

PBL BIOCHEMISTRY
CARBOHYDRATE HOMEOSTASIS PART – 2
DIABETES MELLITUS – DIAGNOSIS AND MONITORING

What are some of the metabolic functions affected by Insulin?

- ❑ Metabolic functions enhanced are:
 - Glucose uptake in muscle and adipose tissue
 - Glycogenesis
 - Glycolysis,
 - Protein synthesis,
 - Cellular uptake of ions, especially Potassium and Phosphate ions

- ❑ Insulin stimulates biosynthesis of:
 - Glycogen, Fats, Proteins,
- ❑ Insulin inhibits degradation of:
 - Glycogen, Fat, Proteins
- ❑ Insulin affects the uptake of Glucose into:
 - Muscle cells, Adipose tissue, Connective tissues, White blood cells
- ❑ Insulin DOES NOT affect uptake of Glucose into:
 - Brain, Liver, Kidneys
- ❑ Insulin counter regulatory hormones, such as Glucagon, Epinephrine, Glucocorticoids, and Growth hormone oppose the actions of Insulin

What is the Insulin feedback loop?

- ❑ Insulin feedback loop is: Action of Insulin and Insulin Counter Regulatory Hormones in regulating blood glucose level
- ❑ Homeostatic regulation of blood glucose is the result of balance between action of Insulin and Insulin Counter-regulatory Hormones: **INSULIN FEEDBACK LOOP**
- ❑ Failure of Insulin feedback loop affects homeostatic regulation of blood glucose
- ❑ Failure of part of the loop can cause increase in blood glucose level,
 - Glucose cannot get into cells that use or store it
 - Excess Glucose may be dumped into the urine resulting in “Sweet Urine”
(Diabetes Mellitus!!!!)

DIABETES MELLITUS (DM)

What is the definition of Diabetes Mellitus?

- ❑ Precise definition of Diabetes Mellitus is very difficult
- ❑ DM: a disease characterized by derangements in Carbohydrate, Fat and Protein metabolism
- ❑ DM: a syndrome characterized by Hyperglycaemia due to:
 - An absolute or relative lack of Insulin and/or Insulin Resistance

What are the major types of Diabetes Mellitus?

- ❑ **Primary DM** is generally sub-classified into:
 - **Type 1 DM {Insulin Dependent Diabetes Mellitus (IDDM or)}**
 - **Type 2 {Non-Insulin Dependent Diabetes Mellitus (NIDDM)}**
- ❑ **Secondary DM:** may result from a number of causes including:

- Pancreatic disease, Endocrine disease such as Cushing's syndrome, Drug therapy, Insulin receptor abnormalities, Gestational diabetes

What are some of the possible causes of Type 1 DM?

- Type 1 DM, also called Juvenile-Onset Diabetes because it usually appears in childhood or in teenagers
 - Type 1 DM is **not only** limited to juvenile patients
- Causes of Type 1 DM include:
 - Inability to produce Insulin, due to either:
 - Defective Beta cells in Pancreatic Islets, or
 - Absent of Beta cells in Pancreatic Islets
 - Autoimmune process causing destruction of Beta cells in Pancreatic Islets
 - Presence of Islet cell antibodies in serum predicts future development of Type 1 DM
 - Islet-cell antibodies act against Glutamic Acid Decarboxylase (GAD)
 - Environmental precipitating factors:
 - Viral infection, Dietary factors (Anti-metabolites)

What are some of the characteristics of Type 1 DM?

- Type 1 DM is usually characterized by:
 - Deficiency in Insulin and the consequent Hyperglycaemia
- Hyperglycaemia causes blood glucose level to exceed Renal Threshold of 200mg/dl or 11.0mmol/L, Resulting in Glucosuria
- Following sequence of events occur:
 - Sugar is excreted in urine (**Glucosuria**)
 - Water follows the sugar due to osmosis (**Osmotic Diuresis**)
 - Large volume of urine is passed out (**Polyuria**)
 - Patient becomes thirsty, thus drinks a lot of water (**Polydipsia**)
 - Lack of Insulin: Thus, Muscles, Adipose tissue, Connective tissues and White Blood Cells cannot utilize Glucose present in blood (Starvation in the midst of plenty),
 - Patient become hungry and eats a lot (**Polyphagia**)
 - Due to continuous lack of Insulin, glucose cannot enter Muscle and other tissues, thus patient may start to loose weight (Wasting)
 - Patient may develop Ketoacidosis (**Why?**)

What are some of the consequences if Type 1 DM is not controlled?

- **Hyperglycaemia:**
 - Partly due to inability of Insulin-dependent tissues to take up glucose from blood (Starvation in the midst of plenty!! (**Why?**))
 - Increased Hepatic Gluconeogenesis, and
 - Depressed Glycolysis resulting from low glucose levels in cells
- **Hyper-Lipoproteinemia (Chylomicrons and VLDL):**
 - May be due to low Lipoprotein Lipase activity in Adipose tissue,
 - Insulin is required for biosynthesis of Lipoprotein Lipase
- **Ketoacidosis:** Increased production of Ketone bodies:

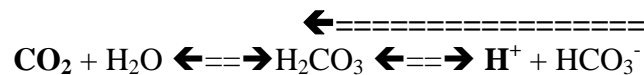
- Acetone, Acetoacetic acid, and β -Hydroxybutyric acid

Why is insulin used to control Type 1 DM?

- Administration of insulin does not cure Type 1 DM, but it alters the clinical course of the disease
- Insulin promotes Glucose uptake and restoration of normal metabolism
- When the hypoglycemia is corrected:
 - Loss of water and electrolytes ceases
 - Formation of Ketone bodies ceases, allowing the Acid-Base balance to return to normal
 - Metabolism of Glucose via Glycolysis and TCA Cycle also allows the Acid-Base balance to return to normal
 - Changes in plasma Bicarbonate levels during treatment serve as a guide to monitor the success of treatment

What are some of the consequences of DKA?

- Decreased Glucose transport into tissues leads to **Hyperglycaemia**, which gives rise to **Glucosuria**
- Increased Lipolysis leads to formation of **Ketone bodies**,
 - Resulting in **Ketonemia**, and **Ketonuria**
 - Acetone is exhaled in Lungs and manifests itself in breath
 - **Acetoacetic acid** and **β -Hydroxybutyric acid** causes **acidosis**
- HCO_3^- concentration in blood falls (Metabolic acidosis) and more Carbonic acid (H_2CO_3) is formed,



- Carbonic acid is converted to CO_2 , which then stimulates respiratory center to remove excess CO_2
- Increased removal of CO_2 causes rapid deep breathing (**Hyperventilation**) observed in patients with DKA
 - Hyperventilation (**Kussmaul breathing**) is a response by the lungs to compensate for Metabolic Acidosis, by removing excess CO_2
- Glycosuria causes Osmotic Diuresis, which leads to:
 - Loss of water, Loss of Electrolytes, Loss of Calcium, Magnesium, and Phosphate
- Dehydration if severe produces Pre-renal Uraemia and may lead to Hypovolaemic Shock
- Frequent vomiting is usually present and accentuates the loss of water and electrolytes

Take Note:

- Development of DKA is a series of interlocking vicious circles all of which must be broken to aid restoration of normal Carbohydrate, Lipid and Protein metabolism
- Correction of DKA requires rapid treatment dictated by severity of the metabolic abnormalities and the associated tissue water and electrolyte imbalance

Why is Insulin essential in the control of DKA?

- ❑ Insulin lowers plasma Glucagon level,
- ❑ Insulin stimulates Glucose uptake into target tissues
- ❑ Insulin antagonizes Catabolic effects of Glucagon on the Liver,
- ❑ Insulin inhibits flow of Ketogenic and Gluconeogenic substrates (free fatty acids and amino acids) from the periphery

General occurrence of Type 2 DM:

- ❑ **Type 2 DM** accounts for about 85% of diagnosed cases of DM in PNG
- ❑ **Type 2 DM:** Formally called:
 - Non-Insulin Dependent Diabetes Mellitus (NIDDM)
 - Maturity-onset diabetes mellitus (most common in middle-age obese individuals, can occur in non-obese middle-age individuals, can occur in any age group)

What are some of the possible causes of Type 2 DM?

- ❑ May be due to any of the following:
 - ❑ Resistance of peripheral tissues to Insulin, despite normal or high Insulin level in circulation
 - ❑ Deficiency or defect in Insulin Receptors in target tissues (Relative Insulin deficiency)
 - ❑ Obesity, (most commonly associated clinical feature of Type 2 DM)
 - ❑ Defect in Insulin Receptors is related to increased levels of Tumor Necrosis Factor- α (TNF- α) in Adipocytes
 - ❑ Increase in adipose tissue mass causes increase in production of TNF- α , which then blocks Insulin Receptors
- ❑ Diet can often be used to control Type 2 DM in Obese patient
- ❑ Obese patients that are motivated to lose weight:
 - ❑ Insulin receptors will increase in number, and the
 - ❑ Post-receptor abnormalities will improve, which may result in increased tissue sensitivity to insulin and Glucose tolerance
- ❑ Defects occurring within Insulin-responsive cells at sites beyond Insulin receptors
- ❑ Non-obese individuals:
 - Type 2 DM may be **cause not only by Insulin Resistance, but also by Impaired Pancreatic β -cell function resulting in Relative Insulin Deficiency**

What are the consequences of uncontrolled Type 2 DM?

- ❑ Uncontrolled Type 2 DM is characterized by:
 - Hyperglycaemia, Hyper-Triglyceridemia
- ❑ Hyperglycaemia causes accumulation of glucose in:
 - Eyes (Lens epithelium, Retinal capillaries),
 - Peripheral Nerve cells (Schwann cells),
 - Kidneys (Papillae, Glomerulus)
- ❑ Aldose Reductase and Sorbitol Dehydrogenase in these tissues converts:
 - Glucose to: Fructose, Dulcitol and Sorbitol
- ❑ Sorbitol accumulates and crystallizes causing damage to tissues by causing them to swell

- Resulting in conditions such as:
 - Cataract formation in the eyes (diabetic cataract),
 - Diabetic Neuropathy including loss of sensation
 - Retinopathy (damage to retina)
 - Damage to blood vessels (Vascular disease)
 - Damage to kidneys leading to renal failure
 - Damage to Cardiac tissue (Ischemic heart disease)
- Ketoacidosis is not PRESENT in patients Type 2 DM (**WHY?**)

DIAGNOSIS OF DIABETES MELLITUS:

Is the diagnosis of DM the same as monitoring of DM?

- Diagnosis of DM is not the same as monitoring of DM
- **Diagnosis:** to clinically establish a condition in a patient
- **Monitor:** to follow progress on a condition that has been diagnosed
- Specific biochemical tests and guidelines are used for diagnosis of DM
- Specific biochemical tests and guideline are used for monitoring DM

Some Biochemical tests for diagnosis of DM

- **Glucosuria (Glycosuria):**
 - Glucosuria is a good first-line screening test for DM
 - Glucose usually appears in urine when plasma glucose concentration rises above renal threshold (11.0mmol/L or 200mg/dL)
 - Glucosuria may occur in some individuals with low renal threshold for glucose;
 - Individuals have Glucosuria without DM
 - Conversely, renal glucose threshold increases with age, thus some diabetics may have DM without Glucosuria
 - Glucosuria indicates Hyperglycaemia over the period of formation of the urine, it does not reflect the exact level of blood glucose at the time of testing
- **Ketone in Urine (Ketonuria) or in Blood plasma (Ketonemia)**
 - Ketone bodies (Acetone, Acetoacetate, and Beta-Hydroxybutyrate) may accumulate in plasma and appear in urine in Type 1 DM
 - Ketonuria or Ketonemia is not an automatic diagnosis of ketoacidosis, which is a serious condition
 - Ketonuria or Ketonemia may occur during prolonged fasting
 - Dry reagent strips, which detect Acetoacetate but not Beta-Hydroxybutyrate usually provides an underestimation of Ketonemia or Ketonuria
- **Fasting blood glucose (FBG):**
 - FBG is measured after an overnight fast (about 8 to 10 hours)
 - FBG is better than RBG for diagnostic purposes
 - FBG above 8.0mmol/L on two occasions may be diagnostic of DM
 - FBG between 6.0 to 8.0mmol/L may be interpreted as borderline

- Measurement of FBG on Whole blood, Plasma or Capillary blood have different cut-off points (see Table above)
- **Random blood glucose (RBG)**
 - RBG is one of the major tests required in an emergency
 - RBG of less than 8.0mmol/L is usually expected in non-diabetics
 - RBG higher than 11.0mmol/L in more than one occasion indicates that the individual be investigated more thoroughly for DM

Two Hours Post-Prandial blood glucose:

- Measure blood glucose level 2-hours after consumption of a meal
- It is a better indicator of DM than RBG and FBG
- Individuals with blood glucose above 11.0mmol/L should be investigated more thoroughly for DM

Briefly explain how to perform oral glucose tolerance test (OGTT)?

- OGTT is recommended only if results from RBG and FBG tests cannot be interpreted clearly to justify DM in a patient
- OGTT **must** be carried out under proper clinical supervision
- Patient must be properly briefed before starting the procedure
- Measure FBG and urine glucose of patient after an overnight fast
 - Record both results
- Prepare a solution containing 75.0g glucose in about 300ml water
- Patient is requested to drink the solution within 5 minutes
- Measure blood glucose level of patient every 30 minutes for 2 hours
- Measure glucose in urine after 2 hours

Patient should be sitting comfortably throughout the test, should not smoke or exercise and should have been on a normal diet for at least 3 days prior to the test

- **WHO recommended guidelines for diagnosis of DM**
 - World Health Organization (WHO) published guidelines for diagnosis of DM on the basis of blood glucose results and the response to an Oral Glucose Load
 - Table shows the WHO criteria for diagnosis of DM and Impaired Glucose Tolerance (IGT)

RANDOM GLUCOSE SAMPLE (mmol/L)			
	Diabetes likely	Diabetes uncertain	Diabetes unlikely
Venous plasma	≥ 11.1	5.5 - < 11.1	< 5.5 (99.0 mg/dl)
Venous blood	≥ 10.0	4.4 - < 10.0	< 4.4 (79.2 mg/dl)
Capillary plasma	≥ 12.2	5.5 - < 12.2	< 5.5
Capillary blood	≥ 11.1	4.4 - < 11.1	< 4.4
STANDARDISED ORAL GLUCOSE TOLERANCE TEST (mmol/L)			
		Diabetes	IGT
Venous plasma	Fasting	≥ 7.8	< 7.8
	2hours	≥ 11.1	7.8 - < 11.1
Venous blood	Fasting	≥ 6.7	< 6.7
	2hours	≥ 10.0	6.7 - < 10.0
Capillary plasma	Fasting	≥ 7.8	< 7.8
	2hours	≥ 12.2	8.9 - < 12.2
Capillary blood	Fasting	≥ 6.7	< 6.7
	2hours	≥ 11.1	7.8 - < 11.1

(Note: to convert mmol/L to mg/dl multiply by 18.0)

How do you interpret the OGTT result?

- Use the data obtained to draw a graph of “Time vs. Blood glucose level”
- In Asymptomatic patients, OGTT should be interpreted as diagnostic of DM only when:
 - There is an increased 2-hour glucose level, and
 - Blood glucose was equal to or greater than 11.0mmol/L (200.0 mg/dL) at some other point during the test
- **If patient has normal fasting plasma glucose and only the 2-Hour value is in the diabetic range, the test should be repeated after approximately 6 weeks**
- Impaired Glucose Tolerance (IGT) should be regarded as abnormal because it signals that the patient is at an intermediate stage between normality and DM and is at an increased risk of developing DM
- Such patients should be followed up yearly, and dietary treatment may be used

MONITORING OF DIABETES MELLITUS:

How can a patient with DM be monitored?

(Long-term indices of diabetic control)

Glycation of ECF proteins

- A high concentration of glucose in the extracellular fluid (ECF) leads to its non-enzymatic attachment to the Lysine residues of a variety of proteins.
- This is called Glycation.
- The extent of this process depends on the Blood glucose level.
- It is virtually irreversible at physiological pH concentration and therefore the glucose molecule will remain attached until the protein molecule is degraded.
- The concentration of Glycated protein is therefore a reflection of a mean blood glucose level prevailing in the ECF for the duration of that protein.

Glycosylated Hemoglobin (HbA_{1c})

- In adults about 98% of Hb in the RBC is Hb A₁.
- About 7% of Hb A consists of a type of Hb (HbA₁) that can combine strongly with Glucose in a process called Glycosylation.
- Once Glycosylation occurs, it is not easily reversible.
- HbA₁ is made up of three components (HbA_{1a}, HbA_{1b}, and HbA_{1c}).
- Of these HbA_{1c} is the highest in concentration and it is also the component that most strongly undergoes Glycosylation with Glucose.
- Thus, as the RBC circulates its HbA₁ combines with blood glucose in a non-enzymatic reaction to form Glycosylated Hb (HbA_{1c}).
- The amount of HbA_{1c} formed is dependent on the concentration of Glucose in the blood over the 120-day life span of the RBC.
- Therefore determination of the HbA_{1c} value reflects the average blood sugar level for the 100- to 120-day period before the test.
- The more glucose the RBC is exposed to, the greater the amount of HbA_{1c} formed.
- One important advantage of this test is that the blood sample can be drawn at any time, because it is not affected by short-term variations (e.g., food intake, exercise, stress, hypoglycemic agents, and patient cooperation).
- Very high short-term blood glucose levels can cause elevation of HbA_{1c}.
- The elevation of HbA_{1c} occurs about 3 weeks after sustained elevation in blood glucose.
- It takes at least 4 weeks for the HbA_{1c} to decrease after a sustained reduction in blood glucose.
- Thus the measurement of HbA_{1c} is a good Clinical indicator of the time-average control of blood glucose.
- In normo-glycemic individuals HbA_{1c} represents 4% to 6% of the total HbA.
- In prolonged Hyperglycaemia the concentration HbA_{1c} may rise to as much 12% of the Total Hb.
- Patients with DM have high blood glucose levels and therefore high amounts of HbA_{1c}.

- This test is accepted as a good index of diabetic control and is used routinely in most diabetic clinics to complement the information from single blood glucose levels, or indeed a patient's log of his or her own blood glucose measurements.

What are some of the major uses of the Hb A_{1c} test?

Some of the major uses of HbA_{1c} test include:

- Evaluating the success of diabetic treatment and patient compliance.
- Comparing and contrasting the success of past and new forms of therapy.
- Determining the duration of hyperglycaemia in patients with newly diagnosed DM.
- Providing a sensitive estimate of glucose imbalance in patients with mild diabetes.
- Individualizing diabetic control regimens.
- Providing a sense of reward for many patients when the test shows achievement of good diabetic control.
- Evaluating the diabetic whose glucose levels change significantly day to day (brittle diabetes).
- Differentiating short-term hyperglycaemia in non-diabetics (e.g., recent stress or myocardial infarction) and diabetics (in whom the glucose has been persistently elevated).

Fructosamine:

- Many other proteins in addition to Hb are Glycated when exposed to glucose in the blood.
- An indication of the extent of this glycation can be obtained by measuring Fructosamine, the Ketoamine product of non-enzymatic glycation.
- As albumin is the most abundant plasma protein, Glycated Albumin is the major contributor to serum Fructosamine measurements.
- As this protein has a shorter half-life than Hb, Fructosamine measurements are complementary to Hb A_{1c} providing an index of glucose control over the 3 weeks prior to its measurement.

Microalbuminuria:

- Microalbuminuria may be defined as an albumin excretion rate intermediate between normality (2.5 to 25 mg/day) and Macroalbuminuria (> 250 mg/day).
- The small increase in urinary albumin excretion is not detected by simple albumin stick tests and requires confirmation by careful quantization in a 24hour urine specimen.
- The importance of Microalbuminuria in the diabetic patient is that it is a signal of early, reversible renal damage.

SUMMARY:

Diagnosis and monitoring of Diabetes Mellitus:

- The diagnosis of DM is made on the basis of blood glucose concentrations either alone or in response to an oral glucose load.
- In Asymptomatic patients the results of an OGTT should be interpreted as diagnostic of DM only when there is an increased 2 h glucose concentration, and the blood glucose is also equal to or greater than 11.0 mmol/L (198.0 g/dl) at some other point during the test.

- The HbA_{1c} and Fructosamine are measures of protein glycation and serve as indices of long-term glucose control.
- Micro-Albuminuria is a measure of early, reversible, diabetic nephropathy.

Hypoglycaemia:

- Hypoglycaemia is a laboratory “diagnosis” which is usually taken to mean a blood glucose level below 2.2mmol/L (45.0 g/dl).
- Hypoglycaemia may be due to a number of underlying conditions including endocrine disorders, liver disease, inborn errors of metabolism and gastrointestinal surgery.
- **The cause (or biochemical basis) is an imbalance between glucose intake, endogenous glucose production and glucose utilization.**
- A low blood glucose level normally leads to the stimulation of Catecholamine secretion and correction of hypoglycemia through suppression of Insulin secretion and stimulation of Glucagon, Cortisol and Growth Hormone.
- The Catecholamine surge accounts for the signs and symptoms most commonly seen in Hypoglycemia, i.e., Sweating, Shaking, Tachycardia, Nausea and Weakness.
- Hypoglycaemia decreases the glucose fuel supply to the brain and may lead to brain damage particularly in infants.

Laboratory Investigation:

The Biochemistry laboratory can confirm hypoglycemia and may also provide some useful clues to the underlying cause.

- **Blood glucose:** The detection of hypoglycemia is by blood glucose testing. Urine testing cannot detect hypoglycemia. **WHY???**
- **Plasma Insulin:** Insulin measurements can lead to the diagnosis or exclusion of Insulinoma. They play no part in the diagnosis of diabetes mellitus.
- **Insulin/Glucose ratio:** In order to make better diagnostic use of Insulin measurements, the ratio of Insulin and Glucose concentrations, measured on the same sample, should be reported.
- **Plasma C-peptide:**
 - Insulin secretion in Insulin-treated diabetics cannot be assessed by the measurement of plasma insulin since the insulin given therapeutically will also be measured in the assay.
 - However, insulin and its associated Connecting-peptide (or C-peptide) are secreted by the Islet cells in equimolar amounts and thus measurement of C-peptide levels together with insulin can differentiate between hypoglycemia due to Insulinoma (high C-peptide) and that due to exogenous insulin (low C-peptide).

Reference:

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