GLUCOSE HOMEOSTASIS/INSULIN PART - II

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State some metabolic functions affected by Insulin?

- Metabolic functions enhanced are:
  - Glucose uptake in muscle and adipose tissue,
  - Glycogenesis,
  - Glycolysis,
  - Protein synthesis,
  - Cellular uptake of Potassium and Phosphate ions;
- Insulin stimulates biosynthesis of:
  - Glycogen, Fats & Proteins,
- Insulin inhibits degradation of:
  - Glycogen, Fat & Proteins

- Insulin affects uptake of Glucose into:
  - Muscle cells,
  - Adipose tissue,
  - Connective tissues,
  - White blood cells;
- Insulin DOES NOT affects Glucose uptake into:
  - Brain,
  - Liver,
  - Kidneys
- Insulin counter regulatory hormones oppose actions of Insulin (counter regulatory hormones are):
  - Glucagon,
  - Epinephrine,
  - Glucocorticoids, and
  - Growth hormone

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 balance between actions of Insulin and Insulin Counter-regulatory Hormones: **INSULIN FEEDBACK LOOP ;**
- Failure of the feedback loop affects regulation of blood glucose;
- Failure of part of the loop causes increase in blood glucose level;
  - Glucose cannot get into cells that use or store it;
  - Excess Glucose may be dumped in urine resulting in "Sweet Urine" (**Diabetes Mellitus**)

DIABETES MELLITUS (DM)

- **What is Diabetes Mellitus (DM)?**
- Precise definition of DM is very difficult;
- Diabetes Mellitus:
  - Disease characterized by derangements in Carbohydrate, Fat and Protein metabolism;
- Diabetes Mellitus:
  - Syndrome characterized by Hyperglycemia 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 I DM: Insulin Dependent Diabetes Mellitus (IDDM);
- Type 2 DM: Non-Insulin Dependent Diabetes Mellitus (NIDDM)

- **Secondary DM**: may be due to:
  - Pancreatic disease,
  - Endocrine disease (Cushing's syndrome),
  - Adrenal diabetes,
  - Drug therapy,
  - Insulin receptor abnormalities,
  - Gestational diabetes,

What are some of the causes of Type 1 DM?

- Type 1 DM, (Juvenile-Onset DM),
- Type 1 DM is **not** limited to juvenile patients;
- Causes of Type 1 DM include the inability to produce Insulin, due to either:
  - Defective Beta cells in Pancreatic Islets,
  - Absent of Beta cells in Pancreatic Islets;
- Autoimmune process causing destruction of Beta cells in Pancreatic Islets,

- Presence of Islet cell antibodies in Serum may predicts future development of Type 1 DM;
- Islet-cell antibodies act against Glutamic Acid Decarboxylase (GAD);
- Environmental precipitating factors of DM:
  - Viral infections,
  - Dietary factors (presence of anti-metabolites in some foodstuffs);

What are some of the characteristics of Type 1 DM?

- Type 1 DM is usually characterized by:
  - Deficiency in Insulin and consequent Hyperglycemia,
  - Hyperglycemia causes blood glucose level to exceed Renal Threshold of 200mg/dl or 11mmol/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, drinks lots of water (**Polydipsia**),
- There is 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 the consequences if Type 1 DM is not controlled?

- **Hyperglycemia**:
  - Partly due to inability of Insulin-dependent tissues to use blood glucose (Starvation in the midst of plenty, (**Why?**)
  - Increased Hepatic Gluconeogenesis,
  - Depressed Glycolysis due low glucose levels in cells;
- **Hyper-Lipoproteinemia (Chylomicrons and VLDL)**:
  - 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,
  - β-Hydroxybutyric acid;

Why can insulin be used to control Type 1 DM?

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

What are some of the consequences of DKA?

- Decreased Glucose transport into tissues leads to **Hyperglycemia**, which gives rise to **Glucosuria,**
- Increased Lipolysis leads to formation of **Ketone bodies**,
  - Resulting in **Ketonemia** and **Ketonuria,**
- Acetone is exhaled in Lungs and passed out in breath,
- **Acetoacetic acid** and **β-Hydroxybutyric acid** causes **acidosis**
- **[HCO_3^-]** in blood falls causing 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

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$$\mathbf{CO_2} + H_2O \leftarrow\!=\!=\!\rightarrow H_2CO_3 \leftarrow\!=\!=\!\rightarrow \mathbf{H^+} + HCO_3^-$$

- 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,
  - Loss of Magnesium,
  - Loss of Phosphate,
- Dehydration if severe produces Pre-renal Uremia and may lead to Hypovolaemic Shock,
- Frequent vomiting may be present and accentuates loss of water and electrolytes,

- DKA is a series of interlocking vicious circles which must be broken to restore normal Carbohydrate, Lipid and Protein metabolism,

- Correction of DKA requires rapid treatment dictated by severity of metabolic abnormalities and 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 Gluconeogenic & Ketogenic substrates (free fatty acids and amino acids) from the periphery;

General occurrence of Type 2 DM

- **Type 2 DM:** accounts for 85% cases of DM in PNG
- Formally called:
  - Non-Insulin Dependent Diabetes Mellitus (NIDDM);
  - Maturity-onset diabetes mellitus,
- 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 blood,
  - Deficiency or defect in Insulin Receptors in target tissues (Relative Insulin deficiency),
  - Obesity, (may have clinical features of Type 2 DM),
  - Defect in Insulin Receptors is related to increased levels of Tumor Necrosis Factor-α (TNF-α) in Adipocytes,
  - Increase adipose tissue mass causes increase TNF-α, which then blocks Insulin Receptors,

- Diet can control Type 2 DM in Obese patient,
- Obese patients that are motivated to lose weight:
  - Insulin receptors will increase in number,
  - Post-receptor abnormalities will improve, resulting in tissue sensitivity to insulin and Glucose tolerance;
- Defects occurring within Insulin-responsive cells at sites beyond Insulin receptors,
- In 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**:
  - Hyperglycemia,
  - Hyper-Triglyceridemia,
- Hyperglycemia 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 eyes (diabetic cataract),
  - Diabetic Neuropathy and loss of sensation,
  - Retinopathy (damage to retina),
  - Damage to blood vessels (Vascular disease),
  - Damage to kidneys causing renal failure,
  - Damage to Cardiac tissue (Ischemic heart disease),

- Type 2 DM does not cause Ketoacidosis (**WHY?)**

DIAGNOSIS OF DIABETES MELLITUS

Is 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 already 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):

- Good first-line screening test for DM,
- Glucose appears in urine when plasma glucose level rises above renal threshold (11mmol/L or 200mg/dL);
- Glucosuria may occur in patients with low renal threshold for glucose;
  - Individuals are said to have Glucosuria without DM,
- Renal threshold increases with age, thus some patients may have DM without Glucosuria,
- **Glucosuria indicates Hyperglycemia over the period of formation of the urine, it does not reflect the exact level of blood glucose at the time of testing;**

Fasting Blood Glucose (FBG):

- FBG is measured after overnight fast (8 to 10hrs),
- FBG is better than RBG for diagnostic purposes,
- FBG above **8.0mmol/L** on **two different occasions** may be diagnostic of DM,
- FBG between **6.0 to 8.0mmol/L** is borderline,
- **(Note: to convert mmol/L to mg/dl multiply by 18.0)**

- **Measurement of FBG on Whole blood, Plasma or Capillary blood have different cut-off points (Table 1)**

Random Blood Glucose (RBG):

- RBG: one of major tests required in emergency,
- RBG 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;
- **Table 1: WHO guideline for diagnosis**

Two Hours Post-Prandial blood glucose:

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

- **Tables 1 & 2** show WHO recommended guidelines for diagnosis of DM;
- 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**)

Table 1: WHO Guideline for diagnosis of DM

RANDOM GLUCOSE SAMPLE (mmol/L)			
	Diabetes likely	Diabetes uncertain	Diabetes unlikely
Venous plasma	≥ 11.1	5.5 - < 11.1	< 5.5
Venous blood	≥ 10.0	4.4 - < 10.0	< 4.4
Capillary plasma	≥ 12.2	5.5 - < 12.2	< 5.5
Capillary blood	≥ 11.1	4.4 - < 11.1	< 4.4

- **Ketones in Urine (Ketonuria)**
- **Ketones in Blood plasma (Ketonemia)**
- Ketone bodies (Acetone, Acetoacetate, Beta-Hydroxybutyrate) may accumulate in plasma and appear in urine in Type 1 DM,
- Ketonuria, Ketonemia is not automatic diagnosis of Ketoacidosis, which is a serious condition;
- Ketonuria, Ketonemia may occur in prolonged fasting,

How is Oral Glucose Tolerance Test (OGTT) performed in a patient, when requested?

- **OGTT is recommended only if RBG and FBG tests cannot be interpreted clearly to justify DM;**
- **OGTT must be carried out under proper clinical supervision;**
- Patient should be sitting comfortably throughout test, should not smoke or exercise and should be on normal diet for at least 3 days prior to the test;

- Brief the patient before starting the procedure;
- Measure FBG and Urine Glucose of patient after an overnight fast;
- Record both results;
- Prepare solution containing **75.0g of Glucose in 300ml water**;
- Patient should drink all the solution within 5 min,
- Measure blood glucose level every 30 min for 2 hrs,
- Measure glucose in urine after 2 hrs,
- Record all the results;

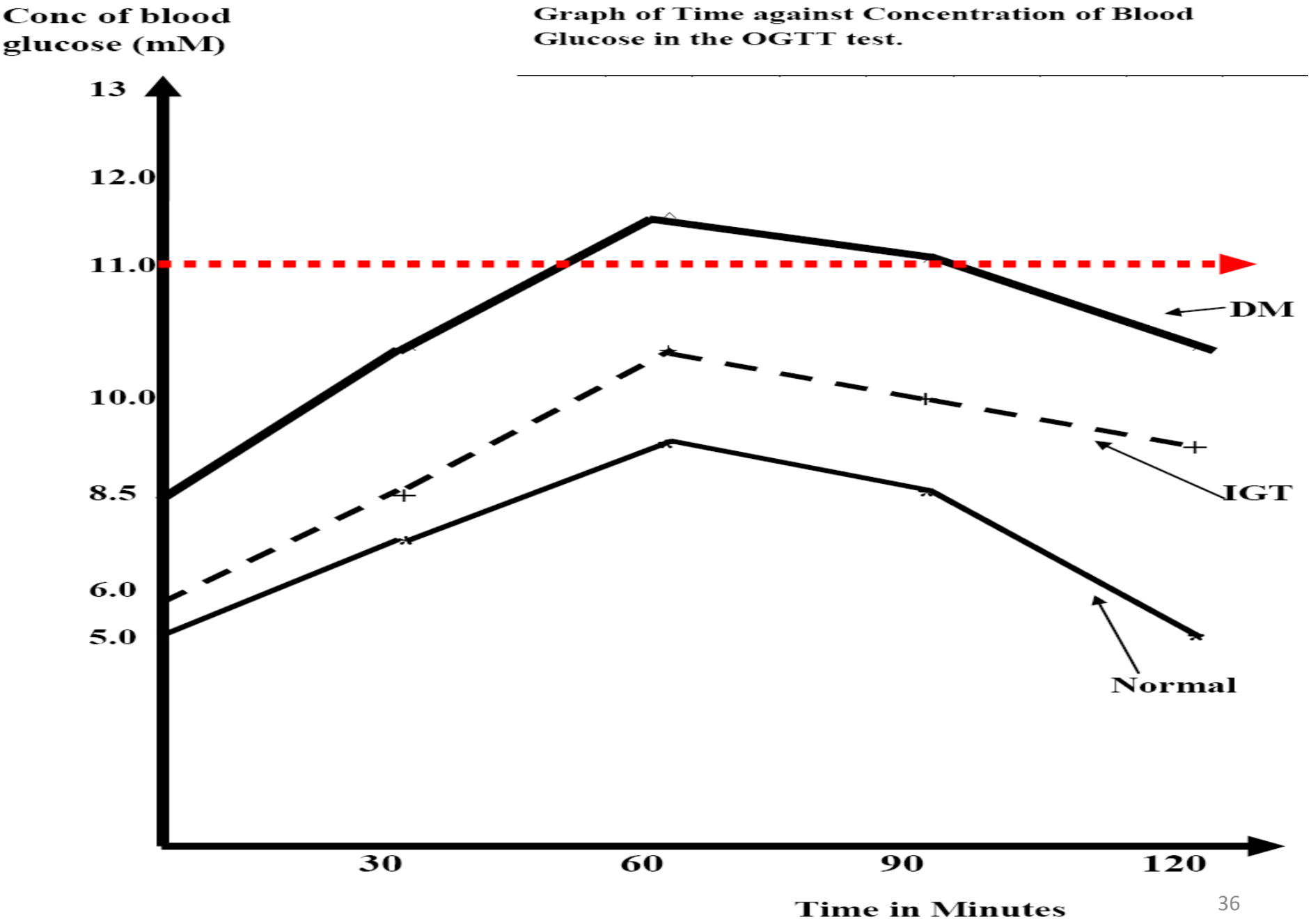
How do you interpret the OGTT result?

- Use results obtained to draw a graph of "Time vs. Blood Glucose level" or Use WHO Guidelines in **Table 2**
- In Asymptomatic patients, OGTT should be interpreted as diagnostic of DM only when:
  - There is an increased 2-hrs Glucose level, and
  - Blood Glucose is equal to or greater than 11.0mmol/L (200.0 mg/dL) at some other point during the test;
- If patient has normal FBG, but the 2hrs value is in the diabetic range, test should be repeated after 6wks;
- IGT is considered abnormal; it is an intermediate stage between normality and DM; an increased risk of developing DM;

Table 2: WHO Guidelines for OGTT diagnosis of DM

		DM (mmol/L)	IGT (mmol/L)
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

Graph of Time against Concentration of Blood Glucose in the OGTT test.



How can a patient with DM be monitored? (Long-term indices of diabetic control)

What is Glycosylated Hemoglobin (HbA_{1c})?

- About 98% of Hb in RBC is HbA_1
- HbA_1 is made up of HbA_{1a}, HbA_{1b}, HbA_{1c}
- HbA_{1c} is highest in amount, and is the component that strongly undergoes Glycosylation with Glucose;
- HbA_1 combines with blood glucose in a non-enzymatic reaction to form Glycosylated Hb (**HbA_{1c}**),
- Amount of HbA_{1c} formed is dependent on amount of Glucose in blood over 120-days life span of RBC;

- HbA_{1c} level reflects the average blood sugar level for the 100- to 120-day period before the test;
- Elevation of HbA_{1c} occurs about 3 weeks after sustained elevation in blood glucose;
- It takes about 4 weeks for HbA_{1c} to decrease after a sustained reduction in blood glucose,
- Measurement of HbA_{1c} is a good Clinical indicator of Glycemic control in a patient on DM medication,
- In healthy person HbA_{1c} is 4% to 6% of total HbA,
- In prolonged Hyperglycemia the level of HbA_{1c} may rise to as much 12% of Total HbA;

What are some of the uses of the HbA_{1c} test?

- HbA1c is a good index of diabetic control, it is used to complement results from single blood glucose level, or as patient's log of own blood glucose measurements;
- Used to evaluate DM treatment and compliance;
- Use to compare past and new diabetic therapy,
- Used to estimate duration of hyperglycemia in patients with newly diagnosed DM,

What is Microalbuminuria (MAU)?

- **MAU** is increase in urinary albumin that cannot be detected during urinalysis with Albustick, Clinistick, Dipstick or Multistick;
- MAU is urinary albumin level between 25 to 250mg/day,
- MAU is may lead to progressive increase in proteinuria resulting in clinical Albuminuria (Macroalbuminuria) and declining Glomerular Filtration Rate,
- Macroalbuminuria may be associated with renal damage leading to end stage renal failure and increased coronary mortality among diabetic and hypertensive patients;
- For a diabetic patient MAU indicates early (Sub-clinical), reversible renal damage;

What is Hypoglycemia?

- **Hypoglycemia** is a laboratory “diagnosis” that means blood glucose level below 2.2mmol/L (40.0 mg/dl);
- Hypoglycemia may be due to:
  - Endocrine disorders,
  - Liver disease,
  - Inborn errors of metabolism,
  - Gastrointestinal surgery,

State some biochemical basis of Hypoglycemia?

- Imbalance between glucose intake, endogenous glucose production and glucose utilization;
- Low blood glucose level leads to Catecholamine secretion and correction of the hypoglycemia via stimulation of Glucagon, Cortisol and Growth Hormone secretion;
- Catecholamine surge accounts for the signs and symptoms most commonly seen in Hypoglycemia, i.e., Sweating, Shaking, Tachycardia, Nausea and Weakness;
- Prolonged hypoglycemia reduces glucose supply to the brain; it may lead to brain damage particularly in infants;
- Consumption of alcohol by infants and young children may lead to hypoglycaemia; (**Why?**)

What lab tests are used to investigate hypoglycemia?

- Biochemistry tests are used to confirm hypoglycemia and may provide useful clues to the underlying cause;
- Hypoglycemia is diagnosed by testing blood glucose;
- Urinary tests cannot detect hypoglycemia, **WHY???**

- **Lab tests for hypoglycaemia:**
- **Insulin/Glucose ratio**:
  - To make diagnostic use of Insulin measurements, the ratio of Insulin and Glucose measured on the same blood sample, should be used;
- **Plasma Insulin**: Insulin measurements can lead to the diagnosis or exclusion of Insulinoma,

Plasma C-peptide:

- Insulin secretion in Insulin-treated diabetics cannot be assessed by measuring plasma insulin, because insulin given therapeutically will also be measured in the assay;
- Insulin and its associated Connecting-peptide (or C-peptide) are secreted by the Islet cells in equimolar amounts,
- Measurement of C-peptide levels together with Insulin can differentiate between hypoglycemia due to Insulinoma (**high C-peptide**) and therapeutically administered Insulin (**low C-peptide**);

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