SIBC511- INTEGRATION OF METABOLISM

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INTEGRATION OF METABOLISM

Energy-containing nutrients
- Carbohydrates
- Fats
- Proteins

Catabolism
- ADP + HPO$_4^{2-}$
- NAD$^+$
- NADP$^+$
- FAD
- ATP
- NADH
- NADPH
- FADH$_2$
- Chemical energy

Energy-depleted end products
- CO$_2$
- H$_2$O
- NH$_3$

Cell macromolecules
- Proteins
- Polysaccharides
- Lipids
- Nucleic acids

Anabolism

Precursor molecules
- Amino acids
- Sugars
- Fatty acids
- Nitrogenous bases
The metabolism of carbohydrate, lipid, and protein are coordinated and well regulated to meet the bodily requirements, especially the energy need, under various conditions.
Major metabolic fuels:
- **GLUCOSE**

![GLUCOSE structure](image)

- **FAT (TRIACYLGLYCEROL)**

![FAT structure](image)
GLUCOSE

- An important fuel for most tissues (e.g. muscle, red blood cells, and particularly neurons*).

- During the fasting state, the glucose level is prevented from dropping too low.

Various cellular and hormonal regulations of the metabolic pathways work together to preserve glucose as a fuel for the important organs (e.g., brain, RBC) and shift the source of fuel in other tissues to fatty acids and ketones.
GLUCOSE

- **An important fuel** for most tissues (e.g. muscle, red blood cells, and particularly *neurons*).

- **During the fed state, the glucose level is also kept from rising too high** (as it can be harmful to the body).

Various cellular and hormonal regulations of the metabolic pathways work together to *accelerate glucose utilization as a fuel and store the excess as glycogen and triacylglycerol.*
INTEGRATION OF METABOLISM

Dietary intake

Fed state

- utilize & restore foodstuff
  (e.g. glycogen, fat, protein)

Glycolysis
Glycogenesis
Lipogenesis
Protein synthesis

Fasting state

- release foodstuff
  (e.g. glucose, fatty acid, amino acid)

Gluconeogenesis
Glycogenolysis
Lipolysis
Ketogenesis, ketolysis
Protein breakdown

INSULIN

glucagon, epinephrine,
growth hormone, cortisol
• Insulin – secreted from β cells in the islet of Langerhan in pancreas

• Its secretion can be stimulated by various compounds (e.g. neuro-transmitters, neuropeptides, amino acids) but the most important one is glucose
Insulin can reduce blood glucose by:

- uptake of glucose in adipose tissue, muscle

* insulin-independent glucose transport in brain, liver, kidney, RBC
GLUT 1, GLUT 3: basal glucose uptake in many tissues, e.g., brain, nerve, RBC

GLUT 2: liver and the islet β cells

GLUT 4: insulin-stimulated glucose uptake in adipose tissue and muscle

* In muscle, more GLUT4 can be recruited to the cell membrane during exercise independent of insulin actions to promote glucose uptake and utilization.
**CARBOHYDRATE METABOLISM DURING FED STATE - GLYCOLYSIS**

**Glycolysis** converts 1 molecule of glucose (C6) into 2 molecules of pyruvate (C3)

**location:** cytosol

**functions:**
- yields 2 ATP, 2 NADH, and 2 pyruvate per one glucose molecule
- Major energy-producing pathway in RBC, brain
- Glycolytic intermediates are important for the biosynthesis of various compounds (e.g. glycerol, acetyl-CoA, 2,3-BPG)
CARBOHYDRATE METABOLISM DURING FED STATE - GLYCOLYSIS

**important enzymes:**

**phosphofructokinase 1** (rate-limiting step)*

**phosphofructokinase 2** (activated by insulin, inhibited by glucagon)

Glucose → Glucose-6-phosphate
Glucose-6-phosphate → Fructose-6-phosphate (PFK1*)
Fructose-6-phosphate → Fructose-1,6-bisphosphate
Fructose-1,6-bisphosphate → Glyceraldehyde-3-phosphate
Glyceraldehyde-3-phosphate → 1,3-bisphosphoglycerate
1,3-bisphosphoglycerate → 3-phosphoglycerate
3-phosphoglycerate → 2-phosphoglycerate
2-phosphoglycerate → Phosphoenolpyruvate
Phosphoenolpyruvate → Pyruvate + ATP + NADH

*Phosphofructokinase 1 (PFK1) is the rate-limiting step in glycolysis.

**Activated by insulin, inhibited by glucagon.**
Mechanisms of enzyme regulation

Allosteric regulation – fructose-2,6-bisphosphate, AMP, ADP, ATP etc.
Covalent modification – phosphorylation (e.g. through actions of hormones)

Allosteric Enzyme
CARBOHYDRATE METABOLISM DURING FED STATE - GLYCOLYSIS

important enzymes:

Three irreversible reactions in glycolytic pathway

- **glucokinase/hexokinase**
- *phosphofructokinase*
- ***pyruvate kinase***

Need new sets of enzymes to operate in the opposite direction, i.e., the gluconeogenetic pathway.
Anaerobic glycolysis

In cells deprived of oxygen supply or in RBC (lacking mitochondria), pyruvate can be converted to lactate by lactate dehydrogenase (LDH) to regenerate NAD$^+$ from NADH to be reused in glycolysis.

Lactate is released into the bloodstream and taken up by the liver to convert it to pyruvate and glucose.
Aerobic glycolysis

When oxygen supply is adequate, pyruvate enters mitochondria and is converted into acetyl CoA by pyruvate dehydrogenase (PDH).

Note

- PDH contains thiamine (vit B1), lipoic acid, CoA, FAD (vit B2), NAD (niacin or vit B3) as coenzymes.

- Disease association
  Wernicke-korsakoff
  B1 deficiency (beri-beri)
CARBOHYDRATE METABOLISM DURING FED STATE - GLYCOLYSIS

**aerobic glycolysis**

- pyruvate
- Pyruvate dehydrogenase
- pyruvate
- acetyl CoA
- Pyruvate carboxylase
- oxaloacetate
- citrate
- NADH
- FADH$_2$
- GTP
- TCA cycle

Important feedback controls of acetyl CoA:
- **activates** Pyruvate carboxylase
- **inhibits** Pyruvate dehydrogenase
**KREBS CYCLE**

**location:** mitochondria

**functions:** - Complete oxidation of acetyl CoA

\[
\text{acetyl CoA} \rightarrow 2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP}
\]
important enzymes:

**isocitrate dehydrogenase** (rate-limiting step – inhibited by NADH)
Krebs cycle intermediates are linked with the metabolic pathways of many important compounds (e.g. amino acids, fatty acids, nucleic acids, etc).
**KREBS CYCLE**

*aerobic glycolysis*

- NADH
- FADH$_2$

→ Electron transport system

- NADH + H$^+$ → NAD$^+$
- FADH$_2$ → O$_2$ H$_2$O

- ADP + Pi → ATP
Excess glucose during the fed state is also stored as glycogen in liver and muscle (Glycogenesis).

- **Glucose** is converted to glucose-6-phosphate.
- **Glycogen synthase** (rate-limiting step) activated by insulin.

- **Location**: cytosol
- **Important enzymes**:
  - **Glycogen synthase** (rate-limiting step)
  - **Activated by insulin**
In addition, excess glucose also enters the pentose phosphate pathway (Hexose monophosphate shunt)

**location:** cytosol

**functions:** generate

- NADPH - *synthesis of fatty acid, cholesterol, nucleotides*
- coenzymes of antioxidant enzymes
- Ribose - *DNA & RNA synthesis*

**important enzymes:***

*glucose-6-phosphate dehydrogenase G6PD* (rate-limiting step)
During the fed state, the increased glucose and insulin levels stimulate the syntheses of fatty acid and triacylglycerol (Lipogenesis).

- Excessive intake of high carbohydrate diet can lead to obesity.
- On average, a 70-kg man stores ~ 12 kg of fat, which can provide enough energy for 2 months during starvation (fat gives 9 kcal/g).
Fatty acid synthesis  
**location:** cytosol

Therefore, acetyl CoA has to be transported into the cytosol as citrate, which is then converted back to acetyl CoA in the cytosol by citrate lyase.
FATTY ACID SYNTHESIS AND LIPOGENESIS

Fatty acid synthesis

**Important enzymes:**
- acetyl CoA carboxylase (rate-limiting step) *activated by insulin* and citrate
- fatty acid synthase (use NADPH derived from the pentose phosphate pathway)
• The newly-synthesized fatty acids are combined with glycerol-3-phosphate (a glycolytic intermediate) to generate triacylglycerol, which is then transported into the bloodstream and peripheral tissues in the forms of lipoprotein VLDL.
During the fed state, the cholesterol synthesis is also increased due to the action of insulin and the availability of acetyl CoA in the cytosol.

**location:** cytosol  
**important enzymes:**  
- **HMG CoA reductase** (rate-limiting step)  
- activated by insulin  
- inhibited by antilipidemic drugs Statin
LIPOPROTEIN TRANSPORT OF LIPID

- Intestine
- Liver
- Reverse cholesterol transport
- HDL
- LDL
- Extrahepatic tissues
- VLDL
- Chylomicron remnants
- VLDL remnants (IDL)
- Lipoprotein lipase
- Free fatty acids
- Mammary, muscle, or adipose tissue
- HDL precursors (from liver and intestine)
AMINO ACID METABOLISM DURING FED STATE

Gut

Amino acid pool

Protein synthesis

Nitrogenous compounds

Amino acid
Examples of important nitrogenous compounds:

- Heme
- Pyrimidine
- Purine
- Creatine
- Epinephrine
- Histamine

**AMINO ACID METABOLISM DURING FED STATE**
AMINO ACID METABOLISM DURING FED STATE

- Unlike glucose or fatty acids, amino acids are not stored.

- After being used for the syntheses of proteins and nitrogenous compounds, the excess amino acids are undergone transamination and deamination.
AMINO ACID METABOLISM DURING FED STATE

A) Transamination

\[
\text{α-Ketoglutarate} \quad \text{L-Amino acid} \quad \text{L-Glutamate} \quad \text{α-Keto acid}
\]

\[
\begin{align*}
\text{COO}^- & \quad \text{COO}^- \\
\text{C=O} & \quad \text{H}_3\text{N}^+ \quad \text{C=O} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{R} \\
\text{COO}^- & \quad \text{COO}^-
\end{align*}
\]

\[
\text{PLP} \quad \text{aminotransferase}
\]

B) Deamination

\[
\text{Glutamate} \quad \text{α-Ketoglutarate}
\]

\[
\begin{align*}
\text{H}_3\text{N}^+ & \quad \text{C=O} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{COO}^- \\
\text{COO}^- & \quad \text{H}^+ \\
\end{align*}
\]

\[
\text{NAD(P)}^+ \quad \text{NAD(P)}^+ \quad \text{H}^+ \\
\]

\[
\text{glutamate dehydrogenase}
\]

\[
\text{NH}_4^+ + \text{H}_2\text{O} \quad \text{COO}^- \quad \text{CH}_2 \\
\text{CH}_2 \quad \text{CH}_2 \\
\text{COO}^- \quad \text{COO}^-
\]
AMINO ACID METABOLISM DURING FED STATE

During the fed state, the carbon skeletons of amino acids can be used for:

- energy production
- synthesis of fatty acid and triacylglycerol

α-keto acids

Intermediates in metabolic pathways (e.g. glycolysis and Krebs cycle)
**FED STATE**

**Glucose**

**Insulin**

**Counterregulatory hormones**

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**Effects of Insulin on Blood Glucose: Uptake of Glucose by Cells and Storage as Triacylglycerols and Glycogen**

<table>
<thead>
<tr>
<th>Metabolic effect</th>
<th>Target enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Glucose uptake (muscle, adipose)</td>
<td>↑ Glucose transporter (GLUT4)</td>
</tr>
<tr>
<td>↑ Glucose uptake (liver)</td>
<td>↑ Glucokinase (increased expression)</td>
</tr>
<tr>
<td>↑ Glycogen synthesis (liver, muscle)</td>
<td>↑ Glycogen synthase</td>
</tr>
<tr>
<td>↓ Glycogen breakdown (liver, muscle)</td>
<td>↓ Glycogen phosphorylase</td>
</tr>
<tr>
<td>↑ Glycolysis, acetyl-CoA production (liver, muscle)</td>
<td>↑ PFK-1 (by ↑ PFK-2)</td>
</tr>
<tr>
<td>↑ Fatty acid synthesis (liver)</td>
<td>↑ Pyruvate dehydrogenase complex</td>
</tr>
<tr>
<td>↑ Triacylglycerol synthesis (adipose tissue)</td>
<td>↑ Acetyl-CoA carboxylase</td>
</tr>
<tr>
<td></td>
<td>↑ Lipoprotein lipase</td>
</tr>
</tbody>
</table>
Glucose

Insulin

counterregulatory hormones

glycolysis

glycogen genesis

lipogenesis

protein synthesis
FASTING STATE

glucagon
epinephrines
growth hormone
cortisol

counterregulatory hormones
During the fasting state, glycogen in the liver is broken down to release glucose into the bloodstream, preventing the blood glucose from dropping too low (Glycogenolysis).

**location:** cytosol

**important enzymes:**
- glycogen phosphorylase (rate-limiting step) activated by glucagon and epinephrine
• Glycogenesis occurs both in liver and muscle.

• However, the muscle lacks the enzyme **glucose-6-phosphatase**, thus unable to release glucose into the bloodstream: i.e. the muscle glycogen is stored and used only within the muscle.
• On average, a 70-kg man stores ~70 g of glycogen in the liver and 400 g in muscle (carbohydrate yields 4 kcal/g). 

  *very little comparing with fat storage (~12 kg in a 70-kg man)*

• Therefore, glycogen is used up only within 12-24 hr after fasting.

• To keep up the blood glucose level, new glucose must be synthesized from other compounds (e.g. amino acids, lactate, glycerol) by a process called **gluconeogenesis**, which is activated by the counterregulatory hormones (glucagon and epinephrine).
Gluconeogenesis is essentially a reverse process of glycolysis except at the three irreversible steps, which require new set of enzymes operating in the opposite directions.

**Location:** cytosol และ mitochondria
CARBOHYDRATE METABOLISM DURING FASTING STATE - GLUCONEOGENESIS

Important enzymes:
1. pyruvate carboxylase and 2. PEP carboxykinase
3. fructose-1,6-bisphosphatase (rate-limiting step)
4. glucose-6-phosphatase

- Glucose
- Glucose-6-phosphate
- Fructose-6-phosphate
- Fructose-1,6-bisphosphate
- Phosphoenolpyruvate
- Pyruvate
- Acetyl CoA
- Oxaloacetate
- Citrate
- Lactate
- Glycerol
- Amino acids

(source - muscle)

(liver & kidney)
Note: acetyl CoA (derived from the beta-oxidation of fatty acid) cannot be used as a substrate for gluconeogenesis.
LIPOLYSIS AND BETA-OXIDATION OF FATTY ACID

- Counterregulatory hormones stimulate lipolysis by activating hormone-sensitive lipase, which releases fatty acids into the bloodstream—a process called Lipolysis. Peripheral tissues such as muscle, heart, and liver can use fatty acids as fuel, preserving glucose for the important organs like brain.
**Beta oxidation of fatty acid**

**Location:** mitochondria

**Important enzymes:**
- **carnitine acyltransferase-1** (rate-limiting step)

Facilitating fatty acid transport into mitochondria.
LIPOLYSIS AND BETA-OXIDATION OF FATTY ACID

\[ \text{Acyl-CoA (Palmitoyl CoA)} \]

\[ \text{β-oxidation of fatty acids} \]

Acyl-CoA shortened by 2C (Myristoyl CoA)

\[ \text{Citric acid cycle} \]
An increase acetyl CoA in the liver from β oxidation and a low oxaloacetate, which is used for gluconeogenesis, lead to a production of ketone bodies. Ketone bodies are more water soluble than fatty acids and can be easily transported to the peripheral tissues.
The liver produces ketone bodies for other tissues (Ketogenesis) but cannot use them (Ketolysis) due to the lack of enzyme beta-ketoacyl CoA transferase).

Neurons cannot use fatty acids directly. However, they can adapt to use ketone bodies during prolonged fasting or starvation.
- The carbon skeletons of amino acids are an important source of substrates for gluconeogenesis. They are derived from the protein breakdown as a result of a counterregulatory hormone corticosteroid action.
The carbon skeletons of amino acids are an important source of substrates for gluconeogenesis. They are derived from the protein breakdown as a result of a counterregulatory hormone corticosteroid action.
During the fasting state, the carbon skeletons of amino acids are used for:

- energy production
- gluconeogenesis
- ketogenesis
AMINO ACID METABOLISM DURING FASTING STATE

- energy production (Fed & Fasting)
- fatty acids and lipogenesis (Fed)
- gluconeogenesis (Fasting)
- ketogenesis (Fasting)
The metabolism of amino acids are coordinated with those of carbohydrate and lipid both during the fed or fasting states.
FASTING STATE

Glucose

insulin

counterregulatory hormones

Effects of Glucagon on Blood Glucose: Production and Release of Glucose by the Liver

<table>
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<th>Metabolic effect</th>
<th>Effect on glucose metabolism</th>
<th>Target enzyme</th>
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<td>↑ Glycogen breakdown (liver)</td>
<td>Glycogen ——&gt; glucose</td>
<td>↑ Glycogen phosphorylase</td>
</tr>
<tr>
<td>↓ Glycogen synthesis (liver)</td>
<td>Less glucose stored as glycogen</td>
<td>↓ Glycogen synthase</td>
</tr>
<tr>
<td>↓ Glycolysis (liver)</td>
<td>Less glucose used as fuel in liver</td>
<td>↓ PFK-1</td>
</tr>
<tr>
<td>↑ Gluconeogenesis (liver)</td>
<td>Amino acids</td>
<td>↑ FBPase-2</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>↓ Pyruvate kinase</td>
</tr>
<tr>
<td></td>
<td>Oxaloacetate</td>
<td>↑ PEP carboxykinase</td>
</tr>
<tr>
<td>↑ Fatty acid mobilization (adipose tissue)</td>
<td>Less glucose used as fuel by liver, muscle</td>
<td>↑ Triacylglycerol lipase</td>
</tr>
<tr>
<td>↑ Ketogenesis</td>
<td>Provides alternative to glucose as energy source for brain</td>
<td>Perilipin phosphorylation</td>
</tr>
</tbody>
</table>
FASTING STATE

- **Glucose**
- **Insulin**
- **Counterregulatory hormones**

**Glucose**

- **Gluconeogenesis** → to increase blood glucose
- **Glycogenolysis** → to increase blood glucose
- **Lipolysis** → to use fat as fuel, thus preserving glucose
- **Ketogenesis/Ketolysis** → to use fat as fuel, thus preserving glucose
- **Protein breakdown** → for gluconeogenesis/energy
SUMMARY: INTEGRATION OF METABOLISM
A 4-month-old baby girl was taken to the hospital because of a high-grade fever and drowsiness. The patient had a history of frequent hypoglycemic episodes since birth, which improved after receiving intravenous injections of glucose.
Physical examination: the patient was drowsy and had an enlarged liver.

Laboratory findings:

- **glucose** 24 mg/dl (normal 40-90 mg/dl)
- **lactate** 6.7 mol/L (normal 0.9-1.7 mol/L)
- **ammonia** 155 umol/L (normal 25-50 umol/L)
- **ketone** 0 mg/L (normal 0.5-3 mg/dl)
- **plasma fatty acid**
- **plasma dicarboxylic acid**
- **plama and urinary acylcarnitine**

Further biochemical investigations indicates the **Deficiency of Medium-chain Acyl CoA Dehydrogenase (MCAD)**
Defects in fatty acid oxidation

Short chain fatty acid = C2-C4, medium chain = C6-C10, Long chain = C12-C26

Less acetyl-CoA is produced.
During the fasting state, lipolysis is stimulated to release fatty acids to be used as fuel by other tissues, thus preserving glucose for the brain.

- Plasma fatty acid (from increased lipolysis and decreased breakdown)
During the fasting state, amino acids, glycerol, lactate are used as substrates for gluconeogenesis.
Furthermore, gluconeogenesis can be stimulated by acetyl CoA because it is an allosteric activator of pyruvate carboxylase, resulting in an increase of oxaloacetate and glucose production.
Defects in fatty acid oxidation -> a decrease of acetyl CoA -> a decrease of oxaloacetate production -> impaired gluconeogenesis

Hypoglycemia
Hyperlactatemia
During the fasting state, ketogenesis in the liver is stimulated to produce ketones to be used as fuel by other tissues, thus preserving glucose for the brain.
- Defects in fatty acid oxidation -> a decrease of acetyl CoA -> impaired ketogeneis

Hypoketotic
Because of the defects in β oxidation of fatty acid and low ketone in the blood, cells have to use glucose as fuel, further depleting blood glucose. Hypoglycemia, drowsiness in the patient.
• Defect in beta oxidation -> accumulation of acyl CoA & acylcarnitine
• **Causes of hyperammonemia**

- Increase protein breakdown and deamination of amino acids to be used as substrates for gluconeogenesis during the fasting state

- Decrease synthesis of N-acetylglutamate*

  *allosteric activator of CPSI – rate-limiting step of urea cycle
INTEGRATION OF METABOLISM – PROBLEM CASE

• Causes of hyperammonemia

- Increase protein breakdown and deamination of amino acids to be used as substrates for gluconeogenesis during the fasting state

- Decrease synthesis of N-acetylglutamate*
  * allosteric activator of CPSI – rate-limiting step of urea cycle

- Decrease oxaloacetate production
  -> decrease aspartate

* The nitrogen atoms of urea come from free ammonia and the amino group of aspartate.
INTEGRATION OF METABOLISM – PROBLEM CASE

**β-oxidation of fatty acids**

**ω-oxidation**

**Fatty acid**

**β-oxidation in peroxisome**

**plasma dicarboxylic acid**

**dicarboxylic acid**
The patient was received intravenous injection of glucose to correct the hypoglycemic symptoms. The mother was advised to frequently feed the baby and not to let her fast for too long (>12 hr). At night, feeding the patient with corn starch was also recommended.

Briefly explain how the above therapeutic measures can resolve the patient’s problems.
### SUMMARY: INTEGRATION OF METABOLISM

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- **Insulin**

- **Counterregulatory hormones**

  • The processes are regulated to ascertain that the opposite processes do not occur at the same time.

  • The metabolism of carbohydrate, lipid, and protein are coordinated and well regulated to meet the bodily requirements, especially the energy need, under various conditions.