose due to either a lack of insulin or insulin resistance. Fasting serum insulin concentrations may be low, normal, or high.[35] Insulin resistance is caused by damaged insulin receptors on the cell membrane

as a result of genetics or exposure to pathogens, or both.[50] If pancreatic beta cell function is normal, insulin resistance will lead to hyperinsulinemia.[50]

Obesity is associated with the prevalence of type 2 DM. Insulin receptors are reduced in number or function as body fat increases.[35] Hyperglycemia stimulates the pancreas to make insulin and eventually exhausts this ability, leading to insufficient insulin supply. Weight loss of 5-10% of total body weight in obese individuals with diabetes improves glycemic control and insulin sensitivity.[51]

Type 2 DM is also referred to as adult-onset diabetes, non-insulin dependent diabetes (NIDDM), or ketosis resistant diabetes.[19] It is treated with diet, exercise, oral hypoglycemic agents, and sometimes with insulin injections.[52] Risk factors associated with the development of type 2 DM are age, obesity, a family history of DM, history of gestational DM, physical inactivity, and race.[35]

Impaired Glucose Tolerance

Impaired glucose tolerance (IGT) is characterized by abnormality in BG levels between normal and diabetic levels, for instance, a fasting blood glucose level between 110 and 126 mg/dL.[35] It is not clinically significant; it is, however, a risk factor for developing diabetes and cardiovascular disease.[35]

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is characterized by impaired glucose control during pregnancy; usually this disappears after delivery.[53] It affects 4% of all pregnant women or approximately 135,000 women annually.[54] Fetal morbidity may be increased; therefore, it is crucial to diagnose and treat GDM. It is usually controlled with diet, however insulin injections may be required. GDM is a significant risk factor for

developing type 2 DM.[119] Obesity, personal history of GDM, and family history of diabetes are each risk factors for GDM. All pregnant women should be tested at 24-28 weeks of gestation.[35]

Syndrome X

Syndrome X is a cluster of conditions including hyperinsulemia, obesity with an android pattern of distribution, some degree of CHO intolerance, hypertension (HTN), and an abnormal blood lipid pattern of increased triglycerides (TG) and decreased level of high-density lipoproteins (HDL).[55] All of these conditions are atherogenic risk factors. Syndrome X is also known as the insulin resistance syndrome and the cardiovascular dysmetabolic syndrome.[55]

Dyslipidemia, insulin resistance, obesity, and elevation of blood pressure are referred to as the "deadly quartet" of Syndrome X, due to the magnitude of their combined risk factors for coronary heart disease (CHD). Other features of Syndrome X include small, easily oxidized low-density lipoprotein particles (LDL), an increase in blood coagulation, and an increase in serum uric acid levels. Central to Syndrome X is insulin resistance. Insulin resistance occurs when cells become insensitive to the secretion of insulin resulting in the decreased uptake of glucose by cells. When insulin resistance begins, pancreatic beta cells increase insulin secretion to help achieve BG levels that are within normal limits. Cells are unresponsive to this increase in insulin; therefore, hyperinsulemia and hyperglycemia result.[55]

Hyperinsulemia affects muscle, liver, and adipose tissue.[55] In muscle tissue, hyperinsulemia disturbs carbohydrate metabolism. Insulin binds to cell receptors on muscle tissue, which results in the translocation of glucose for uptake, phosphorylation,

and storage as glycogen. Muscle glycogen syntheses decreases.[56] Hyperinsulemia triggers the liver to increase uptake of substrates for gluconeogenesis.[57] In adipose tissue, insulin resistance leads to an increase in the concentration of free fatty acids; thus, increasing the risk for coronary heart disease (CHD). Insulin resistance can also affect kidney function. Hyperinsulemia is linked to increased sodium retention and decreased uric acid clearance. This, in turn, results in hypertension and increased uric acid concentrations.[55]

Both dietary and lifestyle factors influence the development of Syndrome X. Animal studies suggest that a high-fat, high-refined carbohydrate diet can trigger insulin resistance.[58] In other animal studies, substituting dietary CHO for saturated fat caused an increase in plasma TG and a decrease in HDL, which are both risk factors for CHD.[59] This suggests that a high-CHO diet should be avoided in insulin-resistant, Syndrome X individuals. Replacing saturated fat with monounsaturated and polyunsaturated fat instead of CHO may be more beneficial to these individuals; however, long-term low-CHO diets have not been proven to be beneficial.[55] The consumption of a low-glycemic index (GI), high-fiber diet has been shown to improve insulin sensitivity in subjects with or at risk for CHD.[60,61]

Lifestyle factors influencing the development of Syndrome X include body weight and physical activity.[56,120] Physical activity can prevent insulin resistance and protect against Syndrome X.[55] Regular exercise increases insulin sensitivity, increases insulin action, and decreases plasma insulin concentrations.[62] Obesity is also related to the development of insulin resistance. Achieving and maintaining a healthy body weight may prevent the development of insulin resistance and Syndrome X.[56]

Management of Impaired Glucose Metabolism Disorders

Assessment of Glycemia

Glycated protein testing, glycated serum protein testing, and self-monitoring of BG levels can all be used to assess glycemic control.[63] Each of these tests is important in the diagnosis and management of DM. Glycated protein testing is an indicator of glycemic control over time. When proteins, such as hemoglobin (Hb), are exposed to glucose, it binds to that protein. The rate of formation of glycated hemoglobin is directly proportional to the glucose concentration over a period of time from 2-3 months.[64] Therefore, glycated Hb tests are a valuable measure of glycemic control. In non-diabetic individuals, glycated Hb should be less than 6%. In diabetic individuals, the goal for glycated Hb is $\leq 7\%$.[63]

Glycated serum albumin tests can be used to evaluate glycemic control over a much shorter time period—1-2 weeks[64]; however, glycated albumin tests are not performed routinely. Long-term and immediate indicators are more valuable in the overall assessment of glycemic control than are intermediate indicators.[63]

The most immediate indicator of glycemia is obtained through self-monitoring of blood glucose (SMBG). It is extremely important for diabetics to self-monitor their BG to identify and avoid potential fluctuations in levels. Hyper- and hypoglycemic states can be identified through SMBG, which allows the individual to plan meals, exercise, or administrate medications to facilitate glycemic control. Diabetics may monitor glucose levels up to seven times per day: upon waking, before each meal, 1-2 hours after meals, and at bedtime. It is recommended that individuals with type 1 DM monitor 4 times per

day: before each meal and at bedtime. Individuals with type 2 DM are advised to monitor 1-4 times per day, 3-4 days per week.[63]

Lifestyle Modification for Improved Glycemic Control

The most important factor for glycemic control in DM individuals is diet. The importance of diet in the management of diabetes has been recognized for almost a century.[65] Prior to 1921, a starvation diet was advised. In 1921, the recommended diet was low-carbohydrate and high-fat. It was recommended that 70% of total energy consumed come from fat, 20% from carbohydrates and 10% from protein. In 1950, the recommended diet was composed of 40% total energy from fat, 40% from carbohydrate, and 20% from protein. In 1971 recommendations included 35% total energy from fat, 45% from carbohydrate, and 20% from protein. Finally, in 1986 a diet much like the one recommended today came into existence, providing less than 30% total energy from fat, up to 60% from carbohydrate and 10-20% from protein. Thus, the diet recommended for individuals with diabetes evolved from high-fat to high-carbohydrate.[65] The type of carbohydrate, in the recommendation of a high-carbohydrate diet, is not specified.

The American Diabetes Association currently recommends a diet adequate in energy, carbohydrate, and fat (< 10% of total energy from saturated fat or polyunsaturated fat) based on usual intakes and the achievement and maintenance of a healthy weight.[66] The amount of carbohydrate each day should be consistent and spread evenly throughout the day.[66] Protein should supply 10-20% of total energy with an increase during periods of physiological stress. Fiber requirements are the same as for the general population, 20-35g per day. Soluble fiber slows absorption of glucose in the small intestine, but only to a small extent.[66] Both soluble and insoluble fibers contribute

to satiety. Individuals with well-controlled diabetes may use alcohol occasionally, in moderation. Alcohol consumption, however, is contraindicated for diabetics treated with oral hypoglycemic agents. These guidelines and precautions must be followed by diabetics to minimize complications. Diabetics' micronutrient needs are the same as the general population; and diabetics are advised to obtain them through a balanced diet.[66]

Diabetics are encouraged to exercise. Regular exercise can improve insulin sensitivity and reduce risk factors for developing vascular complications of diabetes. Blood glucose levels should be monitored before, during, and after exercise in order to establish a regime that supports glycemic control.[67]

Medication

Individuals with type 1 DM must take exogenous insulin. Insulin may also be administered to individuals with type 2 and GDM. The insulin regimen must be tailored to adjust for the effects of growth, illness, stress, food, and physical activity. Each of these components alters insulin requirements.[52]

Individuals with type 2 DM may use oral hypoglycemic agents to achieve glycemic control. Five classes of drugs act to lower blood glucose levels.[68]
(1) Sulfonylureas are an older class of hypoglycemic agents. They stimulate insulin secretion, but may exhaust this capability and lead to weight gain and hypoglycemia. (2) Meglitinide may be taken before meals to improve insulin secretion in response to glucose. (3) Biguanides, such as the widely prescribed Glucophage, suppress gluconeogenesis and lower insulin resistance. (4) Thiazolidinediones, such as Rezulin, decrease resistance to injected or endogenous insulin and enhance insulin action.

(5) Alpha-glucosidase inhibitors, Precose and Glyset, inhibit digestive enzymes required to digest starch, dextrins, maltose, and sucrose. Some type 2 diabetics may benefit from a combination of these oral medications or an insulin-oral medication combination.

Overweight and Obesity

It is estimated that 97 million adults in the United States (U.S.) are overweight or obese.[69] In the past decade, the incidence of obesity in the U.S. increased in every state, in both genders, and across all age groups, races, educational levels.[70] Based on the Third National Health and Nutrition Examination Survey (NHANES III), 63% of men and 55% of women aged 25 years or older were overweight or obese.[71] Overweight and obese individuals have an increased risk of morbidity from hypertension; type 2 DM; stroke; gallbladder disease; osteoarthritis; sleep apnea; respiratory problems; and endometrial, breast, prostate, and colon cancers.[72]

Calculation of body mass index (BMI) is used to determine the presence of overweight or obesity. The BMI is calculated as: BMI = weight [in kilograms (kg)] / height [in meters squared (m^2)]. Overweight is defined as a BMI from 25 to 29.9 kg/m^2. Obesity is defined as a BMI $\geq$ 30 kg/m^2.[72] A person's BMI is not an indicator of body composition; and it does not make allowances for age, gender, or frame size. This compromises the ability of the BMI to assess accurately a person as overweight or obese.[72]

Energy balance is the major determinant of weight status. A positive energy balance, that is more energy is consumed than expended, results in weight gain. Negative energy balance, that is more energy is expended than consumed, leads to weight loss. Obesity develops gradually over a period of several years. This could be attributed to a

small positive energy balance over a prolonged period of time or to episodes of a large positive energy balance.[73] Recent literature reviews suggest that the major factor related to the increase in overweight and obesity is the decline in physical activity rather than an increase in energy consumption.[73,74]

Weight loss reduces the risk factors for chronic disease. Weight loss may help control or prevent the development of diseases, such as DM and CHD, related to overweight and obesity.[72] Weight loss can be achieved through diet, physical activity, behavior modification, pharmacotherapy, surgery, or a combination of these therapies. Weight loss is recommended for overweight and obese individuals to help reduce blood pressure, total cholesterol, LDL, TG, and BG, and increase HDL levels.[72]

The Glycemic Index

The GI ranks foods according to their affect on BG levels after eating.[6] The GI for a food is derived by expressing the response to a test food as a percentage of the response of a reference food, white bread or glucose.[6] In order to compare the responses, the area under the timed blood glucose response curve is calculated. Methods of calculating the area under the response curve are explained below in the "Area Under the Curve" section.

Glycemic Index Methodology

Portions of test foods and a reference food containing 50 g available CHO are fed to subjects on separate occasions after an overnight fast. Capillary finger-prick blood samples are taken from non-diabetic subjects fasting and at 15, 30, 45, 60, 90, and 120 minutes after the subjects begin to eat the food tested, and for diabetic subjects fasting and at 30-minute intervals for 3 hours. For diabetic subjects, the prescribed dose of

insulin or oral hypoglycemic agent, if any, are taken after the fasting blood sample and 5-10 minutes before starting to eat the test meal. The area under the glycemic-response curve for each food is expressed as a percent of the mean response to the standard food within the same subject. The resulting values are averaged to obtain the GI value for the food.[10]

To reduce variability in computing the GI of a particular food, each subject should repeat the reference food at least three times.[10] When the mean of three white-bread response areas is used for calculations of GI, the mean, variability, and skewness of the resulting GI distribution are reduced.[10]

Area Under the Curve

The area under the blood glucose response curve (AUC) can be calculated by 3 methods[11]: total area, incremental area, or net glucose area. (1) The total AUC includes the complete area under the response curve down to a BG level of 0 mg/dL. (2) The incremental AUC includes the area under the response curve to the fasting level. In this method, the AUC could never be less than zero. (3) The net glucose area subtracts the area below the fasting level from the incremental area; thus, the net glucose area could possibly be less than zero. The GI is based on the incremental AUC.[11]

$$GI = \frac{\text{AUC for 50g carbohydrate from test food}}{\text{AUC for 50g carbohydrate from standard food}} \times 100$$

Figure 4 illustrates the calculation of the incremental AUC. The incremental AUC is the sum of the areas of the triangles and rectangles illustrated in Figure 4, calculated geometrically.[75]

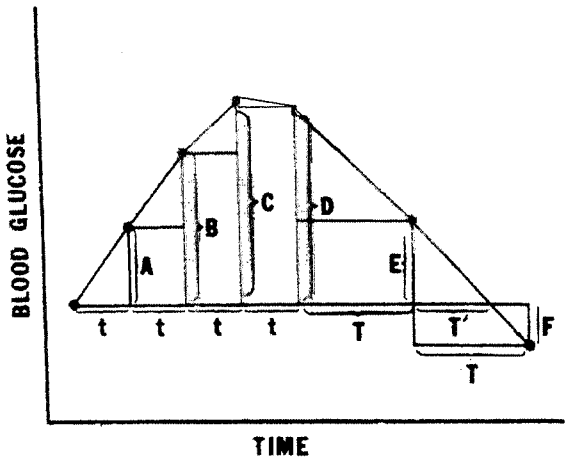


Figure 4. General blood glucose response curve to illustrate the method of calculation of the incremental AUC.[11]

During the development of the GI concept, in the 1980's, researchers proposed that the GI should fulfill four criteria in order to be of clinical utility: (1) consistency of values for the same food across space and time, (2) application in individual subjects, (3) application to mixed meals, and 4) demonstration of clinically significant therapeutic improvements by practical dietary changes.[76,77] Several clinical experiments and epidemiological studies have been performed to address these concerns. However, results of such investigations have produced conflicting results, as discussed in the next section.

GI of Individual Foods

The GI of a food is the mean value of a group of people; therefore, the GI for the same food may vary between groups. The usual number of people in a study to determine the GI of a food is 8-10. Some studies have shown that the GI of foods are not significantly different in different groups of people.[10,11,12] A range of GI values may exist for the same food. Table 2 compares the GI of foods from several different studies.[78]

Whole foods, such as grains and legumes, tend to have lower GI's than processed or refined foods. The GI of a food depends on the reference food to which it is compared, either white bread or glucose. Foods compared to white bread appear to have higher GI's than when compared to glucose.[78] Differences in the nutritional content of the white bread used in different studies could account for the variation in GI values. This leaves the GI highly susceptible to misinterpretation.

Table 2. GI values for common foods, expressed as a range. Adapted from Foster-Powell, 1995. GI ranges are taken from GI values of foods compared to a white bread standard.

Food	GI Range
Whole-meal flour	74-104
All-Bran, breakfast cereal	43-73
Sweet corn	69-89
Ice cream	51-114
Milk, full-fat	15-57
Banana	66-100
Kidney beans	33-66
Carrots	70-131
Baked Potato	80-158

Carbohydrates can be classified as low, medium, or high GI.[19] A low GI is <60. Low GI foods include fructose, apples, applesauce, kidney beans, lentils, peaches, plums, ice cream, milk, and peanuts. A medium GI is between 60 and 85. Medium GI foods include all-bran cereal, bananas, grapes, oatmeal, pasta, rice, whole-grain rye bread, baked beans, potato chips, macaroni, and peas. A high GI is over 85. High GI foods include glucose, sucrose, maple and corn syrups, honey, bagels, candy, corn flakes, potatoes, raisins, white bread, and carrots.[19]

Effects of Carbohydrate Structure on GI of Foods

As stated in the "Complex Carbohydrates" section, because amylopectin is a branched molecule, there are many sites vulnerable to enzymatic attack. Therefore, amylopectin is more rapidly degraded than is straight-chain amylose. Thus, foods with a higher amylose: amylopectin ratio are digested more slowly and have a lower GI.[79,80]

Intact grains retain the outer germ layer, which serves as a physical barrier to enzymatic digestion. Cooking whole grains is only partially effective in removing the germ layer. Thus, unmilled grains generally exert only slight effects on glucose levels, and usually have a lower GI.[79,80]

Conversely, finely milled grains contain little or no germ layer and are rapidly digested. Consequently, starch in solution, partially degraded starch, and finely milled grains cause glycemic and insulin responses similar to that produced by an equivalent amount of glucose. These foods tend to have higher GI's.[81]

Effects of Fiber on GI of Foods

In studies assessing the effect of fiber content on GI, total dietary fiber was found to have a significant inverse relationship to GI.[82,83] Soluble fiber was not significantly related to GI; whereas, cellulose and insoluble fiber were the best predictors of food GI.[83] Legumes have higher cellulose content and lower glycemic responses than cereals. The relationship between cellulose and GI may indicate that foods with low glycemic responses have strong cell walls containing large amounts of cellulose and hemicellulose.[82]

Effects of Protein and Fat on GI of Foods

In studies assessing the impact of protein and fat on the GI of foods have reported conflicting results. The presence of protein and fat in foods has shown a negligible[84,85] or a negative GI[6,75,83,86] effect on food. Jenkins found a significant negative correlation between protein and fat with GI.[6] Later, when proponents of the GI[85] were trying to prove the applicability of the GI to mixed meals, Jenkins found the addition of fat and protein to bread in the form of butter or skim milk cheese did not significantly reduce the overall glycemic response of the bread. Wolever found similar results in a study conducted two years later.[75]

More recent studies have also produced conflicting results. Trout found the presence of protein reduced the GI of foods.[83] Wolever, in another study, found that variation in protein and fat intake appeared to have a negligible effect on postprandial glucose and insulin.[84] Miller found some individual variation in the response to protein.[86] It was concluded, from Miller's study, that the effect of protein on glucose and insulin levels after a meal might depend on the individual's degree of insulin sensitivity.

Effect of Fructose on GI of Foods

Fructose produces a lower glycemic response than sucrose or glucose.[87,88] Therefore, fructose has a lower GI than either glucose or sucrose.[6] In a study assessing glycemic responses to fructose-sweetened cake and ice cream compared to sucrose sweetened counterparts, the fructose sweetened foods produced a "less-pronounced" glycemic response.[89] However, a later study investigated the glycemic effects of frozen desserts sweetened with HFCS and concluded that products made with HFCS are not

necessarily low GI. The authors attributed this to the fact that HFCS contain large amounts of both glucose and fructose.[90]

Glycemic Responsiveness of Individuals

Individuals share common mean GI values for different foods. Significant differences do exist among individuals for the incremental AUCs.[11,12] However, when expressed as the GI, there was no significant difference between subjects.[12] Existing literature has compared the glycemic responsiveness between genders, diabetics verses non-diabetics, and type 1 DM verses type 2 DM, as discussed below. However, possible differences depending on race, age, or body weight have not been adequately explored.

For individuals with type 2 DM who are of the same age, have had a diagnosis of diabetes for a similar amount of time, and have similar body weights, gender does not play an important role in glycemic responses.[91] Individuals with type 1 DM experience greater variation in glycemic response than those with type 2 DM. In a study assessing the glycemic effect of substituting white pea beans into a test meal in place of white bread, the glycemic response of type 2 diabetics was reduced by 50%. However, unlike the results with individuals with type 2 DM, no significant reduction in glycemic response was seen with individuals with type 1 DM.[85] Type 2 diabetics appear to be less variable in glycemic responsiveness from day to day than either non-diabetic or type 1 diabetics.[92]

One study demonstrated that young men had a higher glycemic response than young women to a mixed-protein-rich meal.[93] Differences may be due to higher BMI in men or by differences in hormonal status between men and women.[93] Gannon speculated that there might be possible link between BMI and glycemic responsiveness.

GI of Mixed Meals

The calculation of the GI of mixed meals is based upon the sum of the GI contributions of each carbohydrate component of the meal.[75] The CHO content of each food is determined. The proportion of CHO contributed by each food is then multiplied by its GI. The values are totaled to give the GI of the meal. The GI of a day of meals, the glycemic load, is derived by the same method of the meal GI.[12]

Studies have shown that the GI of a mixed meal can be predicted from the GI of the component CHO foods.[92,94,95] Chew found the GI of meals could be predicted form the GI of the component CHO foods in healthy subjects.[94] Collier showed similar results in individuals with type 2 DM.[95]

Other studies have not validated the method for deriving the meal GI. Coulston and co-workers[96] questioned the clinical utility of the GI reporting, "results are totally disparate from what would have been predicted by previously published values for the 'GI' of the four foods studied".[76,97] These studies assessed the glycemic response, not the GI; it was concluded that the use of the GI as an indicator of glycemic response is misleading. Since the GI is based on an average response, the GI does not allow for individual variation. Coulston tested 3 meals varying only in the type of CHO in non-diabetic and in type 2 diabetics.[97] The meals were expected to yield high, intermediate, and low glycemic responses based on published GI values for the CHO foods tested; however, responses did not vary significantly in either the non-diabetics or the type 1 diabetics.

Clinical Implications of the GI

The author of a recent review article concluded that a low-GI diet appears to have not only a therapeutic role, but also a potential for the prevention of chronic disease.[98] The GI was originally developed in an attempt to design a diet for diabetics that would produce minimal glycemic and insulemic responses in order to minimize the long-term complications of DM.[6] Since then, studies have been conducted in an effort to link DM,[7,8,13,14] as well as CHD[99,100] and obesity[55,101] to GI.

GI and Diabetes

A low GI diet is associated with improved glycemic control in diabetic subjects.[13,14,15] A low GI diet has also been associated with improvements in insulin sensitivity.[16] Proponents of the GI argue that all diabetics should eat a low GI diet.[17,102]

Data from the Health Professionals Follow-Up Study and the Nurses' Health Study, demonstrated a positive association between glycemic load and risk of type 2 DM even after adjustment for BMI, physical activity, family history of diabetes, and total energy intake. Results suggest that the diet with high glycemic load and low cereal fiber content are positively associated with risk of type 2.[7,8]

GI and Coronary Heart Disease

Food frequency questionnaire data from NHANES III were used to examine the relationships between GI and glycemic load and HDL levels. The findings suggested that high dietary GI and high glycemic load are associated with a lower concentration of plasma HDL.[99]

Dietary data from the 1986-1987 Survey of British Adults were analyzed to examine the relationship between serum total cholesterol, HDL, low-density lipoprotein

(LDL), and various other dietary characteristics, including the types of CHO, the GI, and fat intake. Data from this study demonstrated a significant negative relationship between HDL and the GI of the diet for both men and women.[100] No significant relationship was seen between total cholesterol or LDL concentration with any dietary CHO or fat constituent. The authors concluded the GI of the diet is a stronger predictor than total dietary fat intake is of HDL concentrations.[100]

Reduction in the mean GI of diets of twelve hyperlipidemic subjects resulted in a significant reduction in total cholesterol and LDL concentrations.[103] The authors concluded that this study indicates that dietary manipulation to reduce the GI of a diet, but without major alteration in the macronutrient or dietary fiber, content may result in decreases in serum cholesterol and TG levels.[103]

GI and Obesity

Satiety is the link between the GI and obesity.[55,101] It was speculated that low GI foods produce a higher level of satiety than high GI foods. The consumption of low GI foods leads to a feeling of fullness and a subsequent reduction in intake.[104,105,106] Leathwood and Pollet[106] found that the lower BG levels produced after meals with bean puree (a low GI food) were associated with a slower return of hunger as compared to meals with potatoes (a high GI food).

Lavin[104] found that the addition of guar gum to a glucose drink produced a lower glycemic response than that of the glucose drink alone. The reduction in glycemic response was associated with a reduction in hunger and desire to eat and an increase in fullness and satiety.[104] Similarly, Ludwig conducted a study in which twelve obese teenage boys ate three meals on separate occasions. The meals were a vegetable omelet

with fruit (low GI meal), steel-cut oatmeal (medium GI meal), and instant oatmeal (high GI meal). Voluntary energy intake after the high-GI meal was 53% greater than after the medium-GI meal and 81% greater than after the low-GI meal. The authors concluded that consumption of high-GI foods induces hormonal and metabolic changes that limit availability of metabolic fuels and lead to overeating in obese subjects.[105]

In contrast, a recent investigation conducted by Holt found that glycemic responses to bread were not significantly associated with satiety scores.[107] In that study, effects of isoenergetic portions of seven different breads on feelings of fullness and subsequent food intake were compared. The strongest predictor of the breads' satiety score was portion size and energy density, not the breads' glycemic response.

GI and Dietary Recommendations and Popular Diet Plans

Researchers differ in the emphasis of the therapeutic use of the GI. Wolever contends that the GI has clinical utility; however, the role of the GI in patient education is not known.[102] On the other hand, Brand-Miller argues that the GI should be integrated into diabetes education as a means of glycemic control for diabetics. Brand-Miller stated that diabetic diets "...must be based on sound scientific evidence, not on opinion or tradition."[17] However, the GI is not recommended by the American Diabetes Association as a form of diet therapy.[66] Brand-Miller says this criticism reflects an "American bias."[108] She says, "...in Australia, South Africa, and parts of Europe, GI tables are used to fashion healthier diets."[108] The link between GI and reduced appetite and weight loss has lead to the development of fad diets that include GI principles. Such diet books include *Sugar Busters,*[109] *The Zone,*[110] and *The Glucose Revolution.*[111]

Sugar Busters

Sugar Busters[109] is based on the diet philosophy that sugar is toxic to the body and causes release of insulin, which promotes fat storage. Foods allowed on *Sugar Busters'* diet plan include protein foods like eggs, meat, milk; fat, for example, canola oil, olive oil; low GI foods; and alcohol in moderation, specifically red wine. Foods to be avoided include potatoes, white rice, corn, carrots, beets, white bread, and all refined white flour and sugar products. Eating fruit with a meal is discouraged. The diet is composed of 27% protein, 52% carbohydrate, and 21% fat. The diet composition seems reasonable, but when considering the exclusion of many CHO foods, the diet appears difficult to comply with over a prolonged period of time.

The Zone

The Zone[110] is based on the diet philosophy that eating the right combination of foods leads to a metabolic state at which body functions at peak performance, leading to decreased hunger, weight loss, and increased energy. *The Zone*'s diet plan prescribes protein, fat, and carbohydrates to be eaten in exact proportions. Low GI foods are recommended. Alcohol is allowed in moderation. The diet is composed of 34% protein, 36% carbohydrates, 29% fat, and 1% alcohol. A large quantity of vegetables would need to be consumed in order to proportion the meal as suggested, making compliance difficult. The diet also relies on the dieter's ability to estimate equal proportions of food, which leads to error in diet proportioning.

The Glucose Revolution

The Glucose Revolution[111] is based on the philosophy that the modern diet is too high in saturated fat and fast-release carbohydrates; low GI diets can prevent DM and

CHD, control established DM, help people lose weight, lower blood lipid levels, and improve insulin sensitivity; and low GI foods can reduce the GI of the meal as a whole. The authors advise the increased intake of low GI foods. No foods are intentionally excluded from the diet on the basis that a high GI food combined with a low GI food produces a meal with an intermediate GI. Advice from the authors is confusing. The authors maintain that, "Potatoes with a high GI can still be included." Yet, in the sample diets given by the authors, potatoes are replaced with pasta. Similarly, carrots, a high GI but highly nutritious vegetable are not included in the sample low GI diet. Diet compliance would be difficult due to the confusing advice and the fact that commonly consumed foods such as potatoes and white bread are not recommended.

CHAPTER 3

SUBJECTS AND METHODS

This study was approved on 2/20/01 by the Institutional Review Board by Louisiana State University Agricultural Center (LSU) (H-01-02) (Appendix A).

Subjects

To recruit volunteers, flyers and in-class announcements within the LSU-School of Human Ecology were used. Inclusion criteria were that subjects be without diagnosed glucose metabolism impairment, gluten sensitivity, or wheat allergy, not pregnant, without chronic illness, and be either European-American or African-American. The final study population consisted of a convenience sample (n=10). Written informed consent was required from each subject prior to participation in the study (Appendix B). Subjects received an incentive following completion of the study.

Methods

Study Design

To determine intra- and inter-person variability in glycemic responsiveness, subjects consumed three different doses of carbohydrate in the form of white bread. Each dose was tested in triplicate. Subjects were required to complete a total of nine separate glycemic response tests, each on a separate day. Subjects were instructed to fast 12 hours prior to testing and to begin testing periods at consistent times on each separate day. Glycemic tests are explained below in the "Glycemic Response Tests" section.

Subject Information

Subjects completed a "Subject Information Form" (Appendix C) to determine contact information; demographics including age, height, and race; as well as information required for payment of the incentive. Weight and percent body fat were measured using a Tanita Body Fat Monitor/Scale, Model TBF-521. Body mass index (BMI) was calculated using measured weight and self-reported height in the formula:[72]

$$\text{Body Mass Index} = \frac{\text{Weight in kilograms (kg)}}{\text{Height in meters (m)}^2}$$

Finger-Stick Blood Samples

Blood samples were obtained using a finger-stick method in which a lancet device (Accu-Chek™ Softclix®) was used to prick the skin on the tip of a finger to draw a drop of blood. The blood was then placed in a capillary action test strip (Accu-Chek™ Comfort Curve®); and blood glucose levels were determined using a glucometer (Accu-Check™ Advantage®). Subjects performed their own finger-sticks. Biohazardous materials were disposed of according to LSU policy.

Glycemic Response Tests

To assess the subjects' blood glucose response to white bread, subjects consumed a pre-measured portion of bread after a 12-hour fast. The bread was eaten within approximately 15 minutes and finger-stick blood samples were taken at the fasting level (baseline) and 15, 30, 45, 60, 90, and 120 minutes post baseline. Four different doses of carbohydrate were tested in triplicate: 25 grams (g) (54g white bread [enriched white sandwich bread (Dixie Darling, Velva® Sandwich; Winn Dixie)]) (n=10), 50g (108g white bread) (n=10), 75g (162g white bread) (n=10), and 100g (216g white bread) (n=3). In addition to the standard 7 finger sticks, samples were also taken at 150, 180, and 210

minutes after ingesting the bread for the 100g CHO treatment. Each dose was tested on 3 different days. The student investigator was available during testing periods to answer questions or discuss concerns of study participants.

Subjects were allowed to drink water during the test period, but were asked to drink approximately the same amount of water during each test period; however, the amount of water consumed was not measured. After each test was completed, subjects were offered breakfast foods including fruit juice, yogurt, and cereal bars.

Statistical Analyses

Coefficient of Variation (CV) was determined for an assay (2 subjects; 10 replicates) to test the inter-reliability of the glucometer and test strips. CV was also determined for fasting blood glucose levels (10 subjects; 9 or 12 different days). The incremental area under the timed glucose response curve (AUC) was calculated for each subject for each test according to Wolever and co-workers[10] as described in The Review of Literature. Analysis of variance (ANOVA) was used to compare the calculated AUC's and fasting blood glucose (FBG) levels (n=10).

In performing statistical analyses on the AUC's, the calculated AUC for each subject was defined as the dependent variable. Likewise, in performing statistical analysis on the FBG levels, the dependent variable was the measured fasting blood glucose for each subject. The subject number was the independent variable in analyses of both the AUC's and FBG's. Intra-person variability was assessed by standard deviations (SD).

Linear regression analysis was performed for each subject to determine if there was a significant dose response, with the AUC as the dependent variable and the

treatment number as the independent variable. All statistical analyses were performed using SPSS statistical software. Data are presented as mean $\pm$ SD.

CHAPTER 4

RESULTS

Demographics and Anthropometrics

Ten subjects completed the study. Table 3 summarizes subject characteristics.

Table 3. Subject characteristics, including age, gender, race, weight, body mass index, and percent body fat.

Subject	Age (years)	Gender	Race*	Weight (kg)	Body Mass Index	Percent Body Fat
1	50	Female	EA	87	28	43
2	50	Female	EA	68	22	37
3	23	Female	EA	70	27	37
4	23	Female	AA	80	32	43
5	21	Female	AA	56	20	24
6	22	Male	AA	75	22	18
7	31	Female	AA	86	30	40
8	27	Male	EA	108	33	30
9	38	Female	EA	54	24	28
10	49	Female	EA	49	19	14

* EA= European-American
AA= African-American

Coefficient of Variation for Glucose Assay

Coefficient of variation (CV) for the glucose assay (2 subjects; 10 replicates) was 2.22 (subject 1) and 4.48 (subject 2).

Fasting Blood Glucose Levels

Analysis of variance (ANOVA) for fasting blood glucose levels (Table 4) showed no significant difference among subjects.

Table 4. Subjects' fasting blood glucose levels (mg/dL) expressed as the mean ± standard deviation (SD) with the corresponding coefficient of variation (CV). (n=number of tests)

Subject	n	Mean ± SD (span)	CV
1	12	91.33 ± 9.33 (77-109)	10.21
2	12	96.33 ± 10.24 (79-108)	10.63
3	12	89.17 ± 6.26 (79-103)	7.03
4	9	90.33 ± 9.47 (72-100)	10.49
5	9	92.33 ± 11.23 (83-118)	12.16
6	9	88.56 ± 5.27 (80-97)	5.95
7	9	88.22 ± 5.85 (78-95)	6.63
8	9	98.44 ± 6.54 (91-112)	6.64
9	9	90.33 ± 6.22 (80-98)	6.89
10	9	88.77 ± 15.00 (63-120)	16.89

Individual Responses

There were significant differences for the area under the incremental blood glucose response curve (AUC) among subjects for each level of carbohydrate (CHO) intake, except for 100 grams (g). Figures 5-11 show each subject's timed glycemic response to each separate dose of CHO. Table 5 shows the mean AUC ± SD for each subject, for each treatment and the group mean AUC ± SD for each treatment. Figures 12-14 show the glucose area under the curve for each subject at each level of CHO tested (in triplicate).

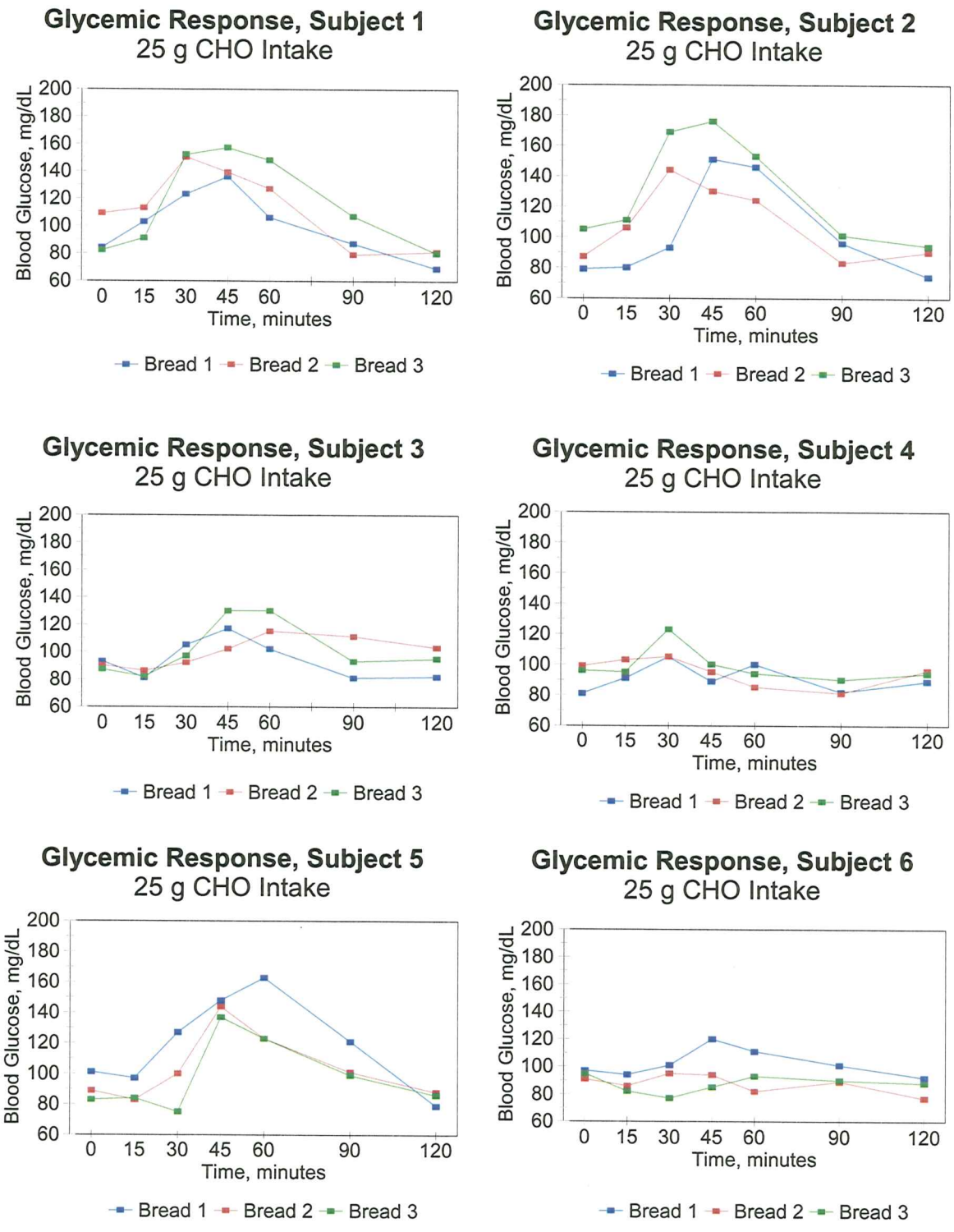


Figure 5. Timed glycemic responses of subjects 1-6 to 3 separate doses of 25 g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

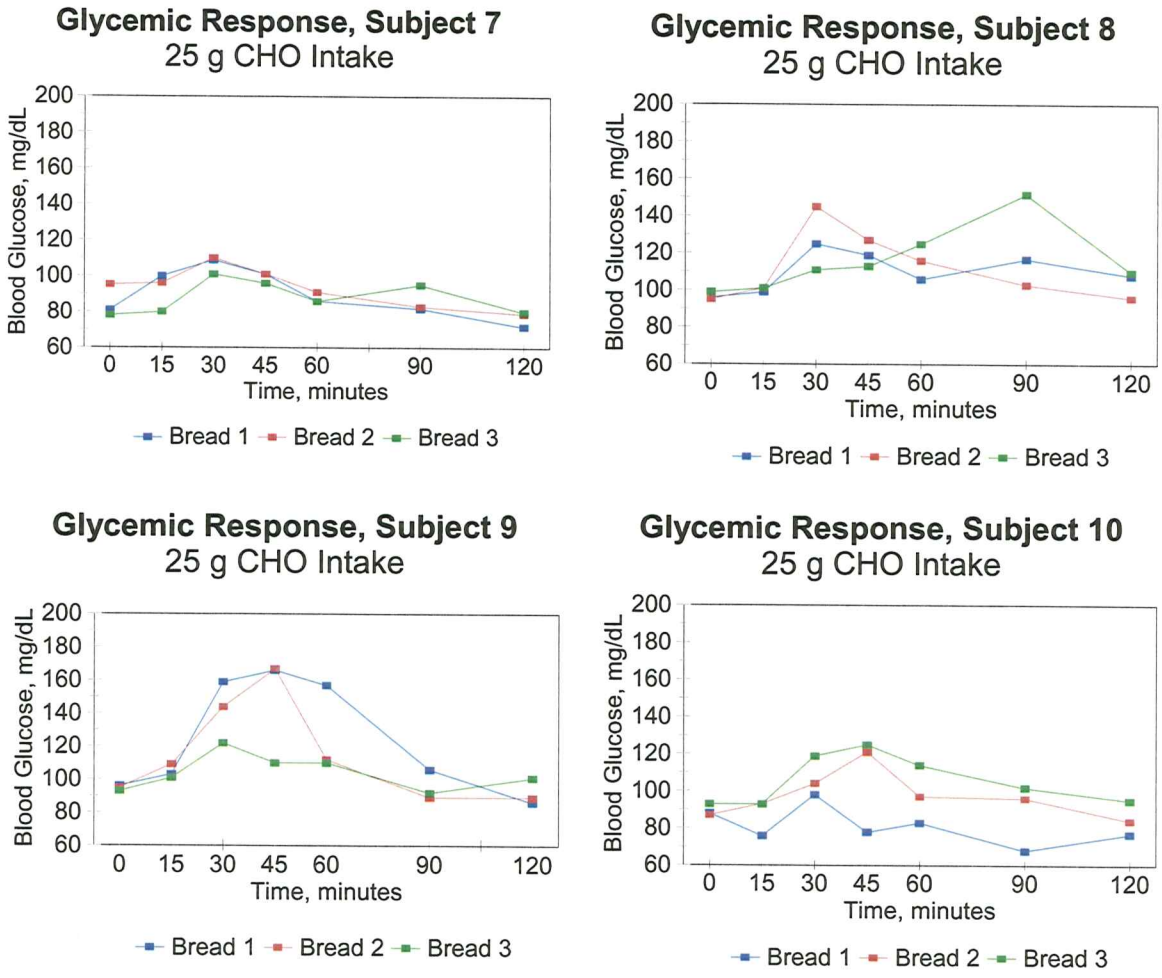


Figure 6. Timed glycemic responses of subjects 7-10 to 3 separate doses of 25g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

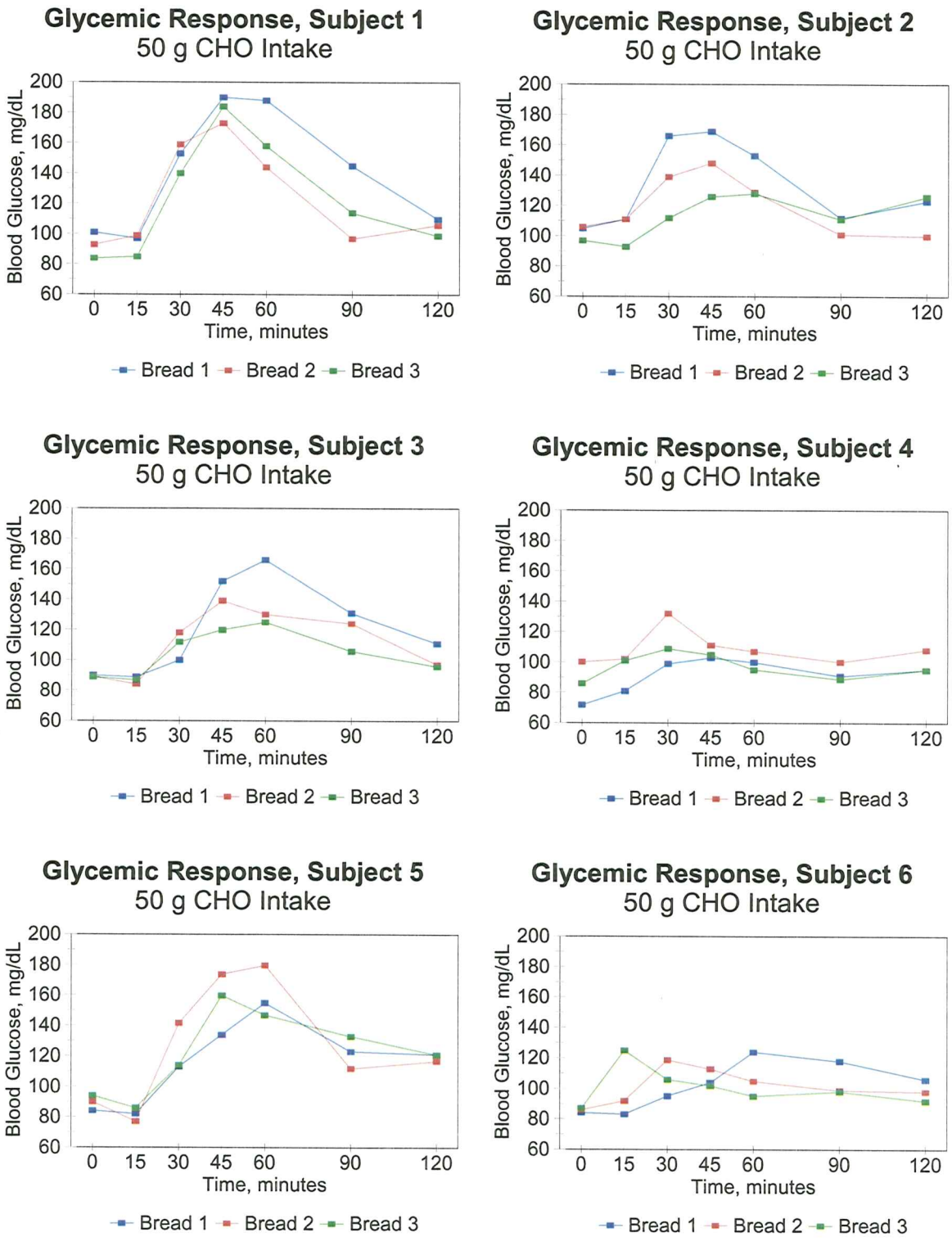


Figure 7. Timed glycemic responses of subjects 1-6 to 3 separate doses of 50 g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

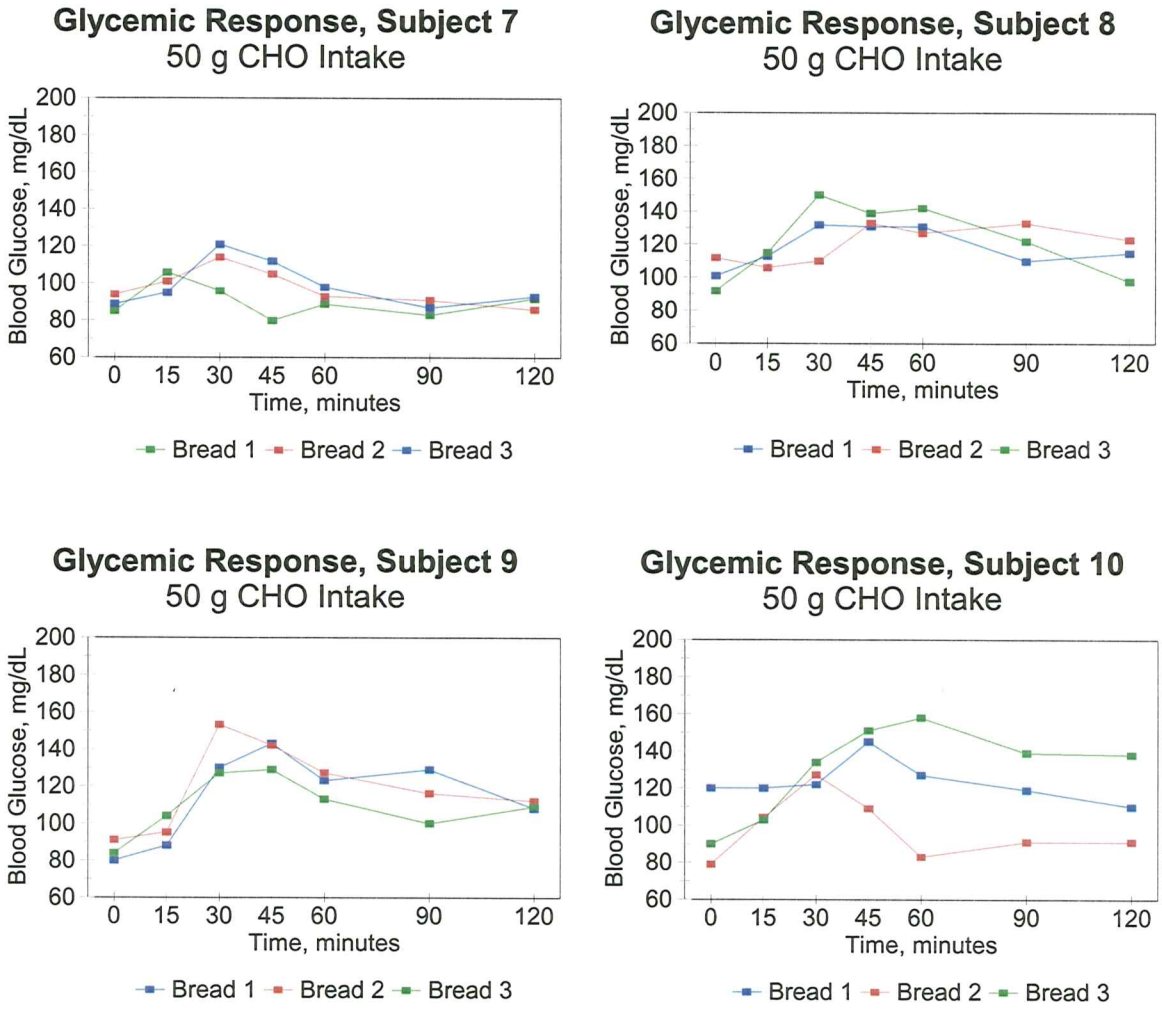


Figure 8. Timed glycemic responses of subjects 7-10 to 3 separate doses of 50g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

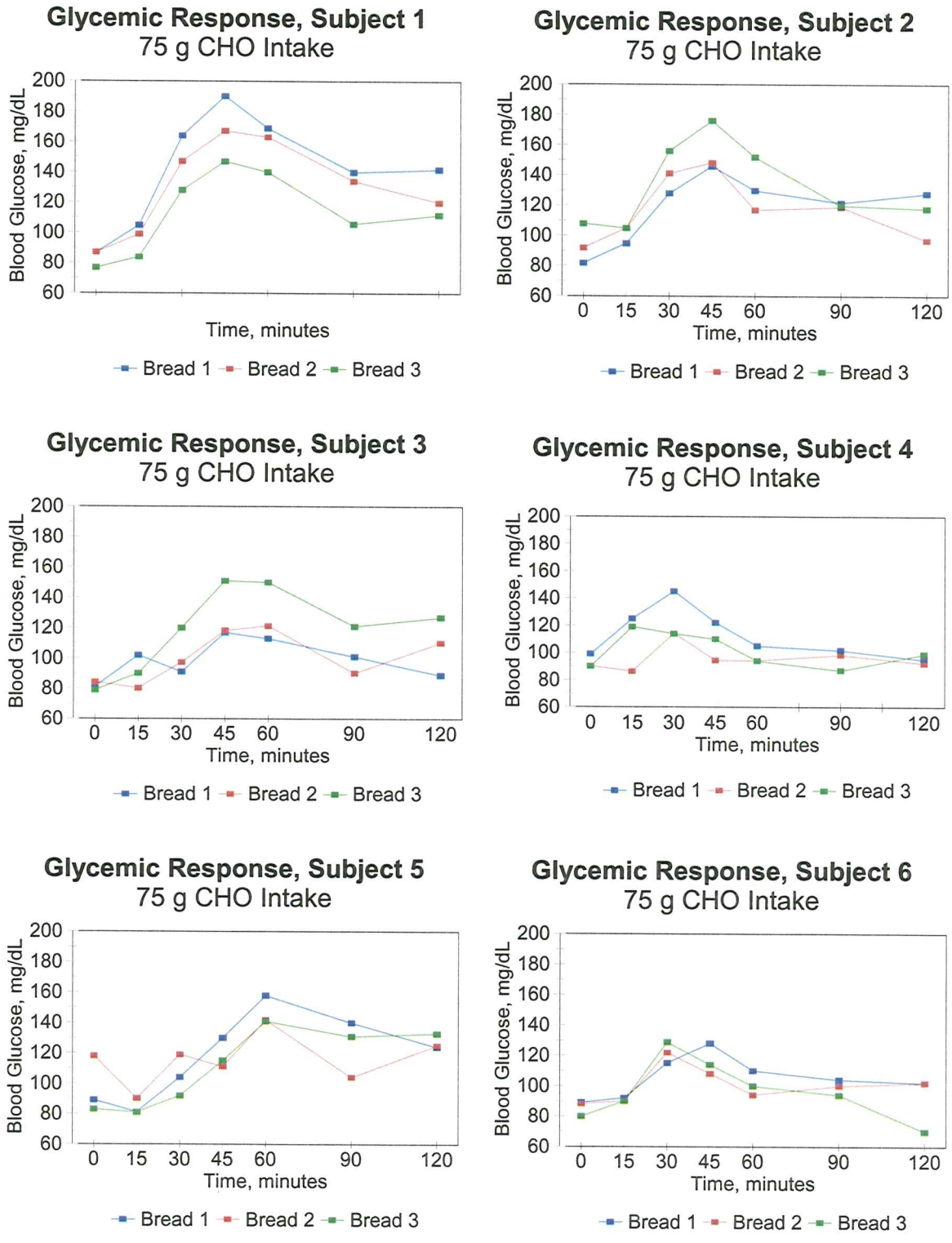


Figure 9. Timed glycemic responses of subjects 1-6 to 3 separate doses of 75 g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

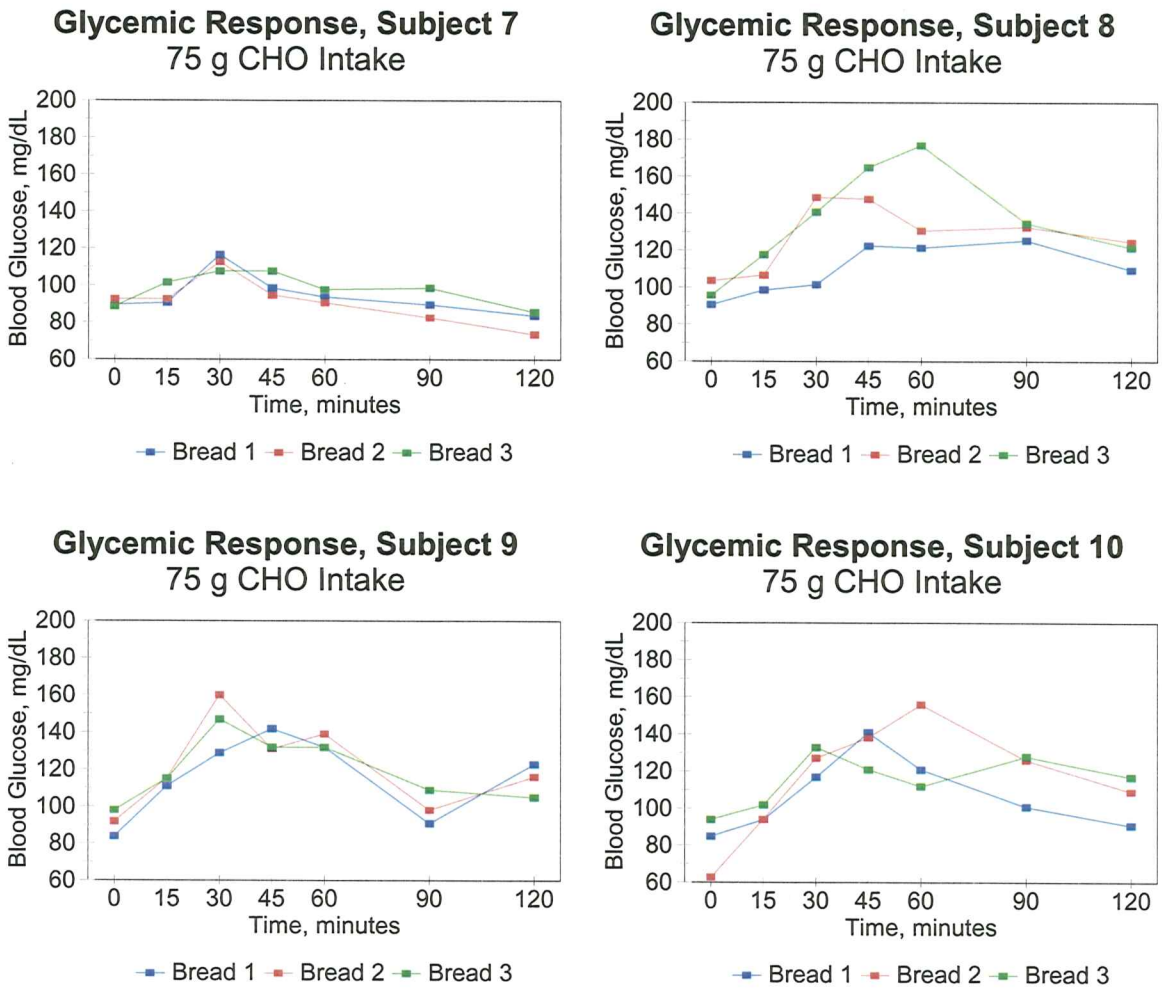
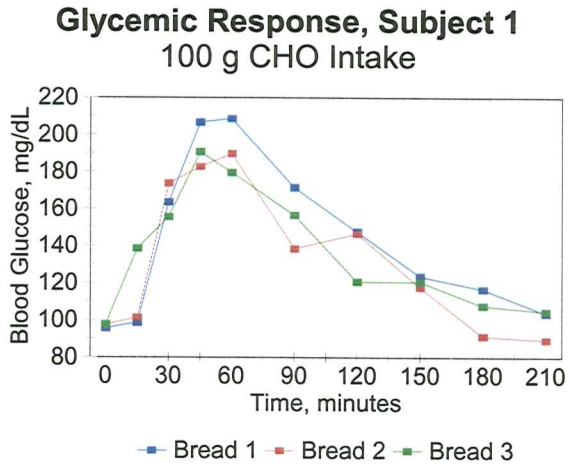
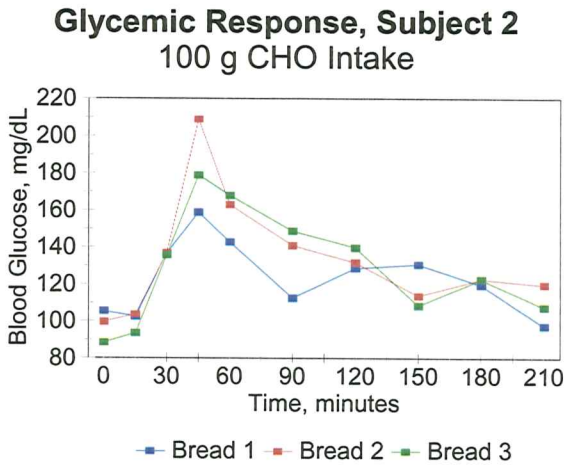


Figure 10. Timed glycemic responses of subjects 7-10 to 3 separate doses of 75g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.





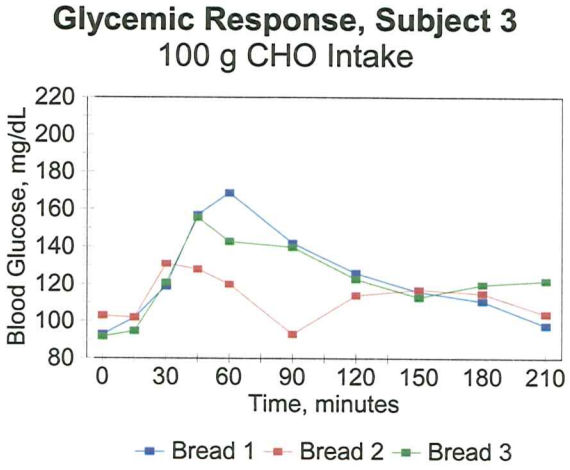


Figure 11. Timed glycemic responses of subjects 1-3 to 3 separate doses of 100 g CHO from white bread. Bread 1, Bread 2, and Bread 3 represent the separate occasions on which the treatment was repeated.

Table 5. Individual subject's, and group total, area under the incremental blood glucose response curve for each treatment tested expressed as the mean ± standard deviation (SD).

Subject	25 g CHO Mean ± SD	50 g CHO Mean ± SD	75 g CHO Mean± SD	100 g CHO Mean ± SD
1	2691 ± 1633	4805 ± 940	5945 ± 1238	7408 ± 912
2	2888 ± 360	2462 ± 963	3748 ± 924	4827 ± 1978
3	1414 ± 724	3283 ± 1050	3322 ± 1841	3606 ± 2140
4	597 ± 553	1595 ± 843	1206 ± 434	
5	2412 ± 454	4533 ± 545	2989 ± 2248	
6	290 ± 440	2121 ± 535	1955 ± 355	
7	947 ± 559	760 ± 314	734 ± 461	
8	2223 ± 475	2575 ± 1394	3798 ± 1420	
9	2441 ± 1209	3755 ± 862	3358 ± 586	
10	982 ± 790	2742 ± 2513	4313 ± 2520	
Group Total	1688 ± 948	2863 ± 1264	3136 ± 1531	5280 ± 1941

AUC for 25-g CHO Treatment

There was a significant difference between subjects for the 25 g CHO treatment ($p<0.004$) ($n=10$). Subjects 1 and 2 had significantly larger responses than did subject 6. The mean AUC for subject 6 was 290; whereas, the mean AUC for subjects 1 and 2 was 2691 and 2888, respectively.

AUC for 50-g CHO Treatment

There was a significant difference between subjects for the 50 g CHO treatment ($p<0.008$) ($n=10$). Subject 7 had a significantly smaller response than either subject 1 or subject 5. The mean AUC for subject 7 was 760. The mean AUC for subjects 1 and 5 were 4805 and 4533, respectively.

AUC for 75-g CHO Treatment

There was a significant difference among subjects for the 75 g CHO treatment ($p<0.009$) ($n=10$). Subject 1 had a significantly larger response to the treatment than did

subjects 4 or 7. Subjects 4 and 7 had a mean AUC of 1206 and 734, respectively. Subject 1 had a mean AUC of 5945.

AUC for 100-g CHO Treatment

There was no significant difference between subjects for the 100 g CHO treatment (n=3).

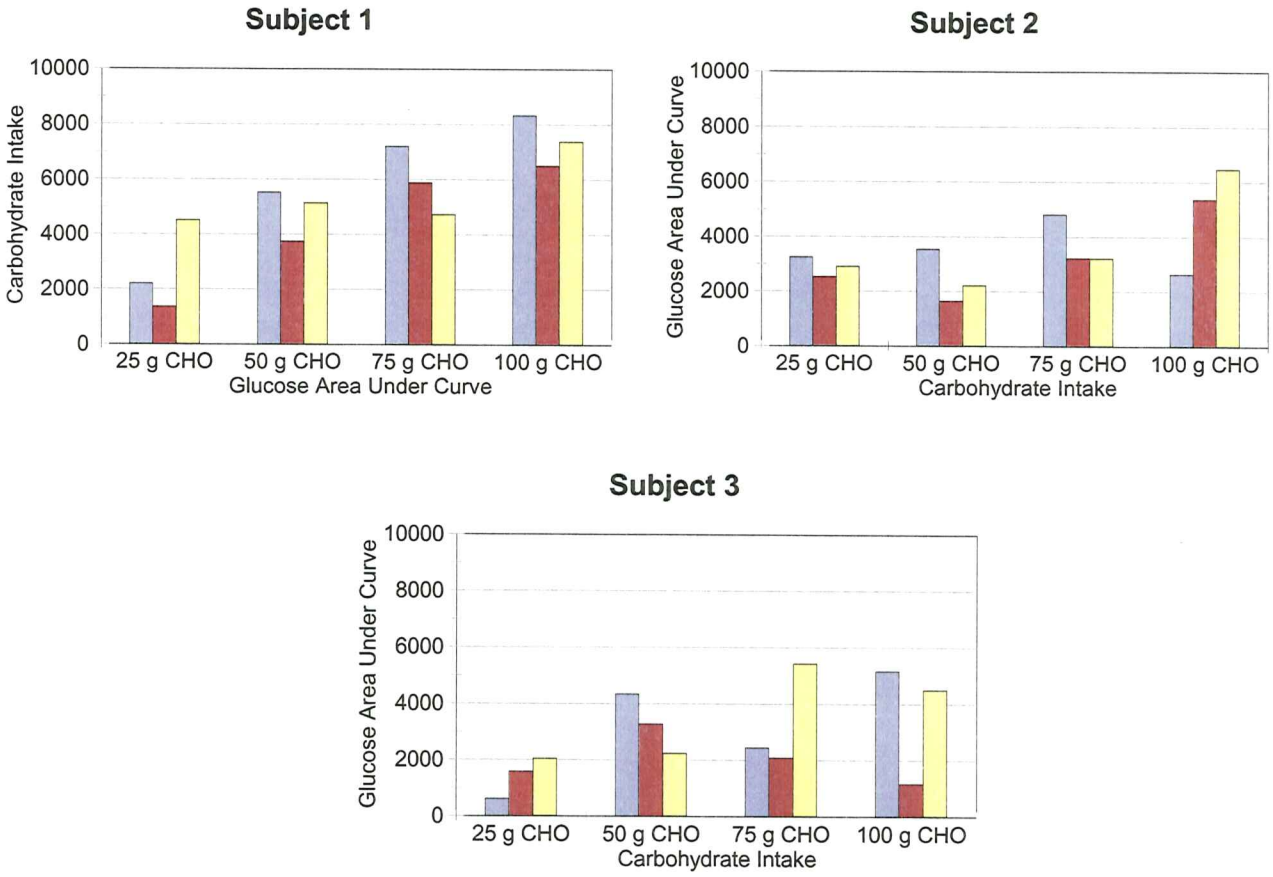


Figure 12. Subjects 1-3's AUC for 25 g CHO, 50 g CHO, 75 g CHO, and 100 g CHO, tested in triplicate. The three separate colors represent the separate occasions on which the treatment was repeated.

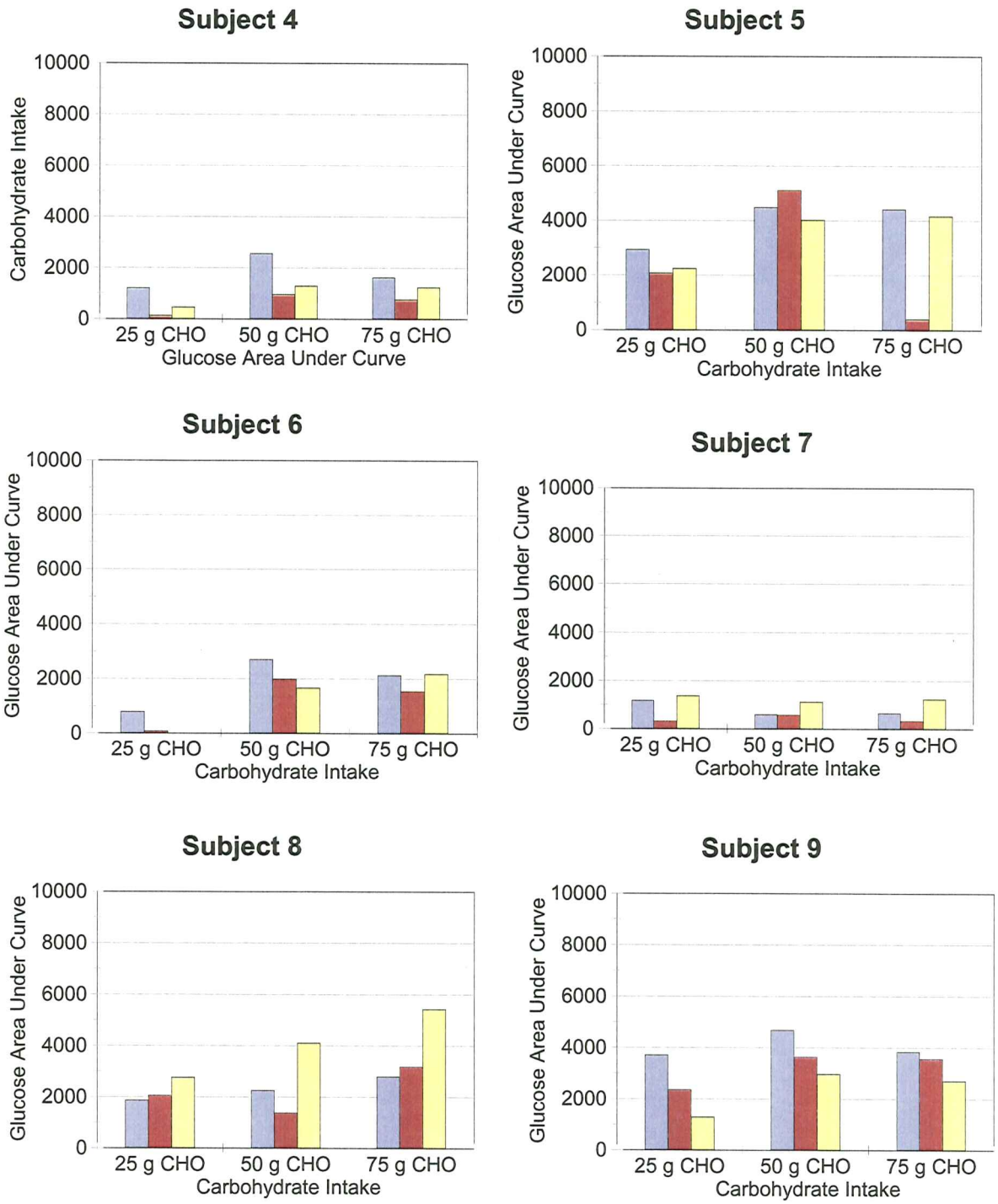


Figure 13. Subjects 4-9's AUC for 25 g CHO, 50 g CHO, 75 g CHO, and 100 g CHO, tested in triplicate. The three separate colors represent the separate occasions on which the treatment was repeated.

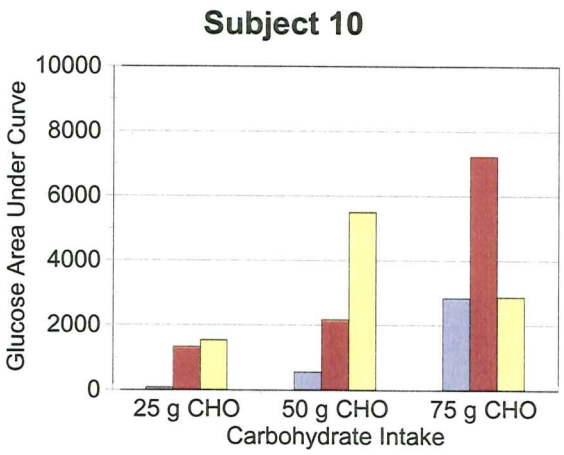


Figure 14. Subject 10's AUC for 25 g CHO, 50 g CHO, 75 g CHO, and 100 g CHO, tested in triplicate. The three separate colors represent the separate occasions on which the treatment was repeated.

Dose Response

Regression analysis demonstrated only subjects 1 (r^2=0.739; $p<0.001$), 2 (r^2=0.352; $p<0.042$), and 6 (r^2=0.565; $p<0.02$) had a significant dose response.

CHAPTER 5

DISCUSSION

A convenience sample of 10 subjects without diagnosed glucose impairment and with varying age, weight, percent body fat, body mass index (BMI), gender, and race completed this study. Glycemic responsiveness to three different doses of carbohydrate (CHO) (25 grams [g], 50 g , 75 g) from white bread was evaluated for all 10 subjects. In addition, three subjects completed three tests with a fourth dose (100 g) of CHO. Coefficient of variation (CV) for the glucose assay (2 subjects, 10 replicates) and fasting blood glucose levels (FBG) (10 subjects; 9 to 12 replicates) was small. No significant differences were found among subjects for fasting blood (FBG) glucose levels. Significant differences for the incremental areas under the timed glucose response curve (AUC) were found among subjects for each level of CHO except 100 g. Only three subjects demonstrated a significant dose response to the different CHO levels.

Coefficient of Variation for Glucose Assay

Coefficient of variation (CV) is a measure of relative variation rather than absolute variation.[112] The CV expresses the standard deviation (SD) as a percent of the mean. The CV of the glucose assay itself was calculated to determine the relative variation in the BG measurements; thus testing the repeatability of the glucometer and test strips used in this study. The relatively small CVs, 2.22 (subject 1) and 4.48 (subject 2), suggest that there was minimal variability between the repeated measures of blood glucose; therefore, the glucometer and test strips, as well as the standard method of testing used in this study produced repeatable measures.

A digital timer was used to time the intervals between blood sampling; and blood volume obtained, for each sample, was adequate to measure glucose levels; thus, the small CV was expected. The student investigator monitored testing periods to confirm the use of consistent methods. The use of capillary blood in these tests was chosen because it requires a relatively non-evasive technique, uses a small volume, and is more sensitive to glycemic responses than venous blood.[10]

Fasting Blood Glucose Levels

Fasting blood glucose levels were within normal limits, with the exception of two subjects on one occasion each, suggesting subjects' reported absence of diabetes or glucose metabolism impairment was correct.[35] Mean fasting blood levels were all within normal limits. Coefficient of variation was determined for all FBG levels of each subject. In this study, half the population (n=5), had a CV >10 and a SD >9, indicating a relatively large daily fluctuation of FBG within subjects. Subjects were asked to fast 12 hours prior to each test, and to begin the test procedure at approximately the same time each day. Consistent behaviors were expected to minimize fluctuation in FBG levels and consequently reduce the variability of FBGs within subjects. The variability of FBGs within subjects, although within normal limits, suggests there is substantial day-to-day variability of blood glucose levels. The variation could be due to unanticipated or uncontrollable changes in activity, stress, unreported or pre-clinical illness, or hormonal changes within the subjects.[113]

According to the Expert Committee on the Classification and Diagnosis of Diabetes[35] a diagnosis of impaired glucose tolerance (IGT) includes: a FBG between 111 and 125 mg/dL, an oral glucose tolerance test (OGTT) with one intervening value >200

mg/dL, or a 2-hour value ≥ 140 mg/dL. All subjects reported no personal history of diagnosis of impaired glucose metabolism; however, two subjects (subjects 5 and 10) each had one documented FBG level of ≥ 110 mg/dL. All other BG levels for each of these subjects were within normal limits; therefore, these isolated values were not considered indicative of impaired glucose metabolism.

Individual Responses—Area Under the Curve

In determining individual responsiveness, different methods of calculating the AUC can produce different results. The validity of the GI has been criticized due to the lack of consistent calculation procedures.[77] In an effort to define the calculation of the GI, Wolever[10] described the three methods for calculating the AUC. Wolever[10] stated only the incremental area under the blood glucose response curve should be used in the calculation of the GI. In 1995, a list of GIs for almost 600 foods was compiled and adjusted to include only the incremental AUC in the GI calculation of each food.[78] To conform with standards in the literature, the incremental AUC was used to compare the glycemic responses of subjects in our study.

Results of our study indicate significant differences among individuals for the AUC, which corresponds with results from previous studies.[11,12] In those studies, variability was thought to be due to day-to-day variations within individuals; however the authors did not discuss possible reasons for this. Investigators found no significant differences among subjects when each subject's response was expressed as the GI. They concluded that glycemic response areas of foods cannot be used as a classification because there are large differences among subjects. However, expressing responses as a

percentage of the response to a standard food, as is done in the calculation of the GI, creates a valid classification.[11,12]

Dose Response

Only three subjects had a dose response to increasing levels of CHO. Subjects 1, 2, and 6 experienced a significant dose response for the calculated mean AUCs. It was expected that all subjects would demonstrate a dose response for the different levels of CHO tested, since total carbohydrate eaten is considered in the calculation of the glycemic load.[82] The day-to-day variation within individuals may have contributed to the lack of a dose response. For each subject, an AUC can be selected for any given treatment that shows no change; however, for subjects 1, 2, 3, 6, 8,and 10 an AUC for can be selected for each treatment that demonstrates a dose response. The lack of a significant dose response might also be due to a leveling effect after 50 g CHO.[6] Jenkins found there was a dose response in non-diabetic subjects for increasing levels of CHO up to 50 g. It was expected that each subject's response would increase from the 25 g CHO treatment to the 50 g CHO treatment; however, the mean AUC for two subjects decreased from the 25 g CHO treatment to the 50 g CHO treatment.

Relationship between Subject Characteristics and Glycemic Status

The prevalence of diabetes increases with age[44] and blood glucose levels tend to increase with age[113]; therefore a higher glycemic response would be expected to occur in older individuals. Evaluation of our results is limited since the oldest subjects were only 50 years of age. In our study, the oldest subjects had some elevated FBG levels, elevated BGs at intermediate points, elevated BGs at the 2-hour time point, and significantly larger

mean AUCs than other subjects for each level of CHO tested, except the 100 g CHO treatment. .

The mean FBG ± SD for subject 2 was within normal limits, but was numerically higher, with the exception of subject 8, than the other subjects. Although subjects were not asked to report a family history of impaired glucose tolerance or diabetes, after the study was completed, subject 2 confirmed a family history in a first-degree relative with type 2 DM. The family history, coupled with age and weight might have contributed to high normal mean fasting levels.

Subject 10, 49 years old, had a documented FBG of 120 mg/dL. In fact, subject 10 had the widest range of FBG (63-120 mg/dL). This was surprising because of her low BMI and low percent body fat. Subject 10 was one of the oldest subjects in the study; however, her level of physical activity may have been a more important factor in determining her glycemic responsiveness. Subject 10 has a strenuous exercise program and might be expected to have tighter glycemic control due to her physical fitness.[35,67] The high degree of variation in fasting levels that was observed may be in part due to unreported changes in exercise patterns or illness, or hormonal changes.[113] This subject started the test protocol earlier in the day than the other subjects did so that she could complete the protocol and continue her exercise program. Circadian variation in corticosteroids and other counterregulatory hormone levels may have been elevated, increasing variability in FBGlevels.[36] Procedural error cannot be ruled out; however, it is unlikely because the subject was familiar with the testing procedures. Further, the subject's variability in FBG's may have contributed to the day-to-day variation observed in her AUC's.

Only three subjects completed the 100-g CHO treatment. Subjects 1 and 2 each had an intermediate point BG level $\geq$ 200 mg/dL during the 100-g CHO treatment. None of the other subjects had blood glucose levels this high any treatment or for any day. Subject 3 did not exceed 200 mg/dL at any point in testing. Subjects 1 and 2 (each 50 years old) are considerably older than subject 3 (23 years old); therefore, age is thought to explain subjects 1 and 2's larger response.

Subjects 1 and 2 had a BG level $\geq$ 140 mg/dL at the 2-hour time point. All other subjects were within normal limits at the 2-hour time point. Subject 1's 2-hour BG was > 140 mg/dL on three occasions: once for a 75g-CHO test, and twice for 100g-CHO tests. None of the fasting BG levels for subject 1 exceeded 110 mg/dL; therefore, these elevated 2-hour BG levels may not indicate of IGT. They may, however, indicate an increased risk of developing IGT. Subject 2 had a 2-hour BG of 140 mg/dL for one of the 100g-CHO tests; because this was an isolated occurrence, and none of her FBGs were $\geq$ 110 mg/dL, this can be speculated as due to uncontrollable variables such as stress, illness, or hormones.[113]

Subjects 1 and 2 had significantly larger mean AUCs for the 25g CHO dose than did subject 6. This higher glycemic response could be explained partially by age. Subjects 1 and 2 are 28 years older than subject 6 (50, 50, and 22 years, respectively).

Subject 1 had a significantly larger mean AUC for the 75g CHO dose than did subjects 4 or 7. Age is thought to be the important factor in evaluating the glycemic responsiveness to this treatment, because gender, weight, BMI, and percent body fat are similar for subjects 1, 4 and 7. Subject 1 is older (by at least 19 years) than subjects 4 or 7.

No significant differences were found among subjects for the mean AUC for the 100g CHO dose. In our study, similar results for each subject (n=3) could be the result of the small sample size, or the extended time period that was allowed to measure the change in BG. The time period, for this dose of CHO, was extended to allow BG to return to baseline. During this extended time period, subject 3 experienced a bimodal response curve; however, this was due to a single low BG, and was not repeated in the other test days. This measurement should have been confirmed by repeat testing. Although the mean AUC of subjects 1 and 3 were not significantly different, subject 1 had a higher initial response. This pronounced response, again, may be due to age and may be indicative of increased risk of glucose intolerance.[35]

Age alone does not account for glycemic sensitivity observed in subjects 1 and 2 because subject 10 is the same age was not as sensitive as subjects 1 and 2. This suggests that other factors, for example, percent body fat and BMI, influence glycemic sensitivity, because subjects 1 and 2 had higher percent body fat and BMI than did subject 10. Further statistical analyses are needed to test this hypothesis.

We are unaware of literature that reports a specific relationship between percent body fat, *per se*, and glycemic responsiveness. Obesity is a risk factor for developing type 2 DM,[35] suggesting that obese individuals might have a higher glycemic response to ingested CHO. Desirable amounts are 12-20% body fat in men and 20-30% body fat in women. Men with over 25% body fat and women with over 30% body fat are considered obese.[19] In our study, percent body fat alone did not appear to be predictive factor of glycemic responsiveness. Subjects 1, 4, and 7 had comparable percent body fat, but were distinguishable by age. The oldest subject was the most sensitive of these three subjects.

Studies have observed a relationship between increasing BMI and the development of diabetes[114,115] and worsening glycemic status.[116] Data from the Iowa Women's Health Study[115] indicated increasing BMI is a strong predicator of diabetes. Similarly, in a study of 2,437 individuals newly diagnosed with type 2 DM, there was a strong inverse linear relation between BMI and age of diagnosis.[114] The chance of early diagnosis of type 2 DM (defined as diagnosis before 45 years of age) increased by 6% for each 1 kilogram (kg) per meter squared (m^2) in BMI. The degree of obesity may determine when diabetes will develop.[114]

To assess the relationship between abdominal adiposity and glycemic variables with obesity and age in 151 Salishan Indians, data from a screening initiative in British Columbia, Canada were analyzed.[116] Subjects were non-diabetic first- and second-degree relatives of individuals with type 2 DM. Findings of this study indicated a strong association between increasing BMI and worsening glycemic status, as assessed by glycated Hb, 2-hour time point BG measure after a 75 g CHO load, and FBG levels.[116] Together these studies suggest that as BMI increases, glycemic responsiveness increases.

Just as in the case of subject 2, the mean FBG $\pm$ SD for subject 8 was within normal limits, but was numerically higher, than the other subjects. After completion of the study, subject 8 confirmed a family history of impaired glucose tolerance (*i.e.* a first-degree relative). Subject 8's FBG is particularly interesting since the relatively small SD, suggests a consistently high-normal FBG. Subject 8 is obese; he had the highest BMI in the study sample. This may account for his high-normal FBG levels and corresponds with data from the Salishan Indians study.[116]

Glycemic responsiveness does not vary significantly between males and females with type 2 DM, when controlling for duration of disease, body weight, and age.[91] Gannon[93] found that young, non-diabetic men and women have similar increases in BG postprandially in response to CHO-rich meals. These studies suggest that glycemic responses are similar between genders. We did not observe a statistical relationship between gender and glycemic responsiveness; however, interpretation of our results is limited due to the fact that there were only two males in the study population.

African-Americans are 1.7 times more likely to have diabetes than European-Americans.[44] This suggests African-Americans may respond to CHO with greater glycemic sensitivity. The United Kingdom Prospective Diabetes Study assessed the relationship of self-reported ethnicity, metabolic control, and blood pressure during treatment of type 2 DM.[117] Glycated hemoglobin (Hb) and FBG were assessed at 3, 6, and 9 years after diagnoses of type 2 DM in 2,999 subjects. The subject population was 82% white-Caucasian, 8% Afro-Caribbean, and 10% Asians of Indian origin. Subjects were divided into two treatment groups: conventional glucose control group—treated with diet alone, and an intensive glucose control group—treated with sulfonylurea, insulin, or metformin. There was no significant difference among groups for changes in glycated Hb and FBG. The authors concluded there are not ethnic differences in glycemic control during the first 9 years after diagnosis of type 2 DM.[117] Data from that study suggest that glycemic responsiveness, at least in subjects with type 2 DM, may be similar among different races.

Results of our study suggest race is not significantly related to glycemic responsiveness; however results are limited due to the small number of African-

Americans in our sample population. Subject 5, a 21 year old African-American female with a percent body fat and BMI within a desirable range, had an isolated FBG of 118 mg/dL. Reasons are unclear, but might include unreported changes in her activities, stress, unreported illness, or hormonal changes within the subject,[113] glucometer error, or an error in the test procedure. The latter is unlikely, however, due to the training effect of subjects. The value was probably spurious and, in retrospect, should have been repeated. All intermediate values and the 2-hour values for subject 5 were within normal limits.

Subject 7 had a significantly smaller mean AUC for the 50g CHO dose than did either subject 1 or 5. Subject 7, an African-American, was classified by BMI and percent body fat as obese. Her low glycemic response was unanticipated, considering her race and classification of obesity increases her risk of diabetes.[35] She was expected to have had a larger response than that of subject 5, also an African-American. Subject 7's young age might account for her decreased sensitivity.

We speculated that African-Americans would have higher glycemic responsiveness; however, the four African American subjects did not display significantly higher responses than did European-American subjects. This could be due to the young age of the African-Americans. Our findings are similar to those of the United Kingdom Diabetes Prospective Study which did not find ethnic differences in glycemic control.[117]

Conclusion

The first of our original hypotheses that individuals would experience similar glycemic responses to a standard food was not supported. Results of this study suggest that glycemic responses within an individual vary on a day-to-day basis.

The second of our original hypotheses was supported. Glycemic responses differed significantly among individuals according to their individual characteristics; however, due to the small sample size, the results cannot be generalized. Taken alone, no single characteristic was a predictor of glycemic response; however, combining characteristics did show a pattern in predicting glycemic sensitivity.

Increasing age coupled with increasing percent body fat and BMI appear to have the largest effect on glycemic responsiveness. Higher glycemic responses to ingested carbohydrates may be due to decreased insulin sensitivity in the subjects with higher percent body fat; obesity and body weight are related to the development of Syndrome X.[56]

The significant differences among subjects of this study question the utility of the GI in planning therapeutic diets. One question that needs to be asked is: How is the classification valid if it does not allow for variation? Hollenbeck,[77] an early critic of the GI stated, "...the more consistent the glycemic response to a given food from individual to individual the greater the likelihood that meal planning based on the GI will be useful." Even though a food is classified as low-GI, it does not necessarily produce a low glycemic effect. It may produce a large or small glycemic response in a person when eaten on separate occasions, due to day-to-day variation in glycemic responsiveness. This is an important fact to consider when planning diabetic diets, since the principal goal of nutrition therapy is to achieve glycemic control.

Further, no diet should be based simply on the "numbers." According to the "International tables of glycemic index,"[78] jelly beans, with a GI of 114, appears to be a better food choice than either a plain baked potato (GI=158) or carrots (GI=131). The GI

reflects a food's potential effect on BG levels, not the entire nutritional content. Jelly beans offer only energy; whereas, carrots and potatoes contain fiber, vitamins, and phytochemicals, which would be more beneficial overall.

Additionally, a person may not realize these "numbers" are based on a portion of food containing 50g of available carbohydrate. To get 50g of carbohydrates from carrots, one must eat 7 medium carrots raw or 3 cups of sliced carrots boiled. Taking the portion size into consideration, one needs to ask: would a person really consume enough carrots to supply 50g of carbohydrates at one sitting? In order to apply to diets of individuals, the GI numbers should be easily applicable to different portion sizes.

A low-GI diet for the prevention or management of coronary heart disease (CHD) does not have clearly proven potential. A diet may be low-GI due to a high fiber content; therefore, it is unclear whether the link between GI and CHD is indeed the GI of the diet or fiber content of the diet.[60,99] Similarly, no long-term studies have determined a statistical relationship between GI and weight loss; but, studies have produced promising results linking low-GI foods with increased satiety.[99,104] Increased satiety is beneficial in planning weight loss diets to help reduce total energy intake; thereby facilitating weight loss.

Future Directions

Results from this study are not be a representative population due to its small size and because it was a convenience sample. For more reliable results, the sample population needs to be increased and selected randomly. Investigations should be conducted to determine possible statistical relationships between glycemic responsiveness and increasing age or percent body fat. Investigations should also be conducted to

determine corresponding glycemic and insulemic responses. Because older individuals are at increased risk for developing diabetes, studies should be conducted to determine the responsiveness of these individuals with the objective of developing meal plans that would produce lower glycemic effects; thereby contributing to glycemic control and prevention or management of diabetes.

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APPENDIX A

HUMAN SUBJECT APPROVAL FORM

LSU Ag Center IRB Correspondence

February 22, 2001

From: Michael Keenan Michael Keenan

To: Carol O'Neil

Re: Protocol H-01-02

Your protocol was reviewed at our meeting on February 20, 2001. We conditionally approved your protocol, Intra- and Inter-Person Variability in Glycemic Responsiveness. We required that you provide three items of information for full approval: 1) the number of anticipated subjects in the study, 2) the role of race in the study, and 3) assurance that gloves will be worn by all investigators who handle blood from finger sticks. With the reception of your e-mail responding to my verbal communication of these items to you, your protocol has received full approval. Your approval number with the Ag Center IRB is H-01-02 and you are approved for a period of three years with yearly review.

APPENDIX B

SUBJECT CONSENT FORM

INFORMED CONSENT

TITLE OF RESEARCH PROJECT: Intra- and Inter-person variability in glycemic responsiveness

The purpose of this study being conducted by the Louisiana State University (LSU) Agricultural Center investigators is to determine your blood sugar levels after consumption of a know amount of carbohydrate. This information will be used to assess the variability in glycemic responsiveness or your response to the carbohydrate. You will be asked to: supply your age, height, and race; have your weight and percent body fat measured; eat a known amount of white bread; and have blood drawn, via a finger stick, prior to eating the carbohydrate or other food that morning (a fasting blood sample), and at 15, 30, 45, 60, 90, and 120 minutes following eating the carbohydrate. On different days, you will be asked to eat 25 grams [g], 50g, and 75 g of carbohydrate in the form of white bread. Eating each amount of bread will be repeated three times, for a total of nine tests, which must be done on nine separate days.

There are no risks associated with this study. Use of finger sticks to assess blood sugar levels is a standard medical test. Blood samples will not be stored at the end of the test and will not be used for tests other than the blood sugar level. Individuals will benefit directly by learning their fasting blood sugar levels and their responsiveness to a know amount of carbohydrate. At the end of the study, participants will receive a check for $100.

Only LSU researchers involved with this project will have access to and be able to review the results of the study. Printed results of the study, including any publications resulting from this study, will not identify individuals by name. Any report, published or other, will present data either in summary form or stripped of individual identifiers. You may withdraw from this study at any time; however, if you withdraw prior to completion of the study you will not receive the above stated stipend.

The study has been discussed with me and all questions have been answered to my satisfaction. I may direct additional questions regarding this study to the principal investigator: Dr. Carol E. O'Neil, LSU, School of Human Ecology, at 225-388-1631. If I have questions about subjects' rights or other concerns, I can contact Dr. David Morrison, LSU Agricultural Center at 225-388-8336 from 8:00AM to 4:30 PM.

With full knowledge of the above information, I voluntarily consent to take part in this study.

Name of participant (please print):_____

Signature of participant:_____ Date: _____

Mailing Address: _____
(Street) (City) (Zip Code)

Phone:_____

Witness (please print):_____

Signature of Witness:_____ Date: _____

APPENDIX C

SUBJECT INFORMATION FORM

Subject Information Form

Name: _____

E-mail address: _____

Phone number: _____

Mailing Address: _____

_____

_____

_____

Social Security Number (for payment purposes):

_____

Age: _____

Gender: _____

Race: _____

Height: _____

Weight: _____