CARBOHYDRATE HOMEOSTASIS II
DIABETES MELLITUS:
DIAGNOSIS & MONITORING

University of Papua New Guinea
School of Medicine & Health Sciences,
Division of Basic Medical Sciences
Discipline of Biochemistry & Molecular Biology,
Clinical Biochemistry Lecture

VJ Temple
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**)
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 1 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
- [HCO₃⁻] in blood falls causing Metabolic acidosis and more Carbonic acid (H₂CO₃) is formed,
• Carbonic acid is converted to $\text{CO}_2$, which then stimulates respiratory center to remove excess $\text{CO}_2$

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

• Increased removal of $\text{CO}_2$ 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 $\text{CO}_2$
• 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 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 $\beta$-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-Triglycerideridemia,
- 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 $8.0\text{mmol/L}$ is usually expected in non-diabetics,
• RBG higher than $11.0\text{mmol/L}$ 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>
<thead>
<tr>
<th>RANDOM GLUCOSE SAMPLE (mmol/L)</th>
<th>Diabetes likely</th>
<th>Diabetes uncertain</th>
<th>Diabetes unlikely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous plasma</td>
<td>≥ 11.1</td>
<td>5.5 - &lt; 11.1</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>Venous blood</td>
<td>≥ 10.0</td>
<td>4.4 - &lt; 10.0</td>
<td>&lt; 4.4</td>
</tr>
<tr>
<td>Capillary plasma</td>
<td>≥ 12.2</td>
<td>5.5 - &lt; 12.2</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>≥ 11.1</td>
<td>4.4 - &lt; 11.1</td>
<td>&lt; 4.4</td>
</tr>
</tbody>
</table>
• Ketones in Urine (Ketonuria)
• Ketones in Blood plasma (Ketonemia)
• Ketone bodies (Acetone, Acetoacacetate, 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>
<thead>
<tr>
<th></th>
<th></th>
<th>DM (mmol/L)</th>
<th>IGT (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Venous plasma</strong></td>
<td>Fasting</td>
<td>≥ 7.8</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td></td>
<td>2hours</td>
<td>≥ 11.1</td>
<td>7.8 - &lt; 11.1</td>
</tr>
<tr>
<td><strong>Venous blood</strong></td>
<td>Fasting</td>
<td>≥ 6.7</td>
<td>&lt; 6.7</td>
</tr>
<tr>
<td></td>
<td>2hours</td>
<td>≥ 10.0</td>
<td>6.7 - &lt; 10.0</td>
</tr>
<tr>
<td><strong>Capillary plasma</strong></td>
<td>Fasting</td>
<td>≥ 7.8</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td></td>
<td>2hours</td>
<td>≥ 12.2</td>
<td>8.9 - &lt; 12.2</td>
</tr>
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<td><strong>Capillary blood</strong></td>
<td>Fasting</td>
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<tr>
<td></td>
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</tr>
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</table>
Graph of Time against Concentration of Blood Glucose in the OGTT test.

Conc of blood glucose (mM)

Time in Minutes

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

What is Glycosylated Hemoglobin (HbA\textsubscript{1c})?

- About 98% of Hb in RBC is HbA\textsubscript{1}
- HbA\textsubscript{1} is made up of HbA\textsubscript{1a}, HbA\textsubscript{1b}, HbA\textsubscript{1c}
- HbA\textsubscript{1c} is highest in amount, and is the component that strongly undergoes Glycosylation with Glucose;
- HbA\textsubscript{1} combines with blood glucose in a non-enzymatic reaction to form Glycosylated Hb (HbA\textsubscript{1c}),
- Amount of HbA\textsubscript{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 Test for HbA₁c?

• 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 2.2 mmol/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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STUDY QUESTIONS

• What are some of the characteristics of Type 1 DM?
• Briefly explain why insulin can be used to control Type 1 DM?
• State some factors that can cause DKO in Type 1 DM patients;
• Briefly outline the consequences of DKO;
• What are some of the possible causes of Type 2 DM?
• What Biochemical tests are used for diagnosis of DM?
• What Biochemical tests are used for monitoring of DM?
• Briefly explain the procedures for carrying out OGTT.
• How are the OGTT results interpretation?
• Briefly explain why Hb A\(_{1c}\) can be used to monitor DM in a patient.