University of Papua New Guinea
School of Medicine and Health Sciences
Division of Basic Medical Sciences
Discipline of Biochemistry and Molecular Biology

CARBOHYDRATE METABOLISM

(ALL FIGURES AND DIAGRAMS ARE IN THE HANDOUT GIVEN TO YOU IN CLASS)

Why is Glycolysis important?

- Glycolysis is major pathway for metabolism of Glucose, Fructose and Galactose in cells
- Glycolysis can occur either in the present of oxygen (Aerobic condition) or in the absence of oxygen (Anaerobic condition)

What are the different types of Glycolysis?

- Anaerobic and Aerobic Glycolysis
- **Anaerobic Glycolysis**:
  - Occurs in the absence of Oxygen,
  - Produces 2 molecules of Lactate and a Net of 2 ATP per molecule of Glucose
  - Lactate is the end product of Anaerobic Glycolysis

- **Aerobic Glycolysis**:
  - Occurs in the presence of Oxygen,
  - Produces 2 molecules of Pyruvate and a Net of 6 ATP per molecule of Glucose
  - Pyruvate is end product of Aerobic Glycolysis

What are the major functions of Glycolysis?

- Major functions of Glycolysis include:
- Production of energy by substrate level Phosphorylation and via supplying substrates to Citric Acid Cycle (Krebs Cycle) and Oxidative Phosphorylation,
- Production of Intermediates for other biosynthetic pathways
- Major biochemical significance of Glycolysis is the ability to provide ATP under Anaerobic condition
- It allows Skeletal muscle to perform at very high level under Anaerobic conditions
- It also allows tissues with significance Glycolytic ability to survive Anoxic Episodes

Give a brief description of the Glycolytic Pathway (Fig. 1)

- Glucose is converted to Glucose-6-phosphate (G-6-P)
  - Enzyme: Hexokinase or Glucokinase,
  - ATP is required, reaction is not reversible
- G-6-P is converted to Fructose-6-Phosphate (F-6-P)
- F-6-P to F-1, 6-Bisphosphate (F-1, 6-BP) catalyzed by Phosphofructokinase (PFK)
  - ATP is required, reaction is not reversible
  - Reaction is the rate limiting step in Glycolysis
  - PFK is the regulatory enzyme of Glycolysis
- F-1, 6-BP is split into Two Triose-sugars by Aldolase
  - Dihydroxyacetone Phosphate (DHAP) and Glyceraldehyde-3-Phosphate (G-3-P)

- DHAP is converted to G-3-P {gives a total of 2 G-3-P}
- G-3-P are converted to 1,3-Bisphosphoglycerate (1,3-BPG)
    - 2 molecules of NAD is converted to 2 NADH + 2 H^+
    - 1,3-BPG is a High Energy compound
- 1,3-BPG is converted to 3-Phosphoglycerate (3-PG)
    - 2 ADP is converted to 2 ATP
    - **Substrate Level Phosphorylation**
- 3-PG converted to 2-PG
- 2-PG converted to Phosphoenolpyruvate (PEP)
    - PEP is a High Energy compound
- PEP is converted to Pyruvate
    - 2 ADP is converted to 2 ATP
    - **Substrate Level Phosphorylation**

What is the fate of Pyruvate during Glycolysis?

- Fate of Pyruvate is determined by **Redox** state of Tissues
- Two possible conditions: Anaerobic and Aerobic conditions
- **Under Anaerobic Conditions**:
    - Pyruvate is converted to Lactate: **Lactate Dehydrogenase (LDH)**
    - **PYRUVATE + NADH + H^+ ⬅====➔ LACTATE + NAD**
        - Reaction is essential step in Anaerobic Glycolysis,
        - It is the anaerobic means of converting NADH to NAD
        - Ensures that NAD required for continuation of Glycolysis is available
    - Lactate is produced in active muscle tissues under Anaerobic conditions
        - Lactate released into the blood, taken up by the Liver, converted back to Glucose by Gluconeogenesis
        - LDH is used mainly for conversion of NADH to NAD,
        - LDH allows Glycolysis to continue, and ATP to be produced under Anaerobic conditions
- **Under Aerobic conditions**:
    - Pyruvate is taken up into Mitochondria and converted to Acetyl-CoA
    - Reaction catalyzed by Pyruvate Dehydrogenase complex
    - Acetyl-CoA enters TCA cycle and is oxidized to CO_2
    - NADH from Glycolysis and TCA cycle are taken up by Mitochondria for oxidation via Electron Transport Chain to produce ATP

What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Anaerobic Glycolysis?

- Amount of ATP molecules used up = **2 ATP**
- Total amount of ATP produced = **4 ATP** (formed at Substrate Level)
- Net amount of ATP produced equals: 4 ATP – 2 ATP = **2 ATP**

What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Aerobic Glycolysis?

- Amount of ATP molecules used up = **2 ATP**
- Amount of ATP produced:
  - At substrate level: **4 ATP**
  - 2 Molecules of NADH produced are transported to Mitochondria
  - In Electron Transport Chain **2 NADH** gives **6 ATP** molecules
- **Total** amount of ATP formed equals: 4 ATP + 6 ATP **= 10 ATP**
- **Net** amount of ATP formed equals: 10 ATP – 2 ATP = **8 ATP**

How is Glycolysis in mammalian RBC different from Glycolysis in muscle tissues (Fig 2)?

- Glycolysis in Red Blood Cell is called 2,3-BisPhosphoglycerate Shunt (2,3-BPG Shunt)
- Anaerobic Glycolysis occurs in RBC
- Mature RBC in mammals do not contain Mitochondria
- Glycolysis in RBC is mainly for production of 2,3-BPG
- Conversion of 1,3-BPG to 3-PG catalyzed by Phosphoglycerate Kinase is bypassed
- 1,3-BPG is converted to 2,3-BPG by **Bis-Phosphoglycerate Mutase** (not in muscle)
- 2,3-BPG is then converted to 3-PG by 2,3-BPG Phosphatase
- High-energy in 1,3-BPG is loss because no ATP is formed

What is the function of 2,3-BPG in RBC?

- 2,3-BPG combines with Hemoglobin (Hb),
  - Causes a decrease in the affinity of Hb for Oxygen
  - Displaces Oxygen from Oxy-hemoglobin (HbO_4)
- Presence of 2,3-BPG in RBC helps Oxy-hemoglobin to unload Oxygen to tissues

What is the function of Pyruvate Dehydrogenase (PDH) Complex?

- PDH complex is located in Mitochondrial matrix
  - It is the link between Glycolysis and TCA cycle, under Aerobic condition
- PDH catalyzes the Oxidative Decaboxylation of Pyruvate to Acetyl-CoA
- Cofactors required are: Thiamine Pyrophosphate (TPP) Lipoic Acid; NAD; FAD; CoASH
- Reaction catalyzed:

  - **Pyruvate + NAD + CoASH =====➔ Acetyl-CoA + NADH + H^+ + CO_2**

- **NADH formed enters ETC to produce 3 ATP**

Give a brief description of TCA cycle (Fig. 3)?

- TCA cycle (Citric Acid Cycle, Kreb's Cycle) is a series of enzymatic reactions responsible for catabolism of Acetyl-CoA
- Acetyl-CoA is an ester of Coenzyme-A, which is the biologically active form of water-soluble vitamin **Pantothenic acid**
- TCA cycle occurs within Mitochondrial matrix under **Aerobic** condition
- Essentially TCA cycle comprises of combination of Acetyl-CoA with Oxaloacetate to give the Six-Carbon Tri-carboxylic acid compound called Citric acid (Citrate)
- Series of reactions then followed during which 2 molecules of CO_2 are given off and reducing equivalents (3 NADH, $FADH_2$) and GTP are formed
- Reducing equivalents enter the ETC to generate energy (ATP) via Oxidative Phosphorylation
- Oxygen is required as the final oxidant of the reducing equivalents
- Absence of Oxygen (Anoxia) or partial deficiency of oxygen (Hypoxia) results in either total or partial inhibition of TCA cycle

What is the significance of TCA cycle?

- TCA cycle is an Amphibolic pathway
    - It is involved in both Anabolic and Catabolic processes
    - Intermediates of TCA cycle are used as precursors in biosynthesis of compounds
    - TCA cycle acts as final common pathway for Oxidation of Carbohydrate, Fats and Proteins
    - Glucose, Fatty Acids and Amino Acids are metabolized to Acetyl-CoA or intermediates of TCA cycle

- TCA cycle plays major role in Gluconeogenesis, Lipogenesis, Transamination and Deamination of most Amino Acids
- TCA cycle provides much of the energy for respiration
- NADH and $FADH_2$ generated in the cycle are transferred to the ETC for formation of ATP via Oxidative Phosphorylation

How many molecules of ATP are produced when One Molecule of Acetyl-CoA goes through the TCA cycle?

- When one Molecule of Acetyl-CoA goes through TCA cycle:
    - **NADH** (Isocitrate Dehydrogenase reaction) to ETC = **3 ATP**
    - **NADH** (Alpha-Oxoglutarate Dehydrogenase reaction) to ETC = **3 ATP**
    - **GTP** (Substrate level Phosphorylation) = **1 ATP**
    - **$FADH_2$** (Succinate Dehydrogenase reaction) to ETC = **2 ATP**
    - **NADH** (Malate Dehydrogenase reaction) to ETC = **3 ATP**

Total equals: 12 ATP molecules

What is Gluconeogenesis?

- Gluconeogenesis is synthesis of Glucose from non-carbohydrates sources
- Gluconeogenesis occurs mainly in the liver and to a lesser extent in the kidney
- Most enzymes of Gluconeogenesis are present in Cytosol, but
    - Pyruvate Carboxylase is located in Mitochondrial matrix, whereas
    - Glucose-6Phosphatase is bound to the smooth endoplasmic reticulum

What is the significance of Gluconeogenesis?

- Gluconeogenesis produces glucose when carbohydrate is not available in sufficient amounts from the diet
- Glucose produced is for maintenance of blood glucose levels during starvation or during vigorous exercise
- Brain and Red Blood Cells depend almost entirely on blood glucose as energy source
    - Supply of glucose is necessary as a source of energy, especially for the nervous system and RBC

- Glucose is required in Adipose Tissues as a source of Glycerol,
- Under Anaerobic conditions glucose is the major fuel for energy production in the Skeletal muscle
- Glucose is the precursor of Lactate in Mammary gland

How is the Gluconeogenic pathway related to Glycolysis?

- Gluconeogenesis is not exactly the reversal of Glycolysis
- Three Irreversible reactions in Glycolysis must be bypassed for Gluconeogenesis to occur
- Three Irreversible reactions are:
    - Hexokinase (or Glucokinase) reaction
    - Phosphofructokinase reaction
    - Pyruvate Kinase reaction

- During Gluconeogenesis only these three reactions are circumvented by special reactions, all the other reactions in Glycolysis are reversible

The following points must be noted about the pathway of Gluconeogenesis:

- Glucose-6-Phosphatase (G-6-Phosphatase):
    - Present in **Liver** and **Kidney**, but
    - ABSENT in **Skeletal Muscle** and **Adipose Tissues**
- G-6-Phosphatase allows tissue to release Glucose into the blood
- Reaction catalyzed by G-6-Phosphatase is as follows:

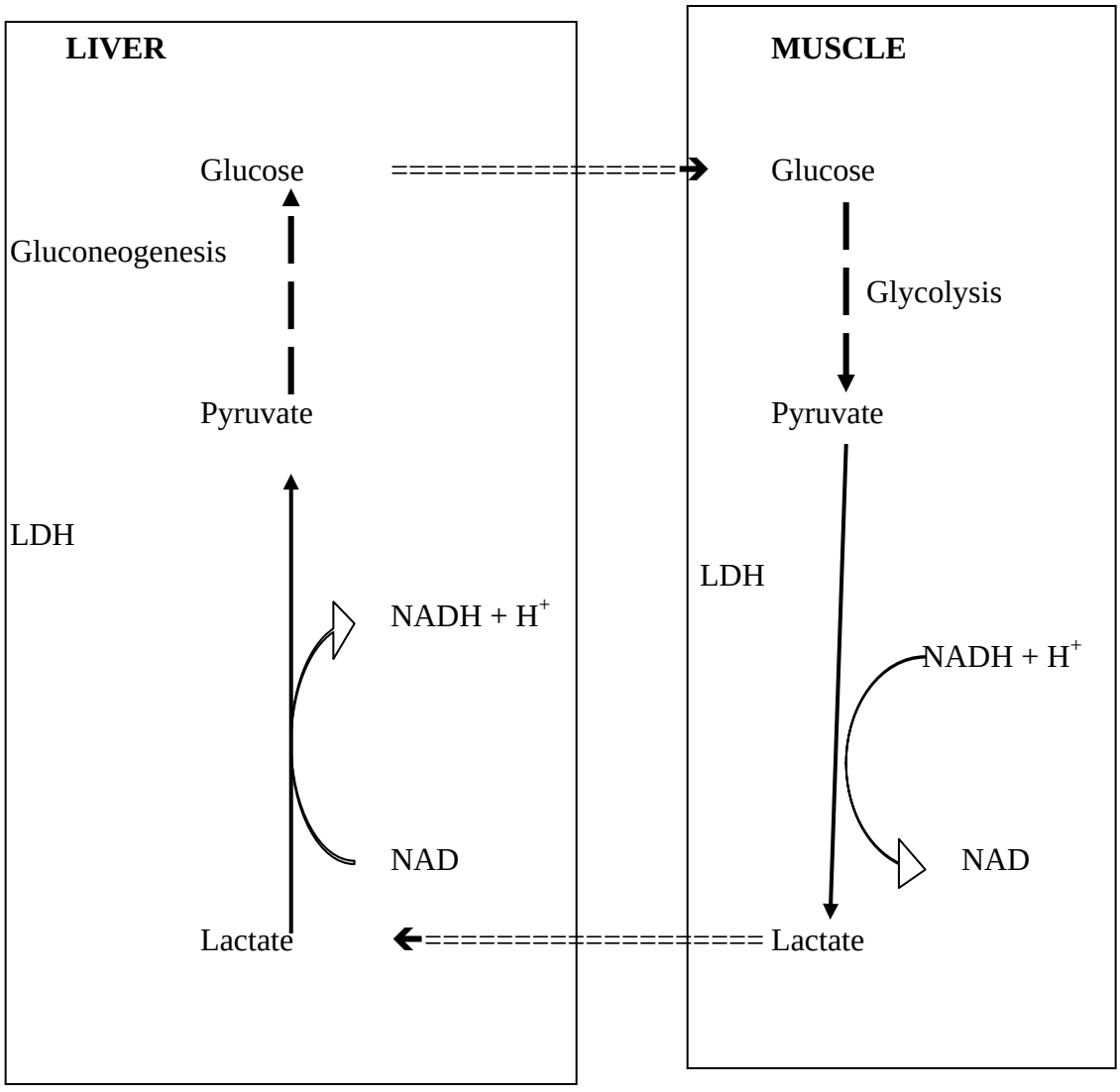
    - **G-6-Phosphate + H_2O =====➔ Glucose + Pi**

- G-6-Phosphatase is present in Liver and Kidney, thus they can both release Glucose into blood
- G-6-Phosphatase in NOT present in skeletal muscle and adipose tissue, thus they cannot release glucose into the blood

- Fructose-I, 6-Bisphosphate is converted to Fructose-6-Phosphate by the enzyme Fructose-1,6-Bisphosphatase
- Fructose-1,6-Bisphosphatase is NOT present in Heart muscle and Smooth muscle
- Some of the substrates for Gluconeogenesis include Pyruvate, Lactate, Glucogenic Amino Acids and Glycerol

What is the Cori cycle (Lactic acid cycle)?

- Cori Cycle also called Lactic Acid cycle is presented in **Figure below**



How is Fructose metabolized?

- Two pathways for metabolism of Fructose
- Metabolism of Fructose in Muscle and Adipose Tissue:
    - Hexokinase Phosphorylate Fructose

Fructose + ATP ======➔ Fructose-6-Phosphate

    - Fructose-6-Phosphate enters Glycolytic pathway
- Metabolism of Fructose in Liver is via Fructose-1-Phosphatre pathway, that utilizes Fructokinase reaction: **See Figure: 4**
- Fructokinase is not affected by fasting
- Fructokinase is not affected by **Insulin:**
    - **Fructose metabolism occurs normally in patients with Diabetic Mellitus**
- Essential Fructosuria (Fructose in urine) is due to:
    - Defect in activity of Fructokinase
- Hereditary Fructose Intolerance is due to:
    - Defect in Fructose-1-Phosphate Aldolase

How is Galactose metabolized?

- **See Figure 5**

What conditions occur when there is defect in Galactose metabolism?

- Galactokinase catalyzes formation of Galactose-1-phosphate
- Defect in Galactokinase causes accumulation of Galactose in blood and tissues
    - Galactose accumulating in Lens is converted to Galacitol (Dulcitol) by Aldose Reductase
    - Dulcitol accumulation causes Cataracts
- **Galactosemia** (Galactose accumulation in blood) is due to
    - Defect in Galactose-1-Phosphate Uridyl Transferase
- Galactose and Galactose-1-phosphate accumulate in blood and tissues

What are some of the consequence of Galactosemia in Children?

- After consuming milk:
    - Severe vomiting and Diarrhea, Enlarged Liver, Jaundice, Cataract due to accumulation of Dulcitol, Mental retardation
- Using Galactose-free diets can control the condition

What is the Pentose Phosphate Pathway (HMP Shunt)?

- Alternative pathway for the metabolism of glucose
- Biosynthetic pathway that does not generate ATP
- **Glucose-6-Phosphate Dehydrogenase (G-6-PD)** catalyzes first reaction
    - **G-6-PD catalyzes the formation of NADPH**
- Produces **NADPH** for reductive biosynthesis of Fatty acids, Steroids
- Produces of **Ribose-5-phosphate** for Nucleotide and Nucleic acid synthesis
- Major route for use of Pentose and their conversion to Fructose-6-phosphate and Glyceraldehyde 3-phosphate
- HMP shunt occurs most actively in Liver, Mammary glands, Adipose tissues, RBC, Testis, Adrenal Cortex
- Core reaction of HMP shunt can be summarize as:

G-6-P + 2 NADP + H_2O ===➔ Ribose-5-phosphate + 2 NADPH + $2H^+$ + CO_2

What is the major role of HMP shunt in Red Blood Cells?

- To provides NADPH for Reduction of Oxidized Glutathione (GSSG) to Reduced Glutathione (GSH)
    - Reaction is catalyzed by Glutathione Reductase
        - **GSSG + NADPH + H^+ ======➔ 2 GSH + NADP**
- **GSH** then removes Hydrogen Peroxide (**H_2O_2**) from RBC
- Reaction is catalyzed by Glutathione Peroxidase that utilizes Selenium
        - **2 GSH + H_2O_2 ===➔ GSSG + 2 H_2O**
- Function of Hb is stops when Hydrogen Peroxide accumulates in RBC
- Hb is converted to MetHb that cannot transport Oxygen

Why does a person with G-6-PD deficiency suffer from hemolytic anemia?

- G-6-PD catalyzes formation of NADPH + H^+ in HMP shunt
- Hemolytic anemia usually occur in individuals with G-6-PD deficiency
- HMP shunt will not produce enough NADPH required to maintain high level of GSH to protect RBC from Oxidative damage
- Hemolysis of RBC will occur because Oxidants will accumulate in RBC and damage the membrane
- Certain drugs and chemicals that act as oxidizing substances (e.g., Aspirin, Sulfonamides and Anti-malarial drug Primaquine) can cause Hemolytic Anemia
- Such drugs or compounds converts GSH to GSSG and increase the demand for HMP shunt to produce more NADPH needed to convert GSSG back to GSH

How is the body store of Glycogen accounted for?

- Liver and Skeletal muscle are major storage sites for Glycogen
- Liver stores about 6.0% by weight of Glycogen
- Skeletal muscle stores about 1.0% by weigh of Glycogen
- Skeletal muscle contains about 3 to 4 times more Glycogen store than Liver
    - Mass of skeletal muscle is much greater than mass of Liver

- Liver Glycogen stores in humans are only adequate for about 12 to 18 hours of fasting, after which the Liver becomes almost totally depleted of Glycogen and require Gluconeogenesis to maintain blood glucose level
- Muscle glycogen is only depleted significantly after prolonged vigorous exercise

What are some of the functions of Glycogen?

- Glycogen in Liver maintains blood glucose level between meals
    - Essential to supply easily metabolizable energy source to particularly the brain, which uses only glucose as the major substrate for energy production
- Muscle glycogen serves as readily available source of glucose for the energy needs of skeletal muscle

Explain how Glycogen is degraded (Glycogenolysis)? (Fig. 6)

- Glycogenolysis is the pathway for Degradation of Glycogen
- Two Stage involved:

Stage 1: Cleavage of terminal alpha-1,4-Glycosidic bond in Glycogen

- Enzyme & Co-enzyme:
    - Glycogen Phosphorylase (**Phosphorylase**) and Pyridoxal Phosphate (B_6PO_4)

Glycogen + Pi ←=====→ Glycogen (n-1 residues) + G-1-P

- Glycogen Phosphorylase catalyzes cleavage of terminal Glycosidic bond in Glycogen to yield
    - G-1-P and Glycogen molecule shorter by one-glucose unit
- Reaction occurs sequentially until it gets close to the branch point in Glycogen

Stage 2: Removal of branch chains:

- Glycogen-De-branching enzyme system catalyzes removal of branch-points in Glycogen
- Catalyzes cleavage of alpha-I, 6-Glycosidic bond in Glycogen
- Combined action of Glycogen Phosphorylase and Glycogen-De-branching enzyme system leads to the complete degradation of Glycogen

What happens to G-1-P?

- Glucose-I-P is converted to Glucose-6-phosphate by Phosphoglucomutase

  - **G-1-P ⬅=====➔ G-6-P**

What happens to G-6-P in Liver, Kidneys and Skeletal Muscle?

- **LIVER and KIDNEYS**:
  - Glucose-6-Phosphatase catalyzes conversion of G-6-P to Glucose

$$\textbf{G-6-P + } H_2O \textbf{ =======➔ Glucose + Pi}$$

  - Free Glucose formed diffuses into the blood to maintain blood glucose concentration

- **SKELETAL MUSCLE**:
- **Glucose-6-Phosphatase is not ABSENT in Skeletal Muscle**
  - Skeletal muscle cannot convert G-6-P to Glucose
- Glycogenolysis in skeletal muscle is to produce energy for muscle contraction and not to increase blood glucose concentration

How is Glycogen synthesized? (Fig. 7)

- Glycogenesis is biosynthesis of Glycogen
- Requires 3 enzymes and "**Glycogen primer** (Glycogenin)"
  - Enzymes required are as follows:
  - **UDP-Glucose Pyrophosphorylase**:

    - Catalyzes formation of UDP-Glucose from UTP and G-1-P

  - **Glycogen Synthase**:
    - Uses UDP-Glucose as substrate to synthesize Glycogen by adding one residue at a time to "Glycogen primer" forming alpha-1 4-Glycosidic bond between Glycosyl residues

  - **Branching enzyme**:
    - Catalyzes formation of branch-points (alpha-1, 6-Glycosidic bonds at the branch points)

How is metabolism of Glycogen regulated?

- Allosteric regulation and Hormones control Glycogenolysis and Glycogenesis
- Glycogen metabolism is controlled by Glycogen Phosphorylase and Glycogen Synthase
  - Both enzymes are partly controlled by cyclic AMP (c AMP)

- Cyclic AMP causes **Activation** of **Glycogen Phosphorylase** and at the same time causes **Inhibition** of **Glycogen Synthase**

- Increasing concentration of cyclic AMP results in Increasing rate of Glycogenolysis and decreasing Glycogenesis
- Adenylate Cyclase catalyzes formation of Cyclic AMP from ATP
  - Epinephrine (Adrenaline), Norepinephrine (Noradrenaline) and Glucagon activate Adenylate cyclase, thus causing it to increasing the formation of cyclic AMP
- Phosphodiesterase catalyzes degradation of Cyclic AMP
- Insulin can increase the activity of Phosphodiesterase in the Liver, thereby reducing the concentration of cyclic AMP

How is Glycogen metabolism regulated in Skeletal Muscle?

- Epinephrine promotes Glycogenolysis and inhibits Glycogenesis (**Why?**)
- Because epinephrine stimulates formation of cyclic AMP by activating Adenylate cyclase

- In an emergency Epinephrine is released, it acts on the muscle cell membrane to increased Glycogenolysis so as to provide energy for muscle contraction and at the same time inhibits the Glycogenesis
- Insulin inhibits Glycogenolysis and promotes Glycogenesis (Why?)
- Because insulin reduces the level of cyclic AMP via the activation of Phosphodiesterase
- Note that insulin enhances the entry of glucose into the muscle ceils

How is Glycogen metabolism regulated in the Liver?

- Glucagon promotes Glycogenolysis and inhibits Glycogenesis (Why)
- Because Glucagon activates Adenylate cyclase, thus increasing the level of cyclic AMP
- Insulin increases Glycogenesis in the Liver by increasing the activity of Glycogen Synthase

QUESTION:
Calculate the Total and Net amounts of ATP formed when One Glucose molecule is metabolized to CO_2 and H_2O under Aerobic condition.

ANSWER:

- Complete metabolism of Glucose to CO_2, H_2O and energy (ATP) involves Glycolysis, TCA cycle and Electron Transport Chain (ETC). Pyruvate produced at the end of aerobic Glycolysis is converted to Acetyl-CoA, which then enters the TCA cycle to produce CO_2 and reducing equivalents that enters the ETC producing ATP and H_2O. Amount of ATP produced is calculated as follows:
  - Glucose is metabolized via Aerobic Glycolysis: **Total of 2 ATP used up**;
  - **Products of Glycolysis**:
    - Substrate level Phosphorylation: **4 ATP** are produced;
    - Reducing Equivalents: **2 NADH via ETC to ATP: 2 x 3 = 6 ATP**;
    - Total amount of ATP produced: 4 ATP + 6 ATP = **10 ATP**;
    - Net amount of ATP produced: 10 ATP – 2 ATP = **8 ATP**;
  - Under Aerobic condition Glucose gives: **2 Pyruvate + 8 ATP**;

- Pyruvate Dehydrogenase reaction (converts Pyruvate to Acetyl-CoA);
    - **2 Pyruvate gives: 2 Acetyl-CoA + 2 NADH;**
    - **2 NADH goes to ETC: 6 ATP produced;**

- TCA cycle and Electron Transport Chain (ETC):
    - Each Acetyl-CoA in TCA gives: **3 NADH, $FADH_2$ and GTP;**
    - Each **NADH** in ETC gives: 3 ATP {thus **3 NADH = 9 ATP**};
    - **$FADH_2$** enters ETC gives: **2 ATP**;
    - **GTP converted to ATP**;
- Total = **9 ATP + 2 ATP + ATP = 12 ATP**;
- Therefore 2 Acetyl-CoA gives: **2 x 12 = 24 ATP**;
- Total ATP = **6 ATP + 24 ATP = 30 ATP**;
- **GRAND TOTAL = 8 ATP + 30 ATP = 38 ATP**

STUDY QUESTIONS:

1. Why is Glycolysis important?
2. What are the different types of Glycolysis?
3. What are the major functions of Glycolysis?
4. What is the fate of Pyruvate (a) Under Anaerobic conditions, (B) Under Aerobic conditions?
5. Give the total and net amounts of ATP formed when One Glucose is metabolized via Anaerobic Glycolysis?
6. What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Aerobic Glycolysis?
7. What is the function of 2,3-BPG in RBC?
8. What is the function of Pyruvate Dehydrogenase (PDH) Complex?
9. What is the significance of TCA cycle?
10. How many molecules of ATP are produced when One Acetyl-CoA goes through the TCA cycle?
11. What is the significance of Gluconeogenesis?
12. What is the Cori cycle (Lactic acid cycle)?
13. What is the major role of HMP shunt in Red Blood Cells?
14. Why does a person with G-6-PD deficiency suffer from hemolytic anemia?
15. What are some of the functions of Glycogen?
16. What happens to G-6-P in Liver, Kidneys and Skeletal Muscle?