BIOCHEMISTRY

ED.08

METABOLISM IN FED AND FASTING STATE

----- Active space -----

Well Fed State

00:00:45

AKA Post prandial state/absorptive phase :

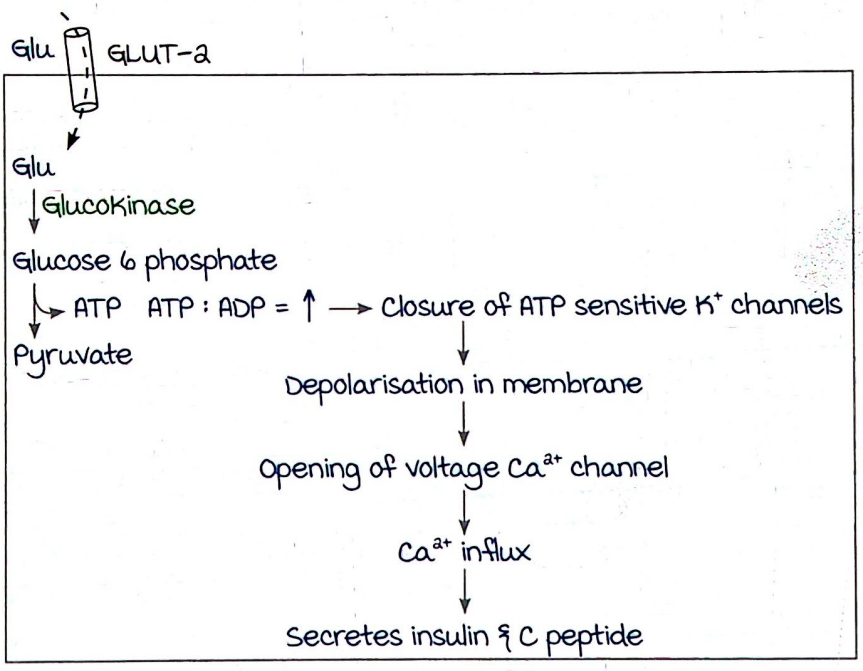
- 2-4 hrs after food.
- Storage metabolism.
- Components of food digested & absorbed in smaller components.
- ↑ in plasma level of glucose, amino acids, fatty acid, TAGs.
- Hormone of fed state is insulin.

Insulin secretion :

- Begins to rise : At blood glucose > 70 mg/dL/> 3.9 mmol/L.
- Assessment of level of C-Peptide = level of insulin.

In pancreas :

β cells of pancreas : GLUT-2 : High Km, low affinity for glucose.



Note : Insulin is synthesized in rough endoplasmic reticulum, packaged in golgi apparatus.

Actions of insulin :

- Favours protein translocation :
  - ↑ GLUT4: Insulin dependent glucose transporter.
  - ↑ Insulin receptor level.
- ↑ Gene transcription of glucokinase.
- ↑ Enzyme activity : Dephosphorylates regulatory enzyme → Phosphodiesterase. / Phosphatase.

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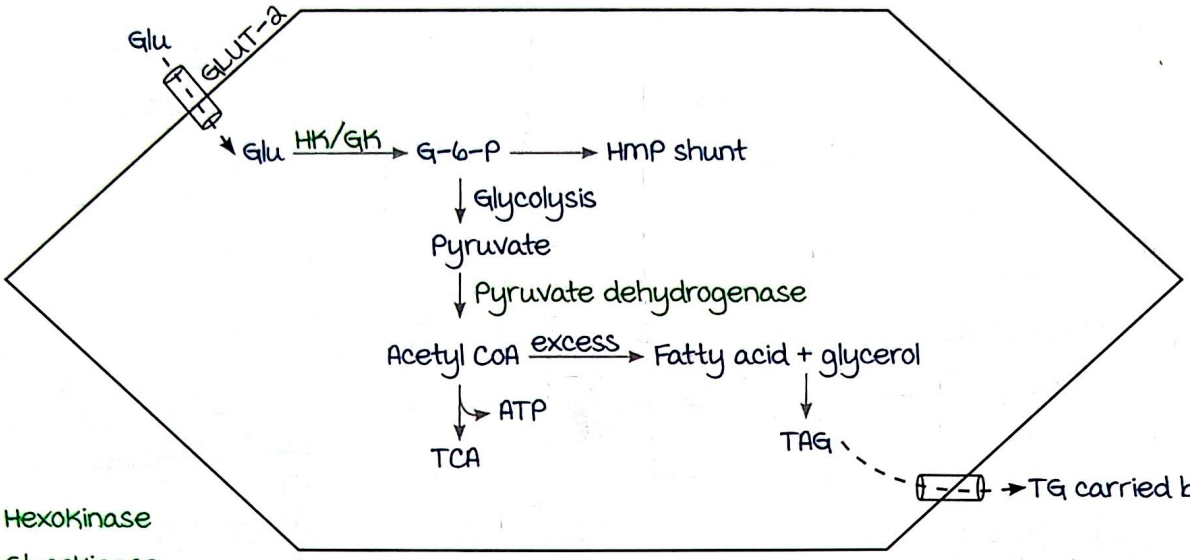
In liver :

Liver : Glucose consumed in fed state.

↑ Blood glucose



HK : Hexokinase

GK : Glucokinase

In fed state :

1. ↑ Blood glucose
   - ↓ Enters via GLUT-2
   - Liver
   - ↓
   - Undergoes glycolysis
   - ↓
   - Pyruvate
   - ↓ In presence of O_2 — PDH/Link reaction
   - Acetyl CoA
   - ↓
   - TCA ---→ ATP

2. In excess glucose → Glycogen synthesis : ↑↑ glucose → G6P → Glycogen.

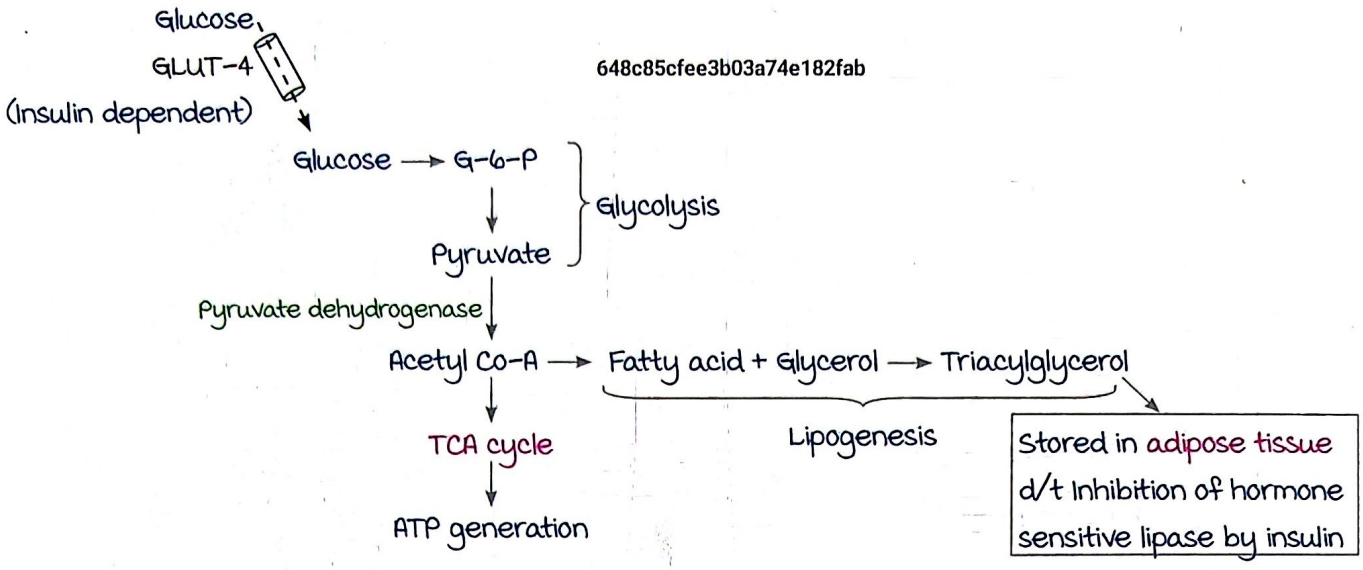
If excess glucose still remains :

↑↑ Glucose

- Pancreas
- HMP pathway
- Fatty acid synthesis

In Adipose tissue :

----- Active space -----



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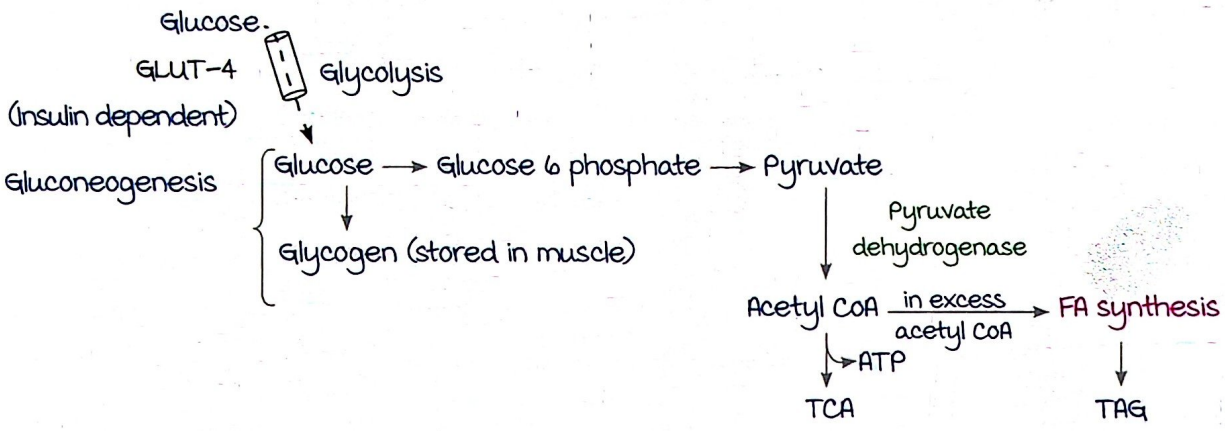
In Skeletal muscle :



Aminoacid in muscles :

- ↑ Protein synthesis
- Transamination
- Oxidative deamination

} Removal of amino group → Carbon skeleton → Anabolic functions.

In Brain :

Glucose in brain :

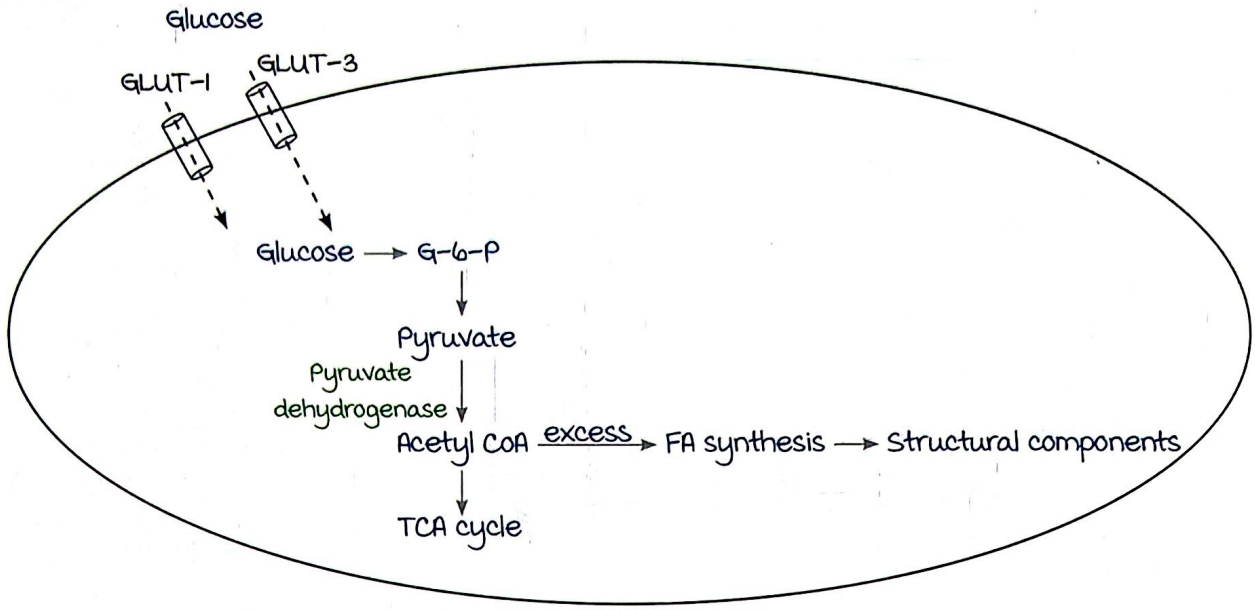
i. Obligatory requirement of glucose.

ii. Oxidative pathway only for energy production.

Feedback

----- Active space -----

Neuron :



GLUT-3 :

- Neuronal glucose transporter.
- Highest affinity, Lowest Km.
- Aerobic glycolysis.

Metabolic fuels in fed state :

Glucose only	Glucose > FFA	FFA > glucose
• Brain • RBC	• Liver • Adipose tissue • Skeletal muscle	• Heart (D/t low glycolytic capacity)

Fasting State/Post Absorptive State

00:28:13

Utilizing stored glycogen & triacylglycerol.

States	Duration without food intake
Early fasting	4-16 hrs
Fasting	16-48 hrs
Prolonged fasting / starvation	2-5 days
Prolonged starvation	>5 days

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Early fasting :

- Hepatic glycogenolysis : Source of glucose.
- Muscle lacks G-6-Pase → Cannot release free glucose directly.
- Depletes in 16-18 hrs.

----- Active space -----

Glycogen $\xrightarrow[\text{glycogenolysis}]{\text{hepatic}}$ G-6-P → Glucose.

Fasting :

Source : Gluconeogenesis (ATP (+)) → Production of glucose from non-carb substrates :

- Glycerol : From TAG.
- Lactate.
- Alanine : Glucogenic aminoacid from muscle.

Gluconeogenesis :

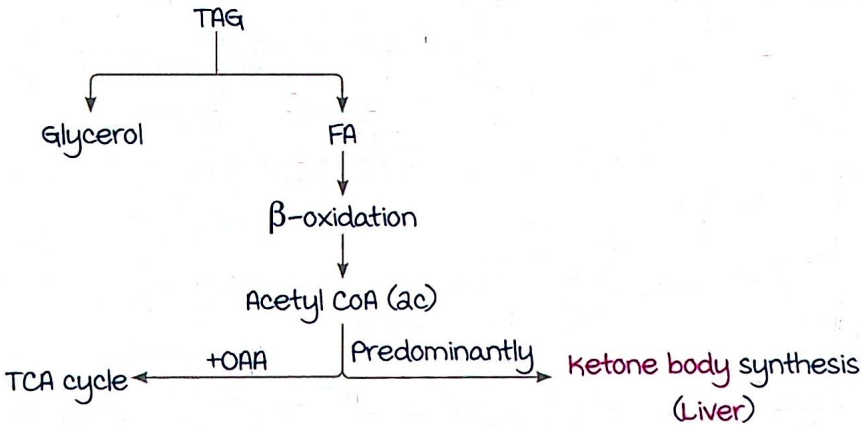
Triacylglycerol (Stored in adipose tissue)

↓ Hormone sensitive lipase ← (+) Glucagon

Glycerol + FA $\xrightarrow{\beta\text{-oxidation}}$ Acetyl coa → TCA cycle → ATP

Starvation/Prolonged fasting :

- ↓ Gluconeogenesis : Non-carb sources are used up.
- Source : Ketone bodies



Note : OAA depleted d/t ↑ gluconeogenesis in fasting state.

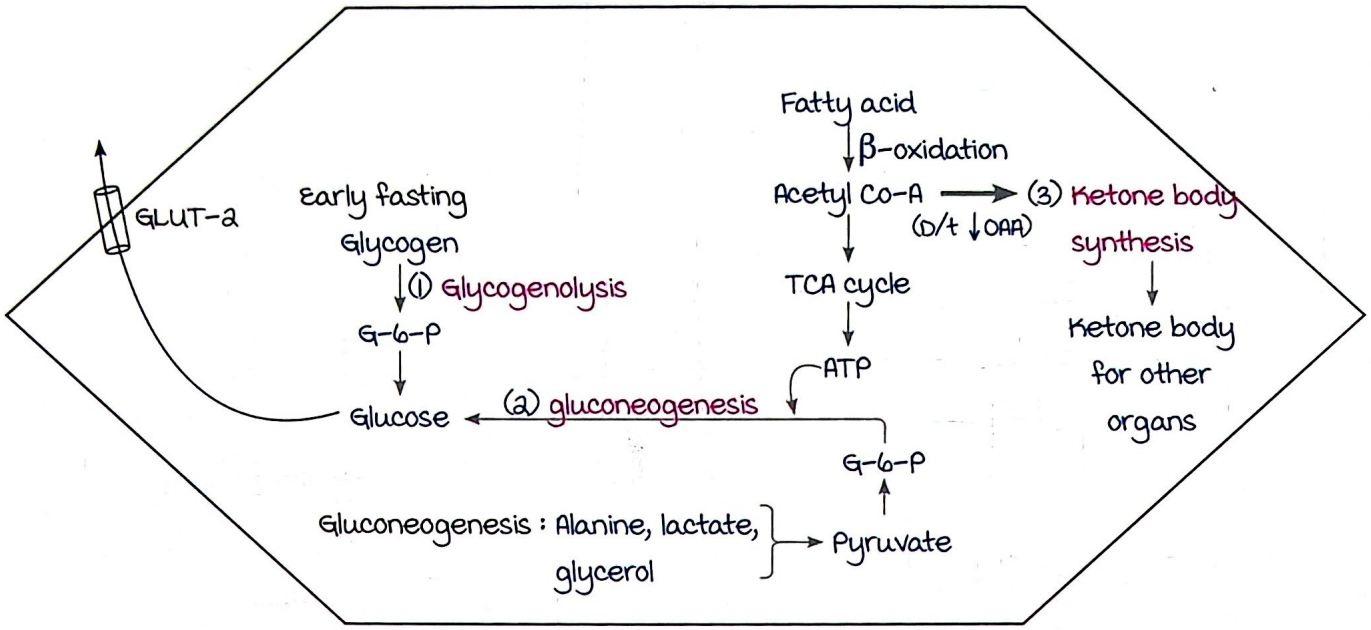
Further Starvation :

Structural muscle proteins
↓ Catabolism
Aminoacid
↓
C-skeleton
↓
Catabolic role (Causes cachexia)

----- Active space -----

FASTING STAGE IN ORGANS

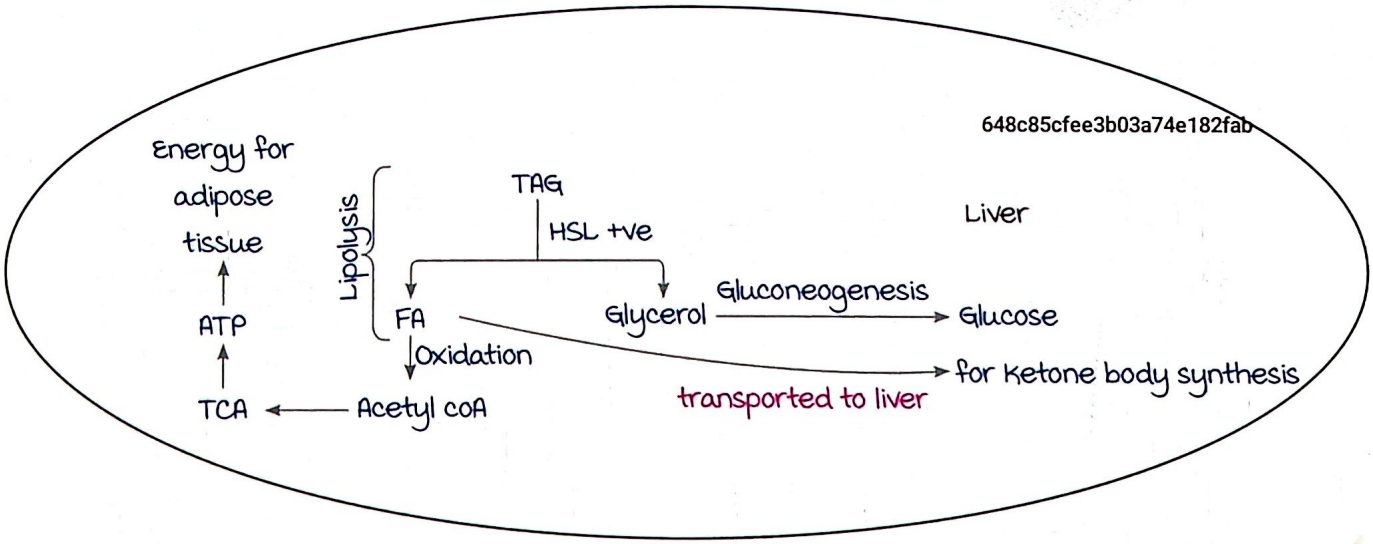
In Liver :



- Liver spares ketone bodies.

In Adipose tissue :

↓ Insulin → ↑ HSL activity.



In Skeletal muscle :

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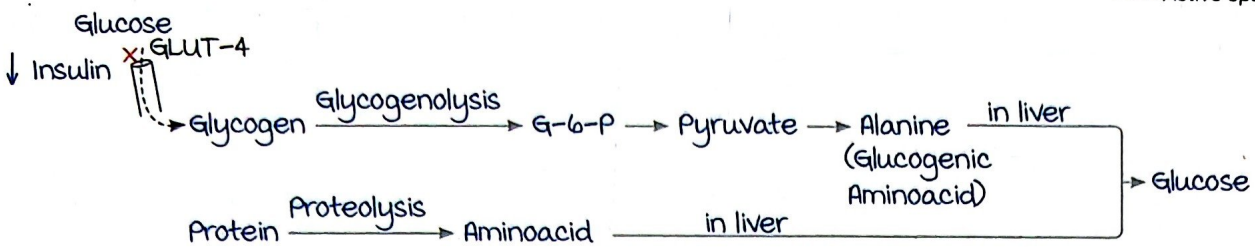

Source of energy in muscle :

1. Fatty acid → Oxidation → Acetyl → TCA → ATP.
2. Ketone bodies from liver.

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In Brain :

During starvation :

- Available glucose transported via GLUT-3 and converted to ATP.
- Ketone body lysis (provides for only 20% energy requirements) → Acetyl CoA → TCA cycle → ATP.

Metabolic fuel in fasting :

Organ	Early fasting/fasting state	Starvation
Brain	Glucose	Glucose/Ketone bodies (20%)
RBC	Glucose	Available glucose Absence of glucose → Lysis of RBC
Liver	Free fatty acid > glucose	Aminoacid, free fatty acid
Adipose tissue	FFA > glucose	FFA / Ketone bodies
Skeletal muscle		
Heart		

----- Active space -----

CONCEPT OF ENZYME REGULATION

Covalent Modification

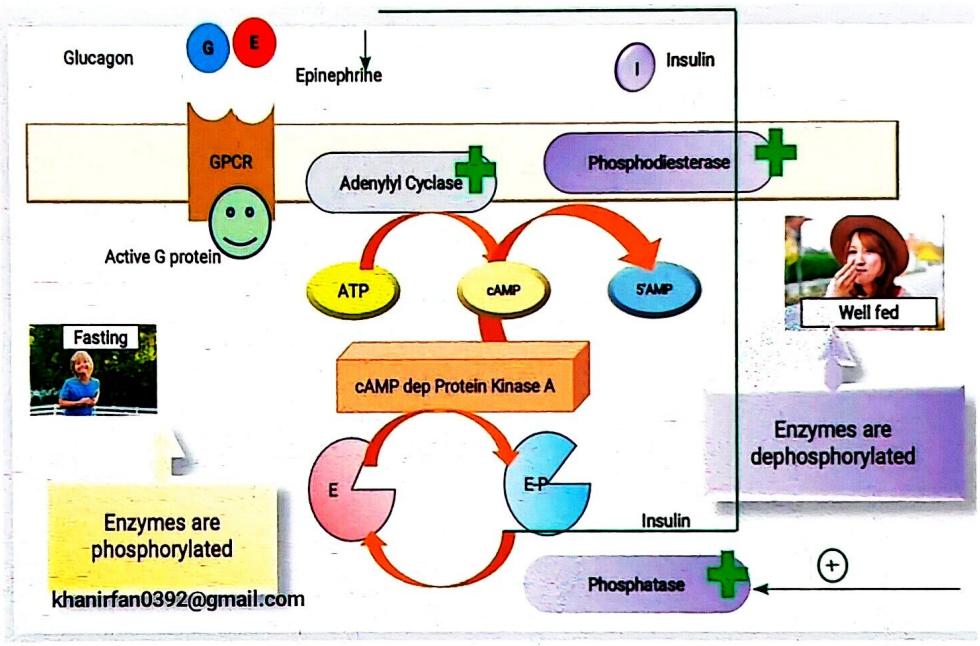
00:01:00

Sites of phosphorylation

Hydroxyl group containing amino-acids:

- Serine (m/c)
- Threonine
- Tyrosine

Mechanism



Examples

Enzyme	Insulin : Glucagon ratio	High activity in
Phosphofructokinase (in glycolysis)	High	DP state
Fructose-1,6-bisphosphate (in gluconeogenesis)	Low	P state
Glycogen synthase	High	DP state
Glycogen phosphorylase (in glycogenolysis)	Low	P state
Pyruvate dehydrogenase (link between glycolysis & TCA cycle)	High	DP state

Feedback

Enzyme	Insulin : Glucagon ratio	High activity in
Acetyl CoA carboxylase (Fatty acid synthesis)	High	DP state
HMG CoA reductase (Cholesterol synthesis)	High	DP state
Hormone sensitive lipase (hydrolysis of stored TAG in adipose tissues)	Low	P state

Allosteric Regulation

00:16:31

Feed Forward reaction

↑[Substrate] $\xrightarrow{\oplus}$ Forward reaction

Feedback inhibition

↑[Product] $\xrightarrow{\ominus}$ Forward reaction

Enzyme	Allosteric activator	Allosteric inhibitor
Phosphofructokinase (In glycolysis) Fructose-6- phosphate → Fructose-1,6-bisphosphate (Product)	Substrates in glycolysis • 5' AMP • Fructose-6-phosphate	Products of glycolysis: • ATP • Low pH (d/t lactic acid) • Citrate (formed from acetyl CoA)
Acetyl CoA carboxylase Acetyl CoA → Malonyl CoA	Citrate (substrate)	• Malonyl CoA (product) • Acyl CoA (fatty acid product)
ALA synthase	-	Heme (product)

----- Active space -----

INTRODUCTION TO ENZYMES

Enzymes

Specialised proteins that can act as biological catalysts.

Exception :

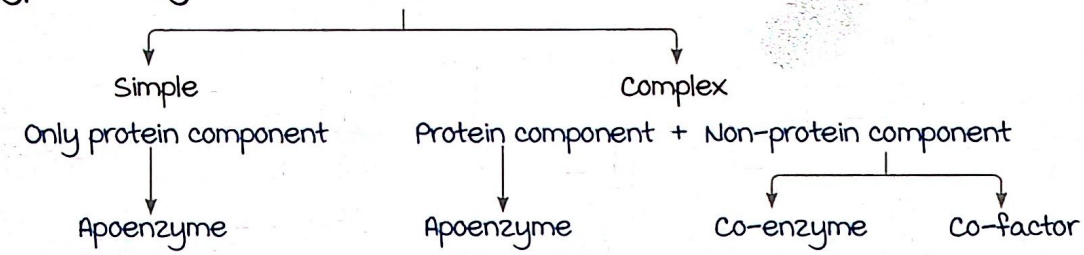
Ribozyme : RNA acts as enzymes.

Ribozymes	Function
Ribosome • 28S rRNA • Peptidyl transferase	Peptide bond synthesis.
Sn RNA	Splicing of exons : post-transcriptional modification of mRNA.
Group II introns	
Ribonuclease P	Post-transcriptional modifications of tRNA.

Enzymes, Co-enzymes and Co-factors

00:05:27

Types of enzymes :



Properties of enzymes :

Enzymes are proteins.

- Nitrogen : 16% by weight.
- Heat labile.
- Precipitated by protein precipitating agents.

Co-enzyme :

- Second substrate or co-substrate.
- Mostly B-complex vitamins.

Properties of co-enzyme :

----- Active space -----

- Heat stable.
- Low molecular weight organic molecule.

Examples :

	Active form	Reactions involved.
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	• Oxidative decarboxylation. • Transketolase.
Riboflavin (B_2)	Flavine adenine dinucleotide (FAD) FMN	• Oxidative decarboxylation. • Redox reaction : – Complex I of electron transport chain (ETC). – Predominantly FMN.
Niacin (B_3)	Nicotinamide adenine dinucleotide (NAD^+), Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	• Oxidative decarbozylation : Predominantly NAD^+ • Oxidative-reduction reaction (dehydrogenase)
Pantothenic acid (B_5)	Co-Oenzyme A	Transfer of acyl group
Pyridoxine (B_6)	Pyridoxal phosphate (PLP)	• Transamination. • Trans-sulfuration.
Folic acid (B_9)	Tetrahydrofolate (THFA)	One carbon transfer.
Cobalamine (B_{12})	Methyl B_{12}	Homocysteine methyl transferase
	Adenosyl B_{12}	Methyl-malonyl CoA mutase
Lipoate	Lipomide – Oxidised form – Reduced form	Oxidative phophorylation
Ascorbic acid (C)	Ascorbate	Hydroxylation reaction

Co-factor :

- Inorganic molecules.
- Predominantly minerals.

----- Active space ----- Types

Metalloenzyme :

- Metal & apoenzyme tightly integrated.
- Eg :
  - Cu in tyrosinase.
  - Zn^{2+} in — Carbonic anhydrase. / Carboxy peptidase.

Metal activated enzyme :

- Metal not tightly integrated with enzyme.
- Presence of metal is required for enzyme action.
  - Eg: Ca^{2+} required for action of lipase.

Prosthetic group :

Co-enzyme/ metalloenzyme (Co-factor) tightly integrated to enzyme.

Holoenzyme

00:17:29

- A type of complex enzyme.
- Apoenzyme + co-enzyme/ co-factor.

Examples :

Metals	Enzyme	Function
Zinc	Carbonic anhydrase.	Transport of CO_2.
	Carboxypeptidase A & B .	Digestion of proteins.
	Alcohol dehydrogenase.	Retinol $\rightleftharpoons$ Retinal (Vision).
	Alkaline phosphatase.	Removal of phosphate in alkaline medium .
	ALA dehydratase.	Synthesis of heme.
	Adenosine deaminase.	Purine catabolism.
	Cystolic superoxide dismutase (SOD).	Free radical scavenging : Anti-oxidant.
	Lactate dehydrogenase.	Anaerobic glycolysis.
Magnesium	• Kinase. • Phosphatase. • Mutase. • Enolase.	Transfer of phosphate.
Iron	Heme iron : Complex of III & IV of ETC (present in cytochrome).	-
	- Nitric acid synthase. - Peroxidase, catalase.	• Synthesis of nitric oxide. • Free radical scavengers.

----- Active space -----

metals	Enzyme	Function
Iron	Tryptophan dioxygenase	-
	Non-heme iron : Complex I & II of ETC (found as Fe-S complex)	-
Manganese	• Kinase, phosphatase • Arginase • Ribonucleotide reductase • Mitochondrial SOD	-
Molybdenum	Xanthine oxidase	Purine catabolism - End product: Uric acid - Deficiency of molybdenum: Hyperuricemia.
Potassium	• Pyruvate Kinase • Na^+-K^+ ATPase	-
Copper	Tyrosinase 648c85cfee3b03a74e182fab	Melanin synthesis - Deficiency of copper : Hypopigmentation.
	Complex IV ETC	Energy productions
	Lysyl oxidase	Collagen synthesis - Deficiency of copper: Bleeding manifestations.
Nickel	Urease	Not seen in humans
Calcium	• Lecithinase • Lipase	-
Selenium	Glutathione peroxidase	Free radical scavenger (Anti-oxidant)
	Thioredoxin reductase	-
	Deiodinase	Thyroid hormone synthesis
	Selenoprotein P	-

Feedback

----- Active space -----

CLASSIFICATION OF ENZYMES

OVERVIEW

Trivial name of enzyme :

- Named after reaction mechanism (m/c) /substrate.
- Can be common for 2 enzymes.

IUBMB Classification of Enzyme :

(International Union of Biochemistry and Molecular Biology)

Enzyme commission/class/code number : 4 digits.
Eg : 1 1 1 1 (Alcohol dehydrogenase).

1st digit :	2nd digit :	3rd digit :	4th digit :
Class	Subclass	Subsubclass	Unique number for every enzyme

Classes of enzymes :

7 Classes → mnemonic : Operation Theatre Has Low Intensity LighT

- Oxidoreductase.
- Transferase.
- Hydrolase.
- Lyase.
- Isomerase.
- Ligase.
- Translocase (added on August 2018).

Class I: Oxidoreductases

00:08:07

Enzyme that catalyze oxidative reduction reactions.

SUBCLASS 1 : DEHYDROGENASES (DH)

- Catalyze transfer of hydrogen elements (H^+, H^-, H_2) & electrons to an acceptor in a coupled oxidation-reduction reaction.

Acceptors (Co-enzymes) :

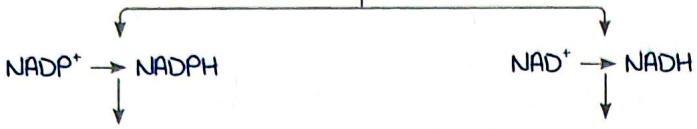
Flavoproteins

- FAD → $FADH_2$
- Catalyzing enzymes

- Acyl CoA DH (in β-oxidation).

- Succinate DH (in Tricarboxylic acid cycle).
- Glycerol-3-P D H (mitochondrial).

Nicotinamide



Catalyzing Enzymes
- enzymes in HMP shunt pathway
  - Glucose-6-phosphate DH.
  - 6-Phosphogluconate DH.
- Cytoplasmic Isocitrate DH.
- Malic enzyme.

Catalyzing enzymes
- most other enzymes.

SUBCLASS – 2 : OXIDASES

Substrate $\xrightarrow[\text{(catalyze } H_a \text{ transfer)}]{\text{oxidase}}$ Product + H_aO / H_aO_a
(H_a acceptor is O_a)

Eg :
- Cytochrome C oxidase
  - Complex III of electron transport chain.
  - O_a gets reduced to H_aO.
- Mono amino oxidase.
- L-Amino acid oxidase.
- Xanthine oxidase.

} Produce H2O2

SUBCLASS 3 : OXYGENASE

Add O_a directly to the substrate.

Types :

	Monooxygenase/ Mixed function oxygenase	Dioxygenase
Features	• Enzymes adding 1 atom of O_a	• Enzymes adding both atoms of O_a
Examples	• Most are hydroxylases • Eg : – Phenylalanine hydroxylase – Tyrosine hydroxylase – Tryptophan hydroxylase } aromatic hydrolases – 7α hydroxylase – Cytochromes	• Homogentisate deoxygenase • Tryptophan deoxygenase

Feedback

----- Active space -----

SUBCLASS 4 : HYDROPEROXIDASES

Types :

	Peroxidase	Catalase
Substrate	H_2O_2 > organic peroxide	
Electron acceptor	Eg : Glutathione (GSH) H_2O_2 → H_2O (GSH (reduced) → GSSG (oxidised); Glutathione peroxidase) • Ascorbate • Quinones • Cytochrome C	H_2O_2 $2H_2O_2$ —Catalase→ $2H_2O + O_2$

Class II : Transferases and Class III : Hydrolases 00:23:25

CLASS II : TRANSFERASES

Transfer of functional groups (except hydrogen) to acceptors.

Examples

- Transaminase
- Transketolase

(enzymes with trans prefix)

Kinases

Catalyse transfer of PO_4^{3-} from ATP (organic molecule)

a) Glucose/Galactose —(Glucokinase/Hexokinase; ATP → ADP)→ Galactose/Glucose 6-phosphate

b) Fructose 6-phosphate —(PhosphofructoKinase (PFK); ATP → ADP)→ Fructose 1,6 bisphosphate

Phosphorylases

Glycogen (n) —(Glycogen phophorylase; Pi (inorganic PO_4^{3-}) → Glu-1 PO_4^{3-})→ Glycogen (n-1)

n=number of glucose molecules

CLASS III : HYDROLASES

----- Active space -----

Catalyze hydrolytic cleavage of covalent bonds (C-C, C-N, C-O)

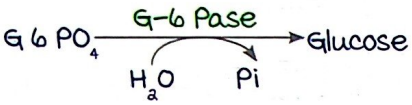
Eg :

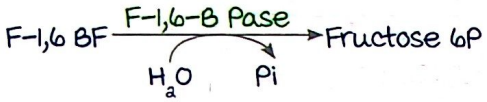
1) Digestive enzymes

Biomolecule	Covalent bond	Digestive enzymes
Carbohydrates	Glycosidic linkage	Amylase, maltase, lactase, sucrase
Protein	Peptide bond	• Protease : Trypsin, chymotrypsin, elastase, pepsin • Peptidase
Nucleic acid	3'-5' Phosphodiester bond	Exonuclease
Lipids	Ester bond	Lipase, esterase

2) Phospholases

3) Arginase (in urea cycle)





Class IV Lyases

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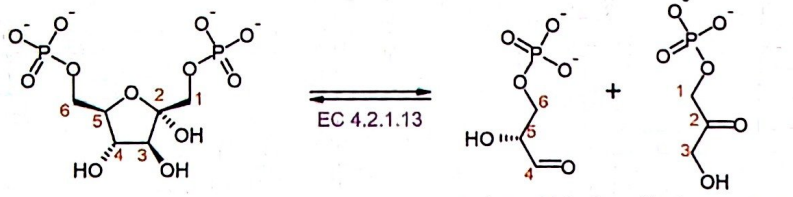
Examples :

1) Cleave covalent bond without adding water

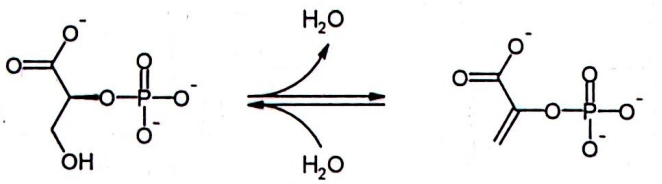
Eg : Aldolase



β-D-Fructose 1,6-bisphosphate — D-glyceraldehyde 3-phosphate — dihydroxyacetone phosphate

2) Form double bonds by atom elimination,

Eg : Enolase



2-phosphoglycerate — EC 4.2.1.11 — phosphoenolpyruvate

3) Add groups across double bonds/form single bond

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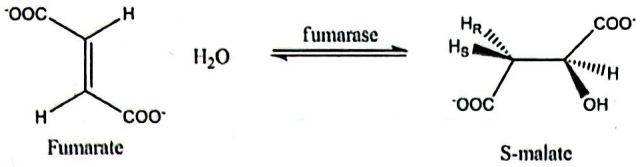
Eg : Fumarase



4) All enzymes with lyase suffixes

Eg : HMG CoA lyase, arginosuccinate lyase, simple decarboxylation enzymes

5) Decarboxylases :

Type of decarboxylation

	Simple decarboxylation	Oxidative Decarboxylation
Enzyme	Class: Lyases Subclass : decarboxylase	Class: Oxidoreductases Subclass : Dehydrogenases • All are multienzyme complexes
Examples	• Histidine $\xrightarrow{PLP}$ Histamine (CO_a) • Tryptophan $\xrightarrow{PLP}$ Tryptamine • Tyrosine $\xrightarrow{PLP}$ Tyramine • Glutamate $\xrightarrow{PLP}$ GABA • Dihydroxy phenylalanine (DOPA) $\xrightarrow{PLP}$ Dopamine → Epinephrine + Norepinephrine	• Pyruvate (3C) → Acetyl CoA (2C) (NAD^+, CO_a, NADH) • α Ketoglutarate (5C) → Succinyl CoA (4C) (NAD^+, NADH, CO_a) • Branched chain Ketoacid (nC) → Corresponding Acyl CoA ((n-1)C) (CO_a, NAD^+, NADH) (n=number of Carbon atoms)
Coenzyme	Vitamin B6 (PLP)	• 5 coenzymes – Thiamine pyrophosphate (TPP) – Coenzyme A – Lipoate/lipomide – FAD – NAD^+
Applied aspect	Pyridoxine (B6) dependent seizures in neonates Pathogenesis : B6 deficiency ↓ Lack of neurotransmitters ↓ Seizures	-

Class V: Isomerases, Class VI: Ligases, Class VII: Translocases 00:48:13 ----- Active space -----

CLASS V : ISOMERASES

- Catalyze structural or geometric isomer formation.

Subclasses

Subclass	Examples
1) Isomerases	• G_6PO_4 (6C) ⟷ (Phosphohexose isomerase) F_6PO_4 (6C) • Dihydroxyacetone PO_4 (3C) ⟷ (Phosphotriose isomerase) Glyceraldehyde 3 PO_4 (3C)
2) Mutase Intra molecular transfer of functional groups.	• G_6PO_4 → (Phosphoglucomutase) G_1PO_4 • 3PG ⟷ (Phosphoglycerate mutase) 2PG (phosphoglycerate)
3) Racemase Create D and L isomers from each other.	• D alanine → L-alanine • D glucose → L-glucose

Note : Kinase: Transfers PO_4^{3-} from 1 substrate to another.

CLASS VI : LIGASES

Coupling of 2 molecules with ATP hydrolysis.

Subclass 1

Biotin dependent carboxylase

- Pyruvate (3C) → (Pyruvate carboxylase; ATP → ADP) Oxaloacetate (4C)
- Acetyl CoA (2C) → (Acetyl CoA carboxylase; ATP → ADP) malonyl CoA (3C)
- Propionyl CoA (3C) → (Propionyl CoA carboxylase; ATP → ADP) methyl malonyl CoA (4C)

- ATP hydrolysis.
- Biotin (B7) dependent.
- CO_2 fixation.

Biotin independent carboxylation :

- Carbamoyl phosphate Synthetase (CPS) 1 – urea cycle.
  CPS II (Pyrimidine synthesis).
- Gamma carboxylation (Vit K required).
- Malic enzyme.
- AIR carboxylase (in de novo purine synthesis).