

Example by StudyDriver

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Glucose Tolerance Test Example

Introduction

Carbohydrate forms the principle source of energy. Usually polysaccharide (starch and glycogen) which are glucose units joined by α -glucosidic links and disaccharides (sucrose and lactose) the main dietary carbohydrate. Carbohydrate absorption must be presented to the intestinal epithelium in monosaccharide form mainly glucose and therefore digestion must precede absorption. Glucose gained a significant importance because brain cells are very dependent on it as it is sole source of energy supply. Red blood cells also depend on glucose to carry out their functions. Therefore the blood glucose concentration must be maintained within relative narrow range. After a carbohydrate-containing meal, glucose is transported in the portal blood to the liver, which takes up 60% of the glucose load. Consequently, a rise in the blood glucose concentration causes the release of insulin which will increase the entry of excess glucose into the liver where it is stored in form of glycogen. The normal plasma glucose concentration remains between 4.5 and 11 mmol/L, despite the intermittent load entering the body from the gastrointestinal tract. The maintenance of plasma glucose concentration below 11 mmol/L minimizes loss from the body as well as providing the optimal supply to the brain. Mayne, (1994). All the filtered glucose through

glomeruli is reabsorbed in the proximal tubules. Therefore no glucose should be detected in urine; significant glycosuria occurs if the plasma glucose concentration exceeds 11 mmol/L. The two most important hormones in glucose homeostasis are insulin and glucagon. Insulin is a 53 amino acid polypeptide, secreted by the β -cells in the islet langerhans of the pancreas in response to a rise in the blood glucose concentration. Insulin stimulates glycogen synthesis and inhibits glycogenolysis through interaction with an exquisitely coordinated control mechanism that is central to the regulation of blood glucose concentration. Glucagon is a 29 amino acid polypeptide secreted by the α -cells of the pancreatic islet. Its secretion is decreased by a rise in the blood glucose concentration. The action of glucagon is opposite those of insulin. It stimulates hepatic glycogenolysis through activation of glycogen phosphorylase, gluconeogenesis, lipolysis and ketogenesis. Marshall, (2000). The world health organization (WHO) defined diabetes on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/L and greater than 11.1 mmol/L two hours after the oral ingestion of the equivalent of 75g of glucose even the fasting concentration is normal. Mayne, (1994). Diabetes mellitus classified in two types; insulin dependent diabetes (IDDM type-1) where there is a defective insulin secretion. This condition presents in childhood or early adulthood (less than 20 years). Because of insulin deficiency, hyperglycaemia is very likely to occur. As a result glucose will leak to urine (glycosuria) because the plasma glucose concentration exceeds the renal threshold (10 mmol/l). Other consequences related to this condition are polyuria (frequent urination), glucose lost in urine draw water with it by osmosis producing osmotic diuresis characterized by polyuria. The excess fluid lost from the body leads to dehydration and thirst which is a compensatory mechanism to counteract the dehydration. One of severe metabolic complication that may occur in this condition is ketoacidosis; there is increased lipid and protein breakdown, enhanced hepatic gluconeogenesis and impaired glucose into cells. Marshall, (2000). In this condition insulin doses are required for the treatment. The causes of the type I diabetes can be an autoimmune where the islet cell antibodies react specifically with the β -cells, or viral infection that destroy the β -cells of pancreatic islet. Individual with certain human leukocyte antigen (HLA) types have been shown to carry a particular high risk of developing type I diabetes. In type II diabetes, non insulin dependent diabetes mellitus (NIDDM), obesity is the biggest risk factor, 90% of type II diabetes are obese and it occurs in the late onset. In this condition β -cells of islet langerhans are normal which means that there will be a normal insulin concentration and sometimes high in the blood. Also the sensitivity of insulin's target cells

reduced. The cause of reduced remains elusive, recent research suggest that adipose tissue cells secrete a hormone known as resistin, which interfere with insulin action in experimental animal. This could be an important link between obesity and insulin resistance. Resistin is distinct from leptin, the hormone secreted by adipose cells that plays a role in controlling food intake. (Kumar & Clark, 2002). Treatment of this condition by dietary control and weight loss, exercise, sometimes oral hypoglycaemic drugs required. Other conditions can lead to Diabetes Mellitus such as absolute insulin deficiency due to a pancreatic disease (chronic pancreatitis, haemochromatosis, cystic fibrosis). Relative insulin deficiency, can cause diabetes mellitus due to excessive growth hormone, glucocorticoid secretion, or increased plasma glucocorticoid concentration due to administration of steroids. Also drugs like thiazide diuretics can cause diabetes mellitus. Mayne, (1994). Materials and method Please refer to medical biochemistry practical book (BMS2). Result: The equation obtained from the calibration curve used to calculate the concentration of glucose in plasma. $Y = 0.018 X$ Where y = absorbance x = glucose concentration

Patient 1: P (fasting) = $0.078 / 0.018 = 4.3 \text{ mmol/L}$ P (2hrs) = $0.105 / 0.018 = 5.8 \text{ mmol/L}$ Patient 2: P (fasting) = $0.113 / 0.018 = 6.2 \text{ mmol/L}$ P (2hrs) = $0.105 / 0.018 = 8.3 \text{ mmol/L}$ Patient 3 P (fasting) = $0.148 / 0.018 = 8.2 \text{ mmol/L}$ P (2hrs) = $0.264 / 0.018 = 14.6 \text{ mmol/L}$ Conclusion: Patient 1 is normal Patient 2 has normal fasting glucose level and high value after 2 hours (9.6 mmol/L), so this patient must be retested before diagnosis. Patient 3 is diabetic

Discussion The glucose calibration graph showed a good linearity which means that Beer's Lambert law is obeyed and the results are accurate. In glucose tolerance test (GTT) the patient is asked to eat normally in the three days leading up to the test and to be fasting for at least 12 hours. At the end of time the patient is asked to collect urine sample and blood sample is collected. After that, the patient drinks 75g of glucose in 300 ml of water within 5 minutes. After 2 hours, the patient is asked to collect another urine sample and blood sample is collected. Normally when the patient is fasting, the glucose level should be $< 5.5 \text{ mmol/L}$ and there is no glucose in urine. After the patient is given the sugar, the glucose level in the blood will increase, but in the normal person the glucose concentration should go back to normal within 2 hours and no glucose can be detected in urine. What is happening in the normal person after given glucose is that insulin is produced in high concentration, the glucose is converted into glycogen and then the glycogen is stored in the liver. Finally, insulin concentration also decreases to normal concentration. Whereas, in the diabetic patient the glucose level stays high because the insulin is insufficient, not produced or present but not functioning due to a defect in the β -cells of pancreas. In normal

condition, the filtered glucose is completely reabsorbed in the proximal tubule. In Diabetes Mellitus the blood glucose is much above the renal threshold (11 mmol/L), reabsorption becomes saturated and it starts to appear in urine. The presence of glucose in urine is called glucosuria. Glucosuria results in osmotic diuresis that increases water excretion and raises the plasma osmolarity, which in turn stimulates the thirst centre. Osmotic diuresis and thirst cause classical signs and symptoms of polyuria (large volume of urine) and polydipsia (excessive thirst). In patient-1, fasting blood glucose (4.3 mmol/L) is within the normal range and no glucose in urine. After 2 hours the blood glucose level is 5.8 mmol/L, which is below 7.8 mmol/L and no glucose in the urine. These mean that this patient is normal. In patient-2, fasting blood glucose is within the normal range and no glucose in urine. After 2 hours the blood glucose level is 8.3 mmol/L which is slightly high but it is within the normal range of impaired glucose tolerance (7.8- 11.1 mmol/L), whereas urine glucose is negative. This means that this patient must be retested before ending to diagnosis of impaired glucose or any other diagnosis. Many people with impaired glucose tolerance progress to develop diabetes, but this condition can be prevented with adoption of a diabetic-type diet and weight loss (if overweight). Whitby, G, et al, (1988). Patient-3 has high fasting blood glucose level (8.2 mmol/L), and in the urine the glucose is not detected. After 2 hours the glucose concentration did not reduce and it went higher up to 14.6 mmol/L. In addition to that, the urine dipstick showed very strong positive reaction (4+), which indicates that this patient is diabetic. In this patient the glucose concentration was high before the sugar was given. This means that there is a defect in insulin secretion which can not breakdown the glucose and bring to the normal level. The high blood glucose level was due to glycogenolysis, gluconeogenesis or high glucose intake. Therefore, this patient may have type-1 Diabetes Mellitus. Questions: What facts should be taken in account when interpreting the results of glucose tolerance test? The facts are:

- Patient should eat normal diet within 3 days before doing the test. The diet should contain at least 250g of carbohydrate.
- Patient should be fast over night at least 10-12 hours and does not eat during the test.
- The 75g of glucose should be dissolved in 300 ml of water and then ask the patient to drink it within 5 minutes after collection of fasting blood sample. A pregnant woman should be given less than 75g of glucose as it may affect the baby. If the amount of glucose given is less than recommended, it will affect the

result as the Oral Glucose Tolerance Test (OGTT) is standardized procedure.

- Patient should rest through the test; smoking is not permitted; drink of water is allowed.
- Blood sample should be collected in container that contains sodium fluoride to inhibit glycolysis.
- The patient must consult the health care provider if he/she is using medication that can interfere with the test result includes Thiazide diuretics (e.g. hydrochlorothiazide), beta-blockers (e.g. propranolol) oral contraceptive and some psychiatric drugs.
- There are interfering factors that affect OGTT. There are acute stresses for example, from surgery or infection, and vigorous exercise.
- Blood glucose rise with age and their renal threshold is increased.
- Time of sample collection is important (morning).

The method we used employed glucose oxidase- name 2 other methods for glucose estimation and describe the principles used. Ortho-toluidine method (mono step): glucose reacts with ortho-toluidine in hot acidic medium to form a green coloured complex. The intensity of the final colour produced is directly proportional to concentration of glucose in the sample. UV-kinetic method: This method also measures the concentration of glucose. The reagent contains ATP, hexokinase, NADP and glucose-6-phosphate dehydrogenase (G6PD) enzyme in ethanol amine buffer (PH 7.5). Why HbA1c a better guide to long term diabetes control than glucose? The determination of plasma and urine glucose provides information about the metabolic status only at the moment. Long term control of glucose can be obtained with relative ease by measuring the amount of particular haemoglobin fraction in red blood cells. The glucose enters the red blood cells and binds the haemoglobin to a very small extent. Although some of the glucose diffuse from the haemoglobin due to formation of covalent bond, but some of the glucose will react with a particular amino acid in the haemoglobin protein. The haemoglobin/glucose complex has different chemical properties from the haemoglobin, thus it can be separated chromatographic or electrophoresis technique. The estimation of Glycosylated haemoglobin (HbA1c) depends on the mean plasma glucose concentration and the life span of red blood cells (RBCs). The normal level of non-diabetic is < 6%. Also because it depends on the plasma glucose concentration, HbA1c in diabetic patient tend to be increased over the previous 1- months. The extent of elevation of HbA1c indicates the overall degree of blood

glucose control; in poorly controlled diabetes it may rise as high as 25%. Whitby, (1998). So the higher percentage of HbA1c indicates more glucose bound to haemoglobin and hence poor control of diabetic. Subsequently this test is used to assess the quality of the long term control of blood glucose in diabetic patient. Also it examines the patient faithfulness with which he/she followed the health care instruction and the effectiveness of the medication prescribed for treatment.