Analysis of blood glucose level | experiment

The body has the extraordinary ability to maintain the blood glucose levels in a specific range, using a feedback system. The failure for physiological systems to maintain blood glucose level could results in homeostatic diseases such as diabetes mellitus.

The Oral glucose tolerance test is a method of checking if one has diabetes. A solution consisting of 250ml of water and 75g glucose is given to patients and their blood glucose levels are monitored (Lee, 2009). The quick rise in blood glucose levels after the consumption of the solution is quickly maintained by normal individuals through the production of insulin by the islet of langerhans. However, diabetics cannot maintain the increase blood glucose levels as they are incapable of producing insulin. This in turn results in improper functionality of the kidney, resulting in excess glucose in the urine.

This experiment aimed to categorise patients (A-C) who have had an OGTT, according to their blood glucose levels before fasting and after 2 hours. Moreover, to categorise patient D who was given a random plasma glucose test (this patient did not fast and did not receive oral glucose tolerance test).

Glucose standards were prepared using the 1. 5ml of stock solution of 300 mg/mL glucose that was provided, Gilson 200µL pipette was used to prepare 500 µL each of a sequence of glucose standards containing 240 mg/mL, 180 mg/mL, 120 mg/mL, 60 mg/mL and 0 mg/mL, respectively. The sets of solutions were duplicated and 200 µL of each solution where place in LP4 tubes.

Samples A, B and C were given OGTT and the blood was collected in fluoride/EDTA anticoagulant and blood cells were separated using a centrifuge.

Both Fasting which was designated as (0) and 2 hour samples (2) where provided, labelling them as A0 and A2, respectively. Samples B, C, D and A0 were used directly without further dilution. Sample A2 was diluted, using a glass pipette, 1ml of sample A2 was added into a LP4 tube along with 2ml of distilled water. Duplicate of sample A-D was pipetted into LP4 tubes using Gilson.

GOD/HRP enzyme reagent where prepared. 2ml of GOD/HRP was added using a glass pipette to the 24 LP4 tubes each containing 200µL of sample or standard solution. Once added the GOD/HRP was allowed to settle in the LP4 tubes for 20 minutes allowing the catalyse to oxidise the glucose producing a blue colour. All the LP4 tubes where measured for the absorbance at 575 nm using a spectrophotometer, after using water as a blank.

Results

Sample Solution

Sample calculation for glucose standard (mg/dL):

C1V1x C2V2 = ((Glucose standard÷ 300) Ã- total volume) = Volume of glucose.

e. g ((240÷300) Ã- 500) = 400 µL of glucose.

Therefore, 400 µL of glucose must be added to 100 µL of water, resulting in 240 mg/dL of glucose stock solution.

Table . 4 – Conversions of glucose standard (mg/dL) to glucose concentration (mmoles/L).

Sample calculation: Mean absorbance’s for each sample where inputted into the graphical equation, replacing Y, which gave the glucose concentration in (mg/dL) e. g

0. 0002x + 0. 0073, replacing mean absorbance for A0 (0. 042) with y. which gives 0. 042 = 0. 0002x + 0. 0073 then solve to find x, giving the glucose concentration in (mg/dL).

The conversion from mg/dL to mmoles/L was deduced by multiplying each glucose concentration (mg/dL) by 0. 055. E. g 267 Ã- 0. 055 = 14. 7 mmoles/L.

This factor is specific to glucose only because 1 mmoles/L = 18 mg/dL (Martin & Blumer 2004).

A2 concentration was multiplied by 3, as the sample was diluted by a factor of 3.

Discussion

The aim of the experiment was to determine and categorise sample (A, B, C and D) into groups to see whether they had normal blood glucose concentration or were diabetics. Diabetes is caused by a homeostatic imbalance leading to kidney complications and blindness (Chiras, 2005). Comparing the samples glucose levels in (mmoles/L) against the reference table, it’s clear to see that sample A’s blood glucose level is above the reference range of a diabetic both at fasting (9. 5mmoles/L) and after 2

hours (18. 7mmoles/L), showing that sample A in a diabetic. Sample B's blood glucose level indicated that the individual is most likely has a impaired glucose tolerance as they have a blood glucose level of 5. 7mmoles/L at fasting and 8. 2mmoles after 2 hours, supporting the case for sample B having IGT. Sample C can be classified as normal, their glucose concentration matches with the reference range for a normal individual. Sample D who did not receive the OGTT, but was given a random plasma glucose test shows signs of having IGT, but because the individual was not fasting before the test, they could have had a meal which could of increased their blood glucose concentration, giving the false impression of IGT, it would be advisable to ask the individual to take a OGTT test.

Graph. 1 shows a linear relationship. Both trials where plotted to give an indication on how precise the data was. The difference between trial 1 and 2 for the glucose standards where relatively small overall, indicating that the results obtained where precise. However, at glucose standard 120 mg/l there was a significant difference to the rest of the data, showing a wider range between the trials. This could have been caused due to human error, such as placing the LP4 tube incorrectly into the spectrophotometer or the incorrect use of a Gilson pipette. R^2 value of o. 9265 shows that there is a very good correlation between absorbance and glucose standard solution as it is very close to one.

The method used had few errors, the errors which had occurred where due to human error or incorrect use of equipment, to enhance the experiment more repeats have to be prepared for glucose standards and samples to give more reliable result.

Additional Question – An enzyme hexokinase catalyzes the phosphorylation reaction between glucose and ATP producing glucose-6-phosphate glucose-6-phosphate dehydrogenase converts glucose-6-phosphate to 6-phosphogluconate, forming NADPH which can be measured at 340nm. The NADPH has a direct correlation to the glucose intake; therefore the more glucose in one's blood the more NADPH produced which is measured by spectrophotometer (Reginald & Grisham 2008).

References

Chiras, D., 2005. Human Biology. 5th ed. Massachusetts: Jones and Bartlett Publishers. Pp. 168- 180.

Lee, M., 2009. Basic Skills in Interpreting Laboratory Data. 4th ed. American Society of Health-System Pharmacists. Pp. 276-278.

Martin, p. Blumer, I., 2004. The everything diabetes book. Cincinnati: Adams Media Corporation. Pp 297.

Reginald, G. Grisham, C., 2008. Biochemistry. Florence: Thomson Brooks/Cole

There were flaws in some aspects of the methodology.

Mention about homeostasis and importance of glucose tests

Mention that sample B had a increase in the absorption between 0 and 2, possible diabetic because individual is unable to ‘ manage’ the glucose. Further testing to confirm ?? in comparison, sample a and b both had a

decrease in absorption from 0 to 2 indicating that they where able to control the glucose solution.

Trends in absorption increases as sample glucose increases, likewise for concentration.

In relation to the aims

Linear relationship between the glucose sample and absorption

This is where you discuss your ‘ findings in light of the material given in the Introduction. The implications of any practical problems constructively discussed and theory related to practice. Evidence of further reading and any inferences drawn from statistical analysis should be included.

You must also show a clear evidence of your own original critical analysis’ (paraphrased; Dr David Perry 2008).

Additional Question – briefly describe one other enzymic method for glucose determination that is used in clinical laboratories.

Hexokinase catalyzes the phosphorylation of glucose in the presence of ATP (Reaction 2. 3). The glucose-6-phosphate formed is converted to 6-phosphogluconate by a second enzyme, glucose-6-phosphate dehydrogenase (Reaction 2. 4). NADPH is formed in the reaction and can be measured at 340 nm.

Reaction 2. 3 glucose + ATP G6P + ADP

Reaction 2. 4 G6P + NADP 6-phosphogluconate + NADPH + H+

HBA1C test