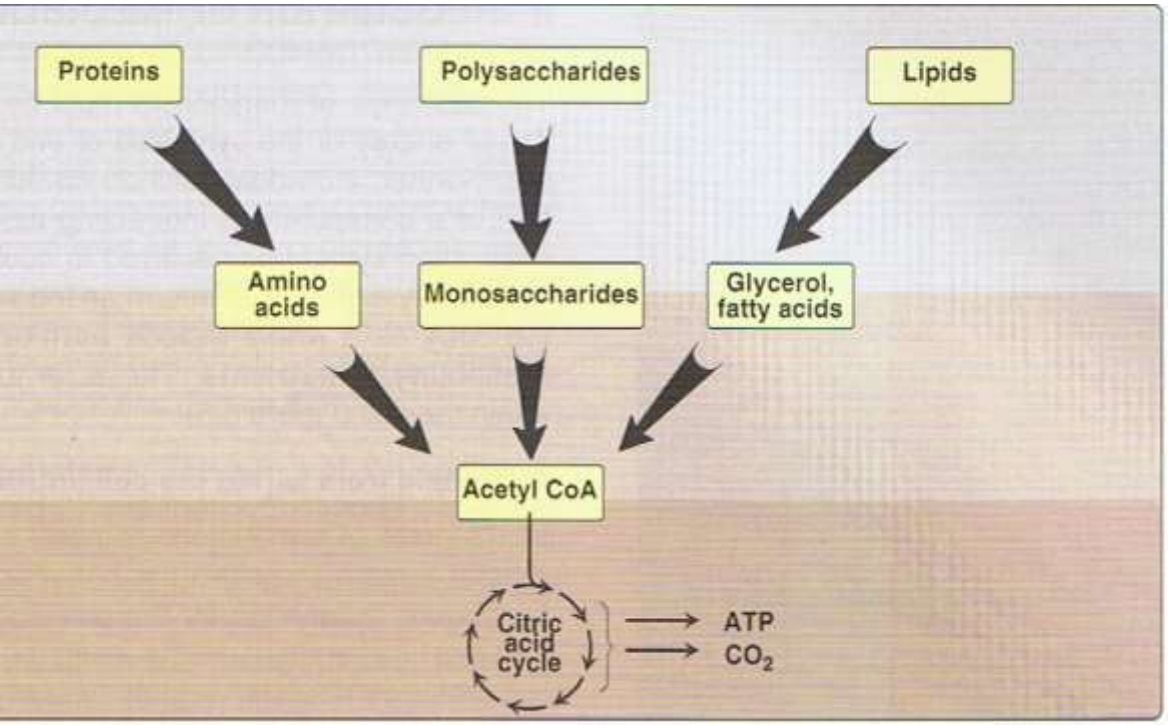
Glucose metabolism

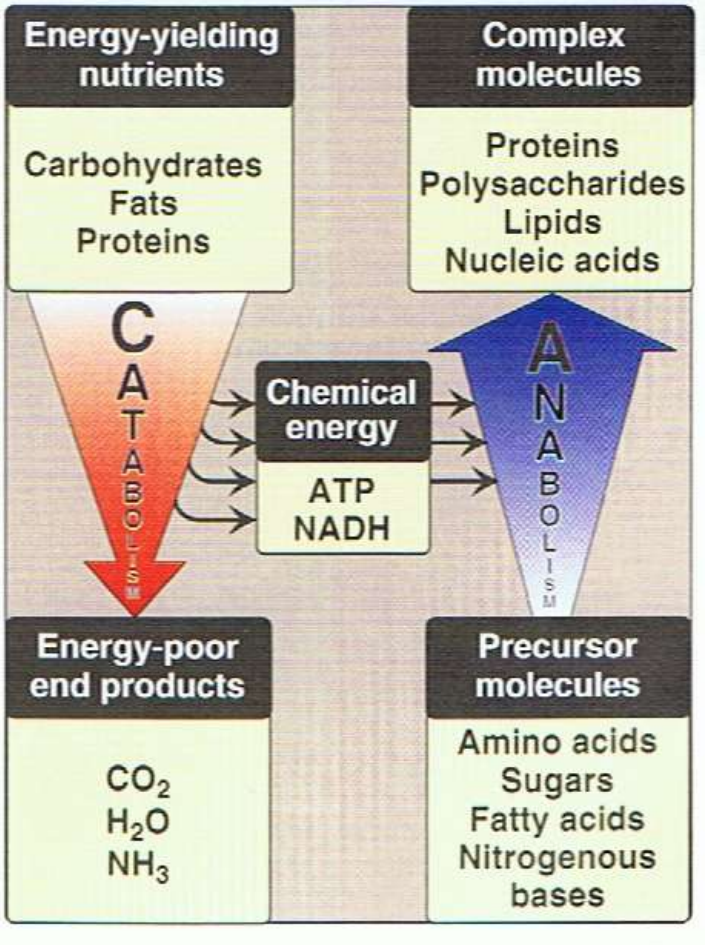
Metabolism

- Most pathways can be classified into:
  - **Catabolism**: degrade complex molecules (proteins, carbohydrate and triglycerides) to few simple products (CO_2, NH_3 and H_2O). Capture chemical energy to form ATP. Considered a **convergent** process (large no. of substances are degraded to few common end products).
  - **Anabolism**: synthesize complex end products from simple precursors. Requires energy which is provided by the breakdown of ATP. Considered a **divergent** process (few starting precursors produce wide variety of complex substances)

المقارنة	Catabolism	Anabolism
المعنى	هدم	بناء
الطاقة	ينتج ATP	يستهلك ATP
الاتجاه	من simple إلى complex	من complex إلى simple
الوصف	Convergent	Divergent

Catabolism


Proteins
Polysaccharides
Lipids
Amino acids
Monosaccharides
Glycerol, fatty acids
Acetyl CoA
Citric acid cycle
ATP
CO_2


Energy-yielding nutrients
Carbohydrates
Fats
Proteins
Complex molecules
Proteins
Polysaccharides
Lipids
Nucleic acids
CATABOLISM
Chemical energy
ATP
NADH
ANABOLISM
Energy-poor end products
CO_2
H_2O
NH_3
Precursor molecules
Amino acids
Sugars
Fatty acids
Nitrogenous bases

Regulation of metabolism

- Signals from within the cell (intracellular)

  The rate of a metabolic pathway may be influenced by the availability of substrates, product inhibition, or alterations in the levels of allosteric activators or inhibitors.

- Communication between cells (intercellular)

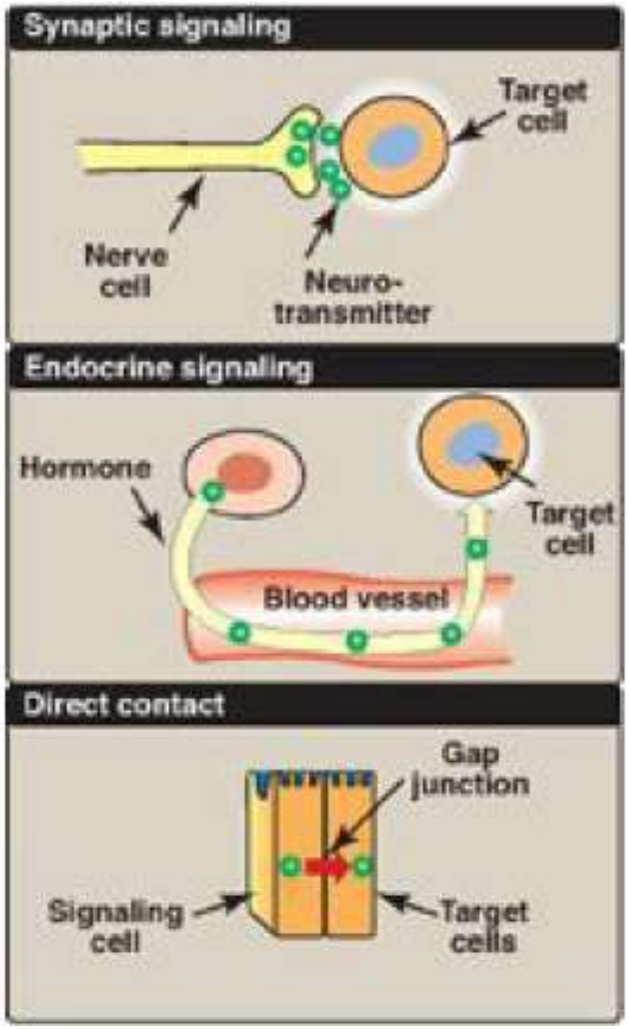
  Can be mediated by surface-to-surface contact, hormones and, in some tissues, by formation of gap junctions

- Second messenger systems

  Two of the most widely recognized second messenger systems are:

  - The calcium/phosphatidylinositol system
  - The adenylyl cyclase system

Communication between cells



Slide 5 — Communication between cells

الصورة تعرض 3 طرق للتواصل:

1. Synaptic signaling

هذا يحدث في الجهاز العصبي.

Nerve cell يفرز neurotransmitter مثل acetylcholine أو norepinephrine، ويرتبط بـ target cell.

2. Endocrine signaling

خلية أو غدة تفرز hormone في الدم، والدم يوصله لخلية بعيدة.

مثال:

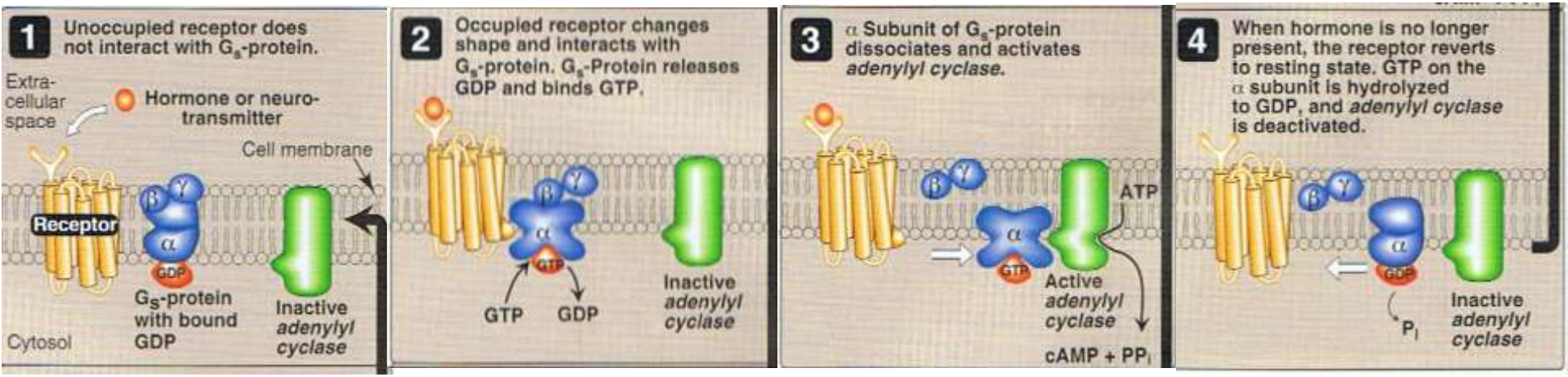
- Pancreas يفرز insulin
- Pancreas يفرز glucagon
- Adrenal medulla تفرز adrenaline

3. Direct contact / Gap junction

الخلايا تكون متصلة ببعض عن طريق **gap junctions**، فتنتقل ions أو small molecules مباشرة.

الفكرة:
تنظيم metabolism مش بس داخل الخلية، بل الجسم كله يتواصل كشبكة واحدة.

Regulation of metabolism
Adenylyl cyclase



1. GTP-dependent regulatory proteins (Gs and Gi-proteins)
2. Protein kinases: phosphorylates different proteins and enzymes
3. Dephosphorylation of proteins: Phosphatases reverse the effect of kinases.

Slide 6 — Regulation of metabolism: Adenylyl cyclase

هذا السلايد مهم لأن glucagon/adrenaline يستخدمون هذا النظام كثيرًا.

خلينا نشرحه خطوة خطوة.

Adenylyl cyclase system

عندك hormone خارج الخلية. هذا الهرمون لا يدخل الخلية غالبًا، بل يرتبط بـ receptor على cell membrane.

بعدها يتم تفعيل **G-protein**.

G-proteins نوعين مهمين:

Gs protein

Stimulatory G-protein
ينشط adenylyl cyclase.

Gi protein

Inhibitory G-protein
يثبط adenylyl cyclase.

ماذا يفعل adenylyl cyclase؟

يحول:

ATP → cAMP

cAMP = cyclic AMP
وهو second messenger.

ماذا يفعل cAMP؟

ينشط:

Protein kinase A / PKA

Slide 7 — cAMP-dependent Protein Kinase A

الصورة تبين كيف cAMP ينشط PKA.

PKA في الوضع الطبيعي تكون inactive لأنها مكونة من:

- Regulatory subunits
- Catalytic subunits

لما cAMP يرتبط بالـ regulatory subunits، يحرر الـ catalytic subunits.

الـ catalytic subunits هي التي تعمل phosphorylation للبروتينات.

تم يحدث:

Protein substrate + ATP → Phosphorylated protein + ADP

بعدها phosphatase ترجع البروتين لحالته الأصلية:

Phosphorylated protein + H_2O → Dephosphorylated protein + Pi

المغزى:
الهرمون خارج الخلية يعمل تأثير كبير داخل الخلية عن طريق cAMP وPKA.

امتحانيًا:
Glucagon increases cAMP → activates PKA → phosphorylates enzymes → inhibits glycolysis in liver and stimulates gluconeogenesis.

ماذا تفعل Protein kinase؟

تعمل **phosphorylation** للبروتينات والإنزيمات.

Phosphorylation يعني إضافة phosphate group.

وهذا ممكن:

- ينشط إنزيم
- أو يثبط إنزيم

حسب الإنزيم.

Dephosphorylation

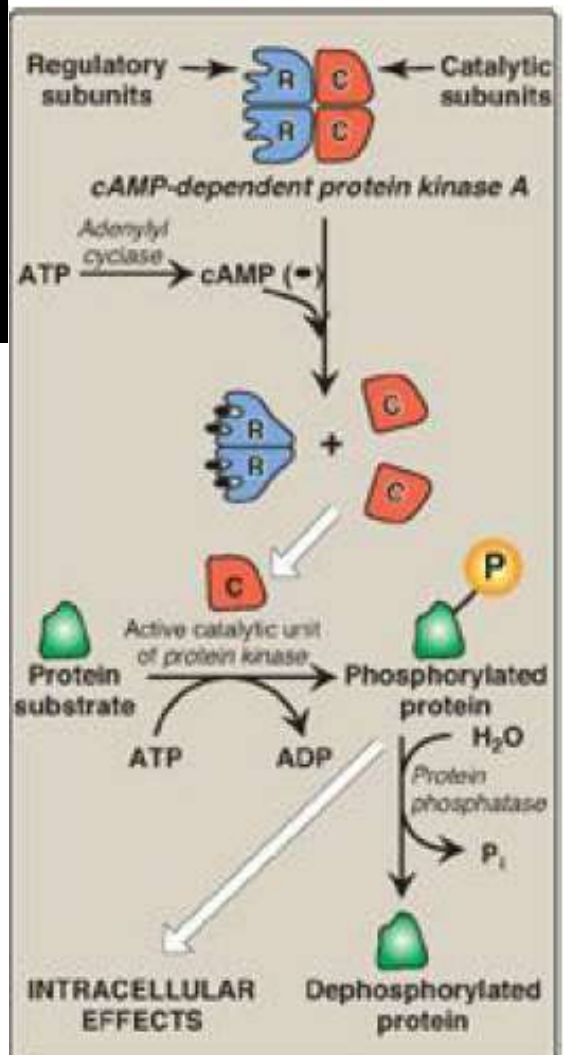
تأتي **Phosphatases** وتشيل phosphate group.

يعني:

- Kinases تضيف phosphate
- Phosphatases تشيل phosphate

قاعدة احفظها:

Kinase adds phosphate. Phosphatase removes phosphate.

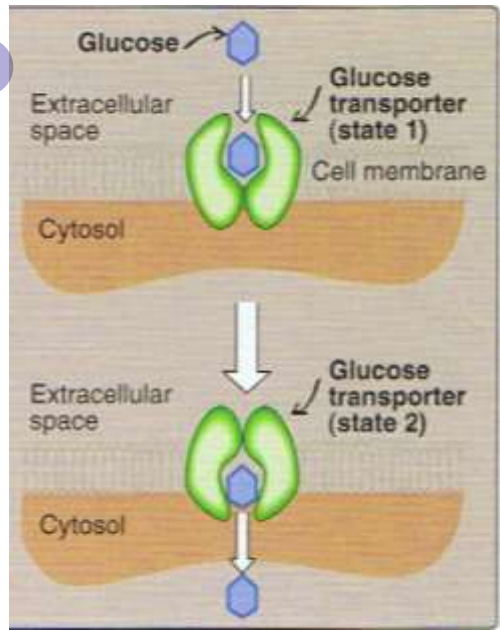


شرح الصفحتين بجوين

Transport of glucose to cells

- Glucose cannot diffuse directly into cells, but enters by one of two transport mechanisms:
- Na-independent, facilitated diffusion transport system
  - In facilitated diffusion, glucose movement follows a concentration gradient
  - Tissue specificity of GLUT gene expression:

1. GLUT-3 is the primary glucose transporter in neurons
2. GLUT-1 is abundant in erythrocytes and brain, but is low in adult muscle
3. GLUT-4 (in adipose tissue and skeletal muscle). Their number is increased by insulin
4. GLUT-2 (in the liver, kidney, and β cells of the pancreas) can either transport glucose into these cells or from it depending on blood glucose levels



Transport of glucose to cells

- GLUT-5 is the primary transporter for fructose in the small intestine and the testes
- GLUT-7 (in the liver and other gluconeogenic tissues) mediates glucose flux across the endoplasmic reticular membrane.

- **Na-monosaccharide cotransporter system:** is an energy-requiring process that transports glucose against a conc. gradient
  - This system is a carrier-mediated process in which the movement of glucose is coupled to the conc. gradient of Na, which is trasported into the cell at the same time.
  - It occurs in the epithelial cells of the intestine, renal tubules, and Choroid plexus.
  - This system is mediated by a family of fourteen glucose transporters in cell membranes (GLUT-1 to GLUT-14)
  - They exist in the membrane in two conformational states. Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

Slide 8 — Transport of glucose to cells

Glucose لا يستطيع دخول الخلية بسهولة لوحده.

ليش؟

لأنه polar molecule وفيه OH groups كثيرة، فلا يذوب في lipid bilayer بسهولة.

لذلك يحتاج transporter.

السلايد يقول glucose يدخل بطريقتين:

1. Na-independent facilitated diffusion

يعني نقل ميسر لا يعتمد على sodium.

هذا يتم بواسطة **GLUT transporters**.

Facilitated diffusion يعني:

- لا يحتاج ATP مباشرة
- يمشي مع concentration gradient
- من high concentration إلى low concentration
- يحتاج carrier protein

يعني لو glucose عالي خارج الخلية، يدخل.

GLUT tissue specificity

كل GLUT له مكان مهم.

GLUT-3

Primary glucose transporter in neurons.

يعني أهم ناقل glucose في neurons.

ليش؟
لأن الدماغ يحتاج glucose دائمًا، فلازم transporter عنده affinity عالي.

GLUT-1

Abundant in:

- Erythrocytes / RBCs
- Brain

لكن low in adult muscle.

RBCs تعتمد على glucose بالكامل لأنها ما عندها mitochondria.

GLUT-4

موجود في:

- Adipose tissue
- Skeletal muscle

وعددها يزيد بواسطة **insulin**.

يعني insulin يخلي GLUT-4 ينتقل إلى cell membrane، فيزيد دخول glucose للعضلات والدهون.

هذه نقطة امتحانية جدًا:

GLUT-4 is insulin-dependent.

GLUT-2

موجود في:

- Liver
- Kidney
- β cells of pancreas

ويمتاز أنه bidirectional.
يعني ينقل glucose للداخل أو للخارج حسب مستوى glucose في الدم.

في liver:

- بعد الأكل: glucose يدخل liver
- أثناء الصيام: liver يطلع glucose للدم

في β cells:

- glucose يدخل حسب مستواه في الدم
- ثم إفراز insulin يساعد الخلية على sensing glucose

Slide 9 — More glucose transporters

GLUT-5

Primary transporter for **fructose** في:

- Small intestine
- Testes

يعني لو الدكتور سألك: fructose يدخل الأمعاء بأي transporter؟
الجواب: **GLUT-5**

GLUT-7

في:

- Liver
- Other gluconeogenic tissues

وظيفته: mediates glucose flux across ER membrane.

Na-monosaccharide cotransporter system

هذا مختلف عن GLUT.

هذا نظام:

- يحتاج طاقة بشكل غير مباشر
- ينقل glucose ضد concentration gradient
- يعتمد على sodium gradient

يعني glucose يدخل الخلية حتى لو تركيزه داخل الخلية أعلى، لأنه "راكب" مع Na^+.

Na^+ يدخل مع **gradient**، وهذا يعطي الطاقة لسحب glucose معه.

هذا يسمى:

Secondary active transport

لأنه لا يستخدم ATP مباشرة في transporter نفسه، لكن يعتمد على Na^+ gradient الذي صنعته Na^+/K^+ ATPase.

أماكنه:

- Intestinal epithelial cells
- Renal tubules
- Choroid plexus

مثال مهم:
في الأمعاء، نحتاج نمتص glucose من lumen حتى لو تركيزه قليل، لذلك نستخدم Na-glucose cotransport.

السلايد يذكر أن GLUT family من GLUT-1 إلى GLUT-14، وأنها توجد بحالتين conformational states:

1. مفتوحة للخارج وتربط glucose
2. تغير شكلها وتفتح للداخل وتنقل glucose

Glycolysis

- Glycolysis occurs in the cytosol of all tissues and cells
- Defined as oxidation of glucose to pyruvic acid (in the presence of O_2, Aerobic) and to lactic acid (in the absence of O_2, anaerobic)
- The catabolism of 1 mol of glucose (6 C) produces 2 moles of pyruvate or lactate (3 C)
- Lactate is produced only in:
  - RBC: as there is no mitochondria
  - Exercising muscles: lack of O_2

glucose-6-phosphate الى glucose أول خطوة في glycolysis هي تحويل
polar أكثر
مش قادر يطلع من الخلية
محبوس داخل الخلية
ليش الجسم يفسفر الglucose؟
لان Phosphorylated sugar molecules لا تعبر membrane بسهولة

Phosphorylation of glucose

- Phosphorylated sugar molecules do not readily penetrate cell membranes (no carriers, too polar to cross)

glucose هيك بس Hexose sugar للأكثر من phosphorylate يعني بستطيع اعمل ①

- Hexokinase has broad substrate specificity and it is inhibited by the reaction product, glucose 6-phosphate

- It has a low Km (high affinity) for glucose. And low Vmax

- Glucokinase (similar broad specificity): In liver parenchymal cells and islet cells of the pancreas

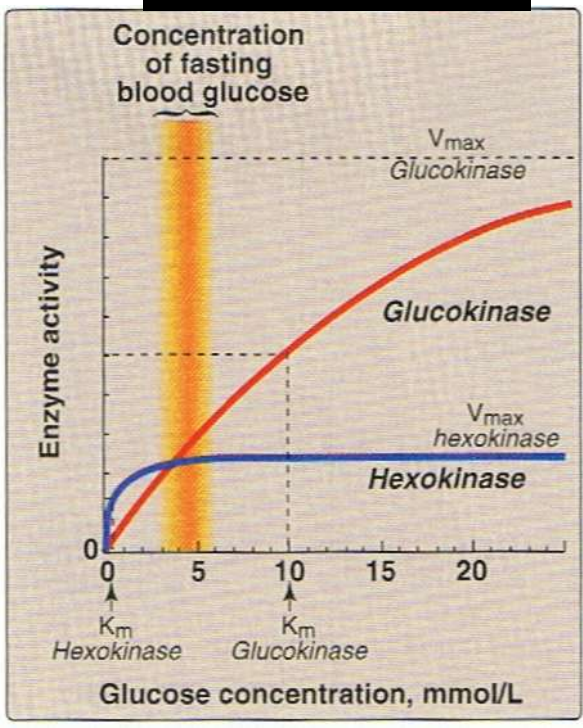
Glut-2

- In β cells, glucokinase functions as the glucose sensor, determining the threshold for insulin secretion. In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia.

- Glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated, such as during the brief period following consumption of a carbohydrate- rich meal

الفرق بين Glucokinase و Hexokinase

المقارنة	Hexokinase	Glucokinase
المكان	معظم الأنسجة	Liver و pancreatic β cells
Km	Low Km	High Km
Affinity	High affinity	Low affinity
Vmax	Low Vmax	High Vmax
يعمل متى؟	حتى glucose قليل	عندما glucose عالي
inhibition by G6P	نعم	لا غالبًا
الوظيفة	يضمن دخول glucose للخلايا	يخزن glucose بعد الأكل ويعمل glucose sensor



Steps of glycolysis

Energy investing phase
ATP يستهلك

Energy generating phase
NADH و ATP ينتج

metabolism الحصول على glucose وتنتج highly exergonic تكون

- Energy investing phase:
  - Step 1: glucose is phosphorylated to glucose-6-phosphate. The reaction is irreversible and is catalyzed by either glucokinase (GK) in liver cells and hexokinase (HK) in other tissues.

1 ATP يستهلك

  - Step 2: glucose-6-phosphate is isomerized to fructose-6-phosphate by isomerase enzyme
  - Step 3: fructose-6-phosphate is phosphorylated to F-1,6-diphosphate. The reaction is catalyzed by phosphofructo-kinase (PFK).

1 ATP يستهلك
irreversible و

  - Step 4: F-1,6-bP is split by aldolase into two trioses (Glyceraldehyde-3-P and dehydroxyacetone phosphate)

Steps of glycolysis

- Step 5: DHAP is isomerized to G-3-P which is catalyzed by isomerase

II- Energy generating phase:

- G-3-P is oxidized phosphorylated forming 1,3-biphosphoglycerate (1,3-BPG) and NADH which is catalyzed by glyceraldehyde 3-P dehydrogenase. NADH produces 2.5 ATP in ETC.
- Step7: 1,3-BPG gives its high energy phosphate to ADP to form ATP converting to 3-PG. This is catalyzed by phosphoglycerate kinase.
- Step 8: 3-PG is converted to 2-phosphoglycerate by mutase

Steps of glycolysis

- Step 9: Enolase enzyme dehydrates 2-PG forming 2-phosphoenol pyruvate (PEP) ⇒ high-energy
- Step 10: PEP is dephosphorylated giving its P to ADP to form ATP and converted to pyruvate. Rxn is irreversible and catalyzed by pyruvate kinase. 1 PEP ⇒ 1 ATP
- Step 11: in RBC's and under anaerobic conditions NADH formed in step 6 is oxidized to give hydrogen and pyruvate which converts into lactate by lactate dehydrogenase

لأن step 6 في NAD^+ تحتاج glycolysis
ولو ما رجع NAD^+ الـ glycolysis تتوقف

Regeneration of NAD^+ هو الأساسي من Lactate تكوين

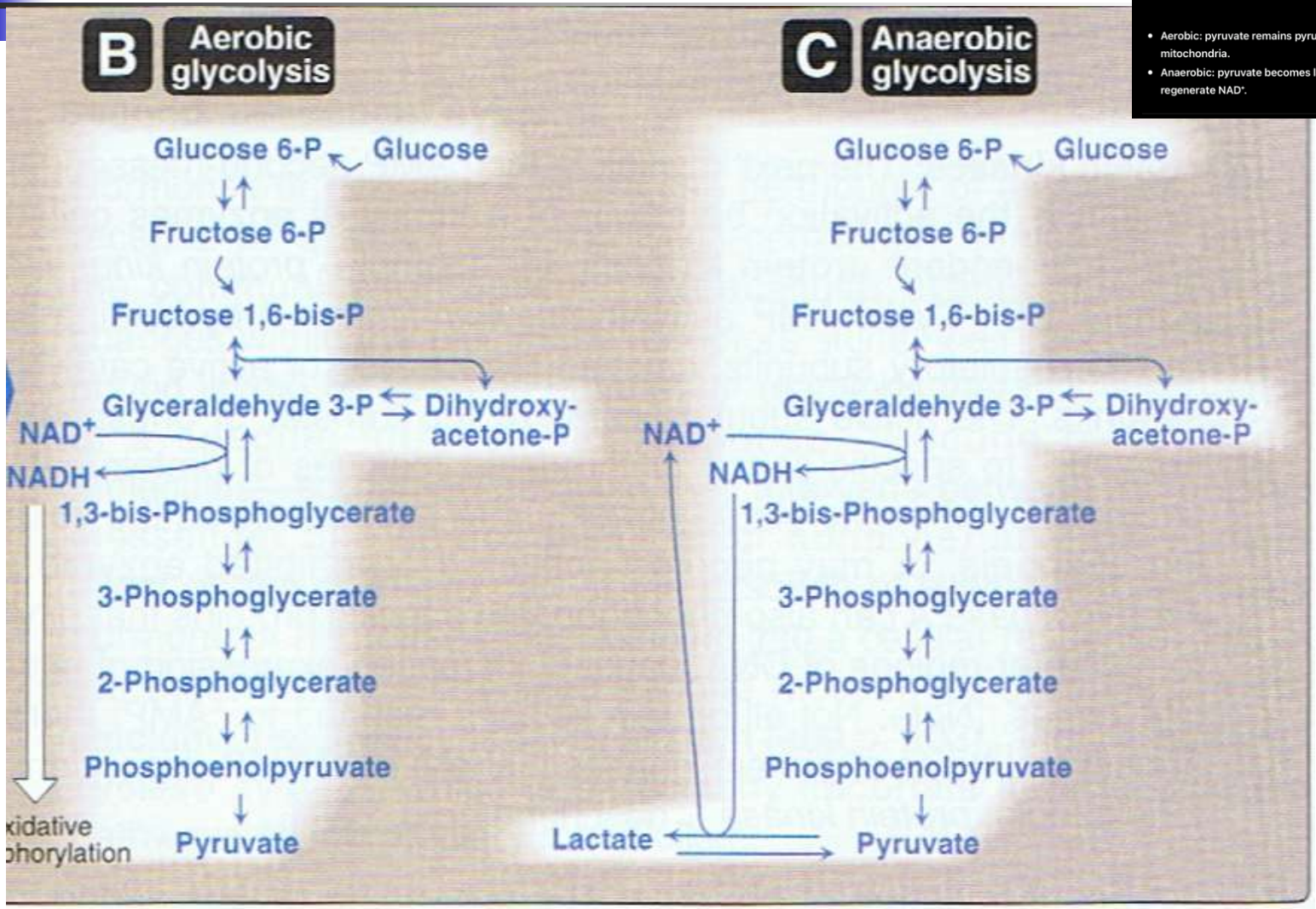
المعادلة:

$$Pyruvate + NADH + H^+ \rightarrow Lactate + NAD^+$$

امتحانيًا:

في anaerobic glycolysis، NADH لا يعطي ATP في ETC لأنه ما في oxygen/mitochondrial oxidation، بل يُستخدم لتحويل pyruvate إلى lactate.

Schematic representation



Slide 15 — Schematic representation

السلايد يقارن بين:

Aerobic glycolysis

Glucose → Pyruvate

في وجود O_2، NADH الناتج من glycolysis يدخل electron transport chain ويعطي ATP.

Pyruvate بعدها يدخل mitochondria ويتحول إلى acetyl-CoA.

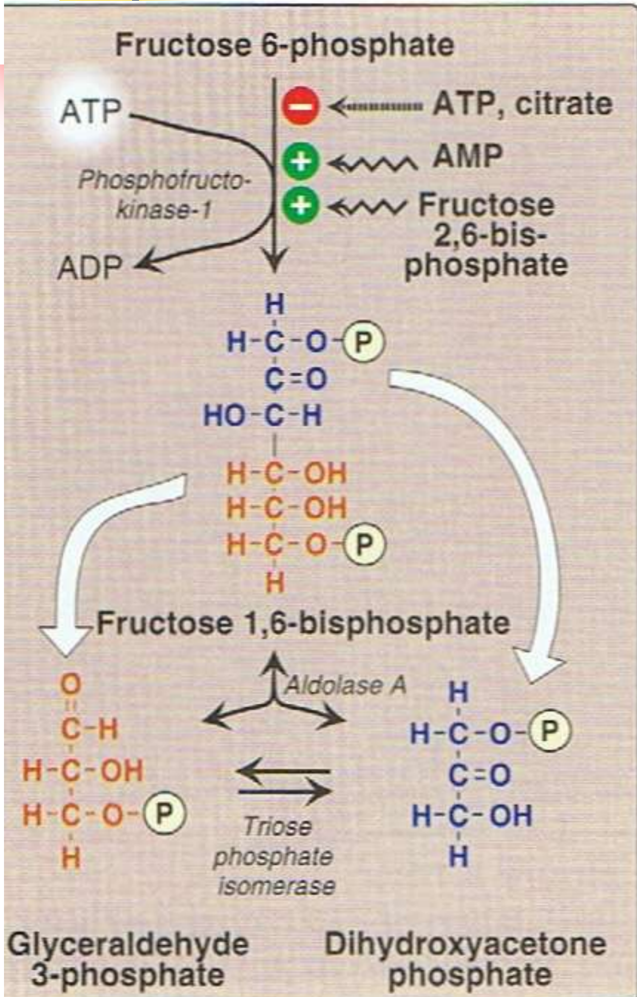
Anaerobic glycolysis

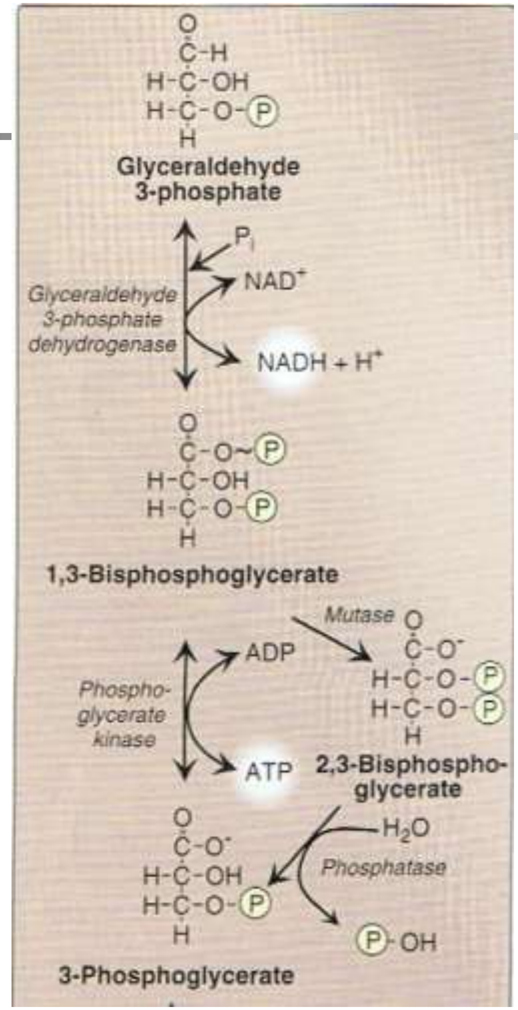
Glucose → Pyruvate → Lactate

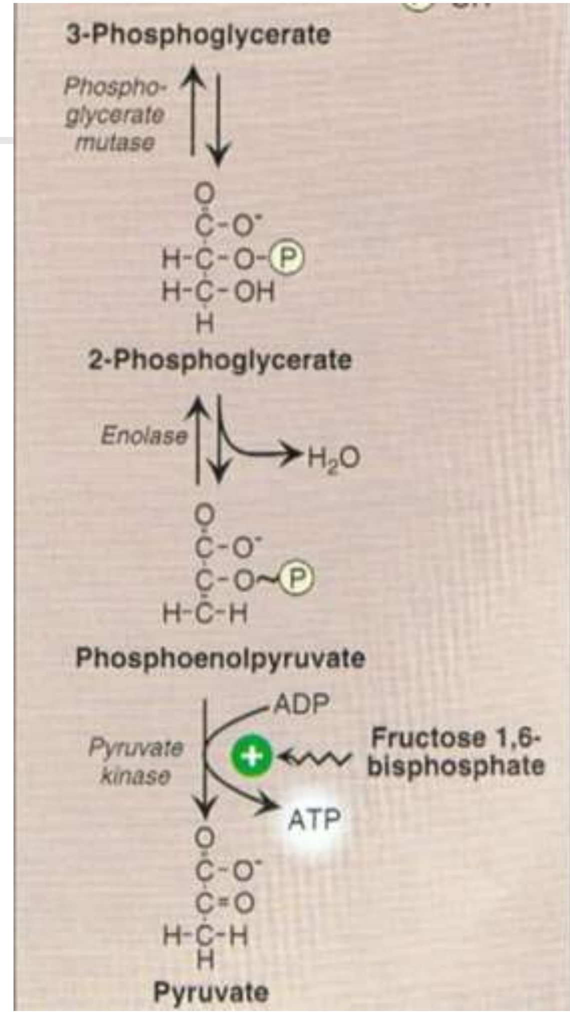
هنا NADH يرجع إلى "NAD بواسطة LDH.

الفكرة الذهبية:

- Aerobic: pyruvate remains pyruvate and enters mitochondria.
- Anaerobic: pyruvate becomes lactate to regenerate NAD⁺.







- regulation [illegible] pathway [illegible]

أهم نقاط التنظيم:

1. PFK-1

Fructose-6-phosphate → Fructose-1,6-bisphosphate

يتأثر بـ PFK-1:

Inhibitors

- ATP
- Citrate

Activators

- AMP
- Fructose-2,6-bisphosphate

شرحهم:

ATP عالي يعني الطاقة كثيرة، فلا داعي لتكسير glucose → يثبط glycolysis.

AMP عالي يعني الطاقة قليلة، الخلية تحتاج ATP → ينشط glycolysis.

Citrate عالي يعني TCA cycle ممتلئ والطاقة كافية → يثبط glycolysis.

Fructose-2,6-bisphosphate أقوى activator لـ PFK-1، وهو الرابط الكبير بين insulin/glucagon وتنظيم glycolysis.

2. Pyruvate kinase

PEP → Pyruvate

ينشط بواسطة:

- Fructose-1,6-bisphosphate

وهذا يسمى:

Feed-forward activation

يعني مادة مبكرة في pathway تنشط خطوة لاحقة لتسريع التدفق.

يثبط بواسطة:

- ATP

هنا كل وحدة كم تعمل ATP وكم المجموع

Energy gain in aerobic glcolysis

Step 1	Glucokinase (GK)	- 1 ATP
Step 3	Phosphofructokinase (PFK)	- 1 ATP
Step 7	Phosphoglycerate kinase	+ 2 ATP
Step 10	Pyruvate kinase (PK)	+ 2 ATP
Step 6	2 NADH	+ 5 ATP
Net gain		+ 7 ATP

احفظ:

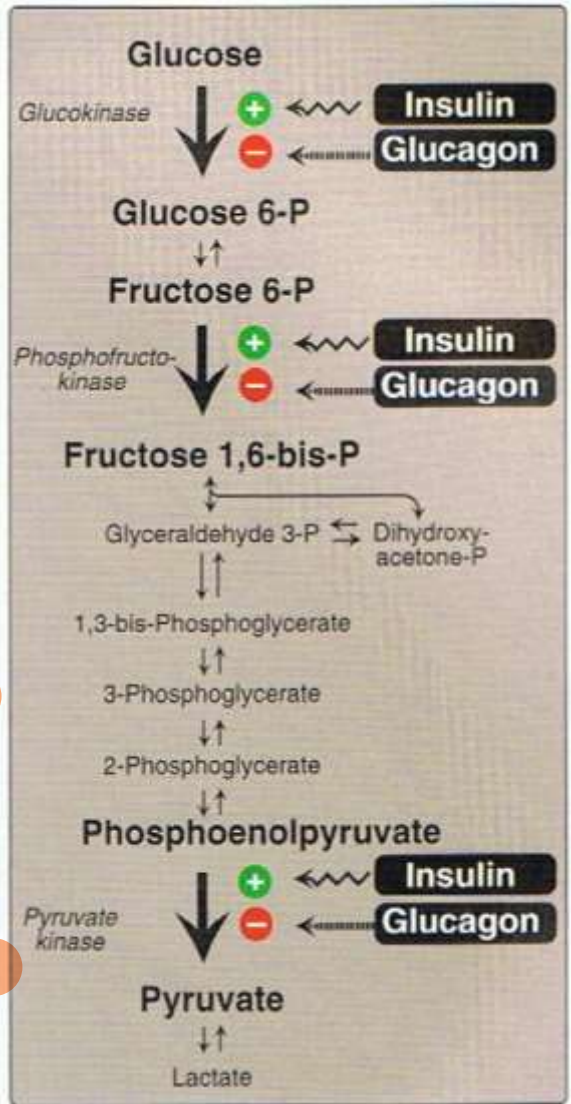
الحالة	الناتج النهائي	ATP net
Aerobic glycolysis	Pyruvate	7 ATP
Anaerobic glycolysis	Lactate	2 ATP

Anaerobic glycolysis

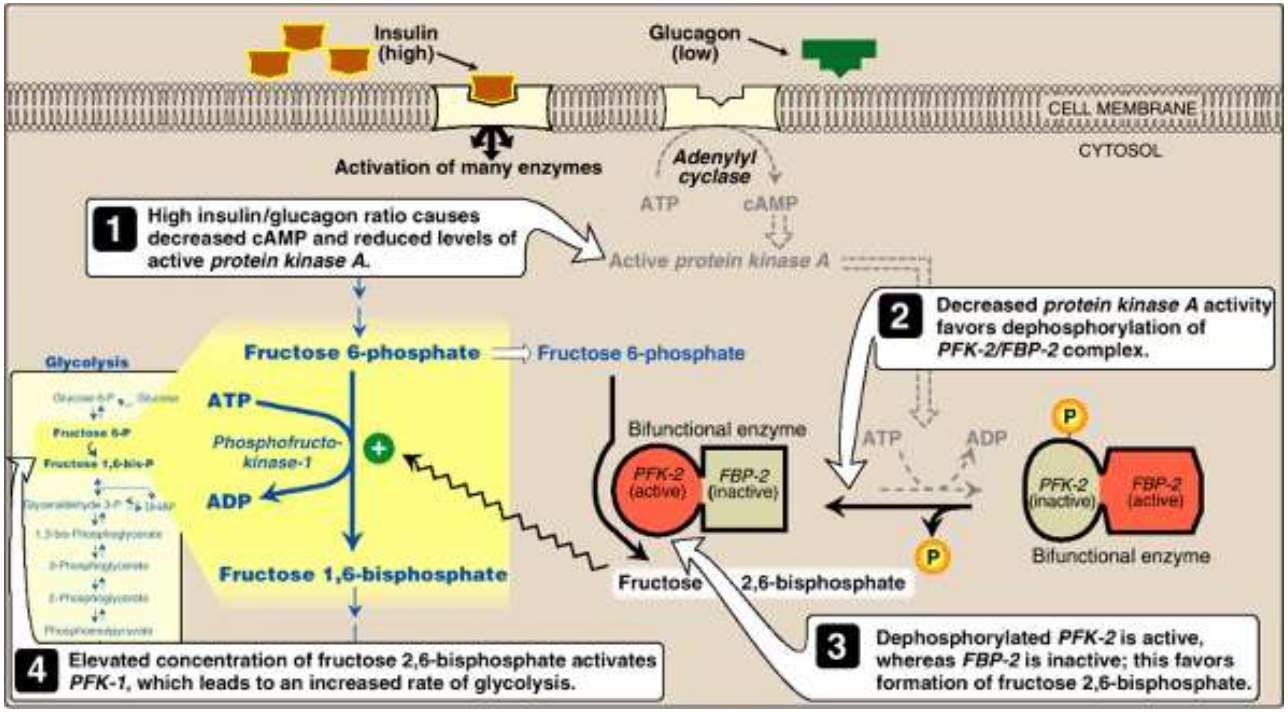
Step 1	Glucokinase (GK)	- 1 ATP
Step 3	Phosphofructokinase (PFK)	- 1 ATP
Step 7	Phosphoglycerate kinase	+ 2 ATP
Step 10	Pyruvate kinase (PK)	+ 2 ATP
Net gain		+ 2 ATP

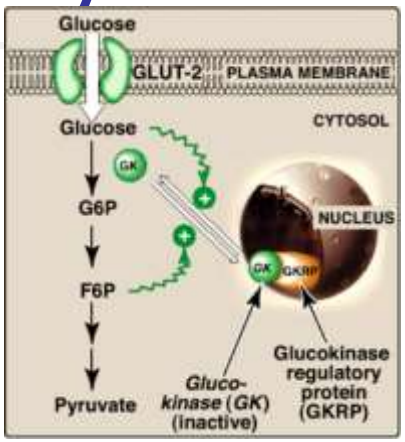
Hormonal regulation of Glycolysis

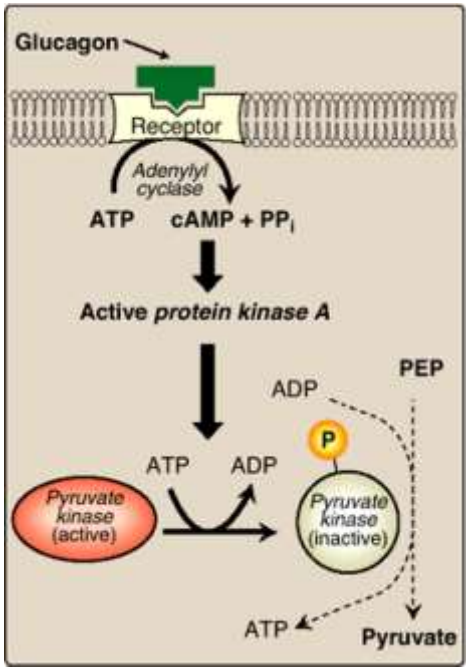
- GK (or HK), PFK and PK are the key enzymes of glycolysis.
- PFK is the most important and considered the rate limiting enzyme.
- Hormones regulate glycolysis according to blood glucose level:
  - After CHO feeding: blood glucose increases, this stimulates insulin secretion, insulin stimulates glycolysis by increasing the synthesis of the three key enzymes: GK, PFK and PK.
  - During fasting: blood glucose level decreases, which inhibits insulin secretion and stimulates glucagon, adrenaline and corticosteroid which inhibit the synthesis of and activity of GK, PFK and PK.



Hormonal regulation of Glycolysis







شرح الرسمة

هذا من أصعب وأهم السلايدات.

المفتاح هنا:

Fructose-2,6-bisphosphate / F2,6BP

هذا molecule ليس intermediate عادي في glycolysis، لكنه regulator قوي.

High insulin / low glucagon

بعد الأكل:

- Insulin عالي
- Glucagon منخفض
- cAMP منخفض
- PKA activity منخفضة

هذا يؤدي إلى activation of PFK-2.

PFK-2 يحول:

Fructose-6-phosphate → Fructose-2,6-bisphosphate

F2,6BP ينشط PFK-1.

وبالتالي:

Glycolysis increases.

High glucagon / fasting

أثناء الصيام:

- Glucagon عالي
- cAMP عالي
- PKA عالي

PKA يعمل phosphorylation لـ bifunctional enzyme.

هذا يفعّل FBPase-2 ويثبط PFK-2.

فتقل F2,6BP.

لما تقل F2,6BP:

- PFK-1 يقل نشاطه
- Glycolysis يقل
- Fructose-1,6-bisphosphatase يزيد
- Gluconeogenesis تزيد

Pyruvate kinase regulation

Glucagon → cAMP → PKA → phosphorylates pyruvate kinase → inactive

يعني liver يوقف تحويل PEP إلى pyruvate أثناء الصيام.

ليش؟

لأن الجسم يريد استخدام PEP لصناعة glucose في gluconeogenesis، وليس تكسيره إلى pyruvate.

امتحاني جدًا:

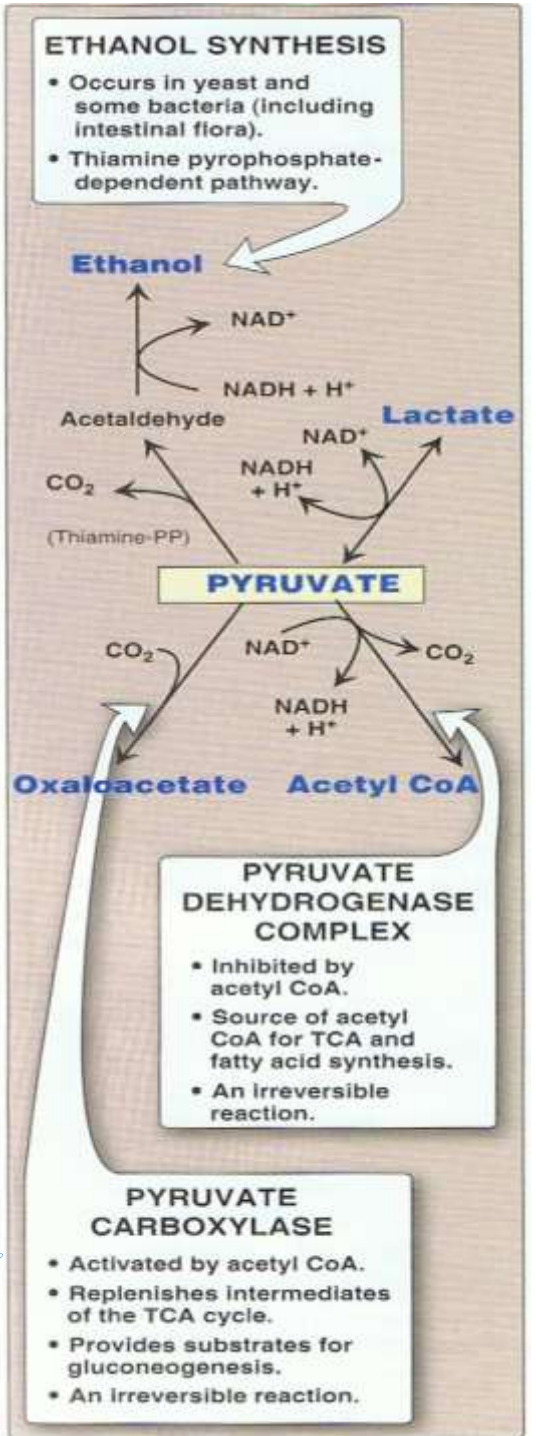
Glucagon phosphorylates and inactivates hepatic pyruvate kinase.

In-vitro inhibition of glycolysis

- Flouride inhibits enolase enzyme (step 8)
  - It is used in toothpastes as it inhibits glycolysis in mouth bacterial flora.
  - It is also used as anticoagulant for blood samples to estimate its glucose content.

Fate of pyruvic acid

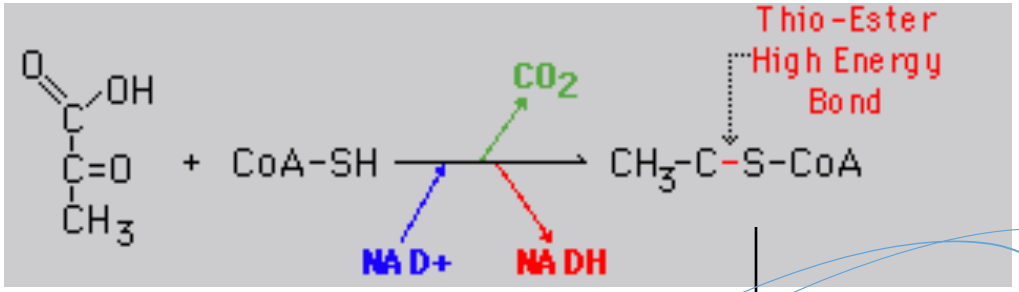
- **Formation of acetyl CoA**
- **Formation of oxaloacetic acid**
- **Formation of lactate**
- **Formation of ethanol (in yeast and some M.O)**



Aerobic phase of glucose oxidation

- Pyruvic acid formed by glycolysis enters the mitochondria where:
  - it will be metabolized to acetyl-CoA by oxidative decarboxylation and
  - then Acetyl-CoA is oxidized in Kreb's cycle

Pyruvate → Acetyl-CoA وفيها
1- CO_2 يخرج
2- NADH يتكون
3- Acetyl-CoA لتأكسيت CoA يرتبط



For 1 acetyl-CoA

Kreb's cycle

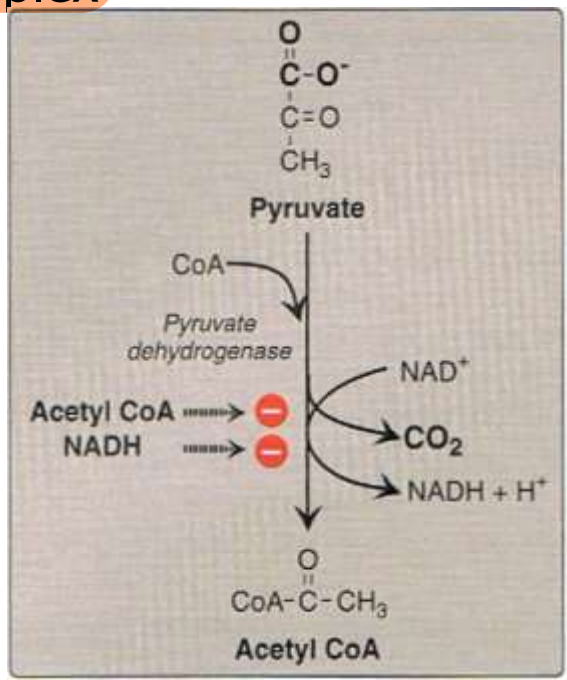
2 CO_2 + 10 ATP

A-Oxidative decarboxylation of pyruvic acid

- Occurs in mitochondria
- Irreversible
- Needs pyruvate dehydrogenase (PDH) complex
- Requires 5 coenzymes
  - Thiamine pyrophosphate
  - 2-lipoic acid
  - CoA-SH
  - FAD
  - NAD^+

TLCFN

تل سفن



Regulation of pyruvate dehydrogenase

PDH يتحكم بدخول pyruvate إلى TCA cycle.

الصورة تعرض حالتين:

Active PDH

PDH يكون active عندما يكون dephosphorylated.

يعني بدون phosphate.

Phosphoprotein phosphatase يزيل phosphate ويفعّل PDH.

ينشط phosphatase بواسطة:

- Ca^{2+}

وهذا مهم في muscle contraction.
لما العضلة تشتغل، Ca^{2+} يزيد، فتحتاج ATP، فينشط PDH لدخول acetyl-CoA إلى TCA.

Inactive PDH

PDH يصبح inactive عندما يكون phosphorylated.

PDH kinase يضيف phosphate ويثبط PDH.

ما الذي ينشط PDH kinase؟

- ATP
- NADH
- Acetyl-CoA

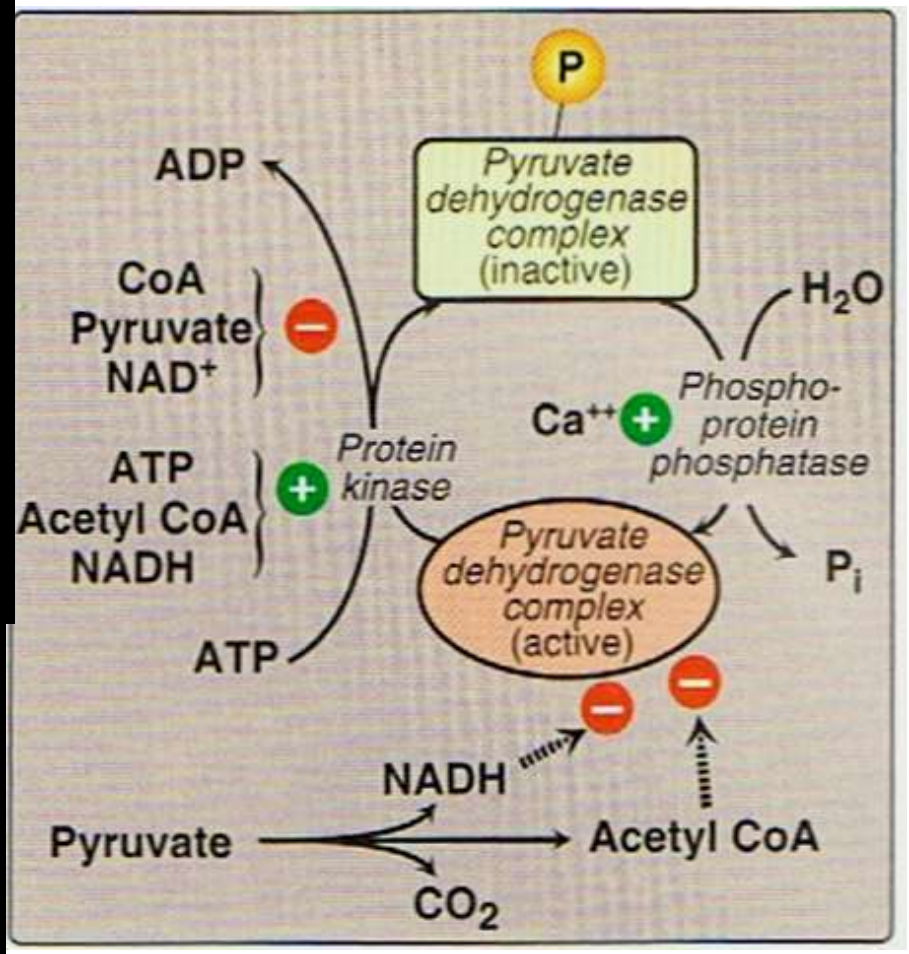
يعني لما الطاقة عالية والنواتج كثيرة، الجسم يوقف PDH.

ما الذي يثبط PDH kinase؟

- ADP
- Pyruvate
- NAD^+
- CoA

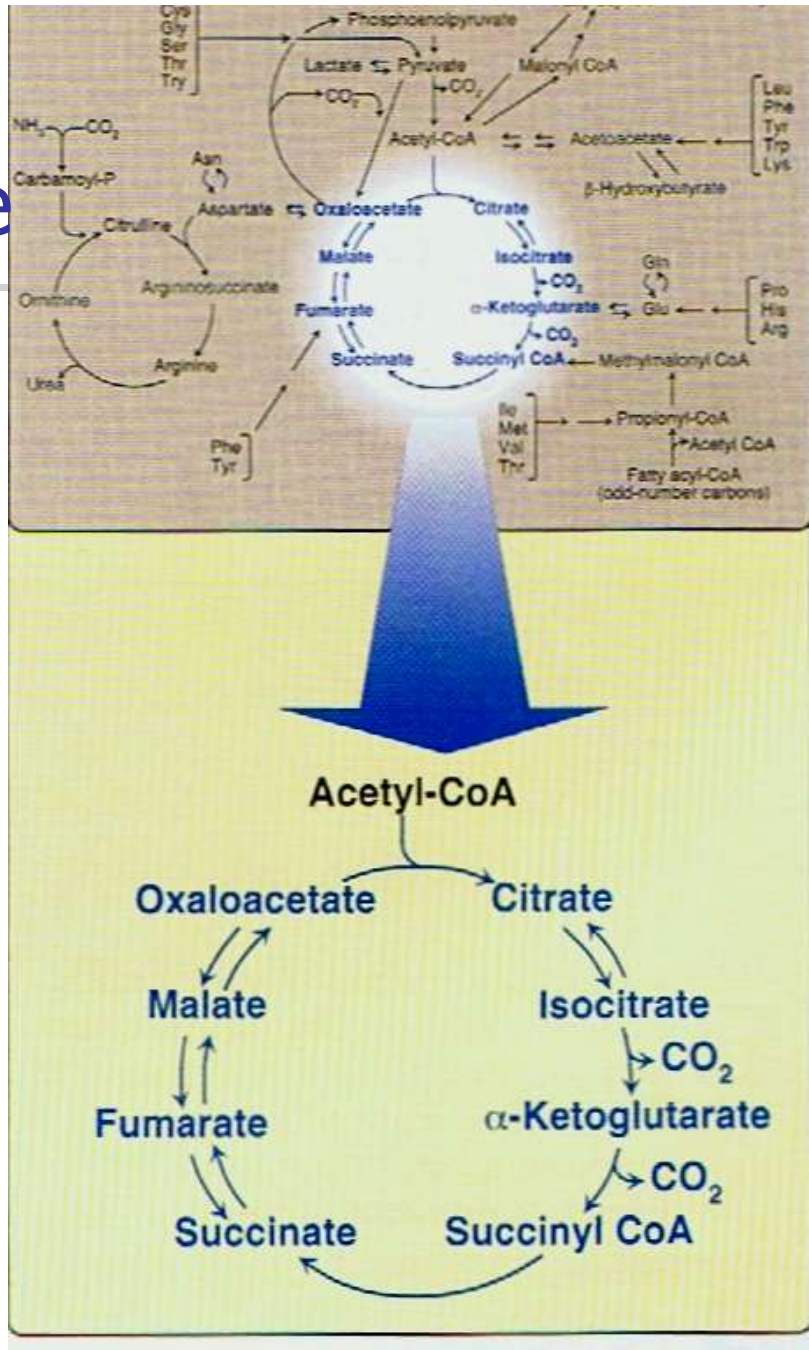
يعني لما substrate عالي أو الطاقة قليلة، PDH يشتغل.

قاعدة:
PDH inhibited by its products: NADH and acetyl-CoA.
.PDH activated when energy is low: ADP, pyruvate



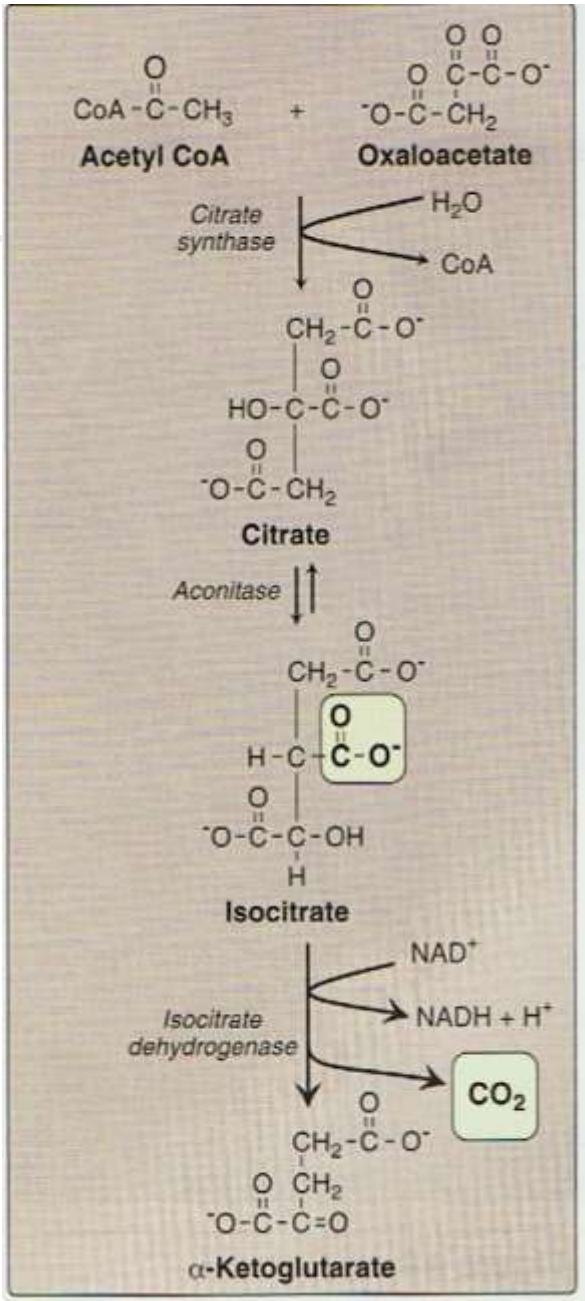
Tricarboxylic acid cycle

- Occurs in the mitochondria of each cell
- Does not occur in RBCs (no mitochondria)
- Considered the final common pathway for the complete oxidation of acetyl-CoA obtained from partial oxidation of CHO, lipids and proteins.



Steps of TCA

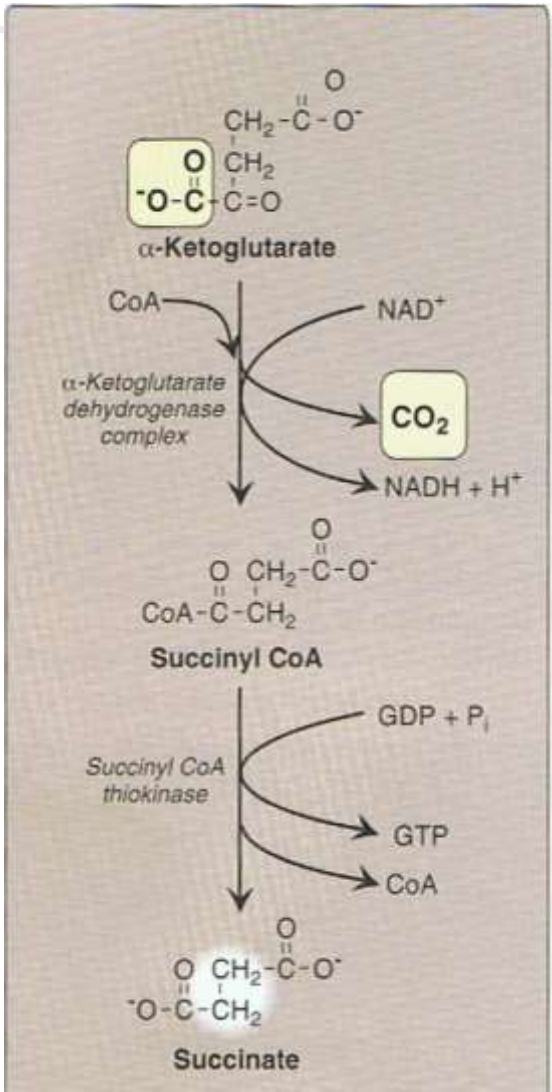
- Step 1: condensation of acetyl-CoA and oxaloacetic acid to form citric acid. Catalyzed by citrate synthase.
- Step 2: Citric acid is converted by isocitrate by aconitase.
- Step 3: Isocitrate is oxidized to α-ketoglutarate by isocitrate dehydrogenase. NADH is produced and CO2 is released.



Steps of TCA

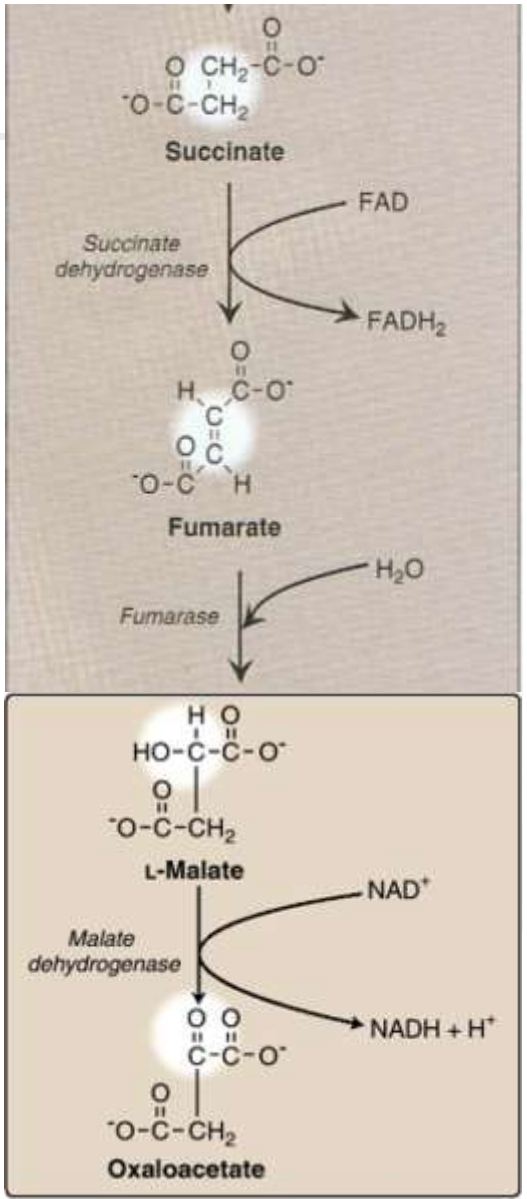
- Step 4: α-ketoglutarate is converted to succinyl CoA. CO_2 is released and NADH is produced. The reaction is catalyzed by α-ketoglutarate dehydrogenase complex. It also requires 5 coenzymes (thiamine pyrophosphate, lipoic acids, CoA-SH, FAD and NAD)

- Step 5: the high-energy, thioester bond of succinyl-CoA is cleaved providing energy for the synthesis of GTP fro GDP and Pi. Succinate is formed and the reaction is catalyzed succinate thiokinase.



Steps of TCA

- Step 6: succinate is oxidized to fumarate by succinate dehydrogenase. FAD is reduced to FADH2.
- Step 7: fumarate is hydrated to form malate by fumarase.
- Malate is oxidized to oxaloacetate by malate dehydrogenase. NAD is reduced to NADH.
- Oxaloacetate will reinitiate the cycle again.



Step 1

Acetyl-CoA + Oxaloacetate → Citrate

الإنزيم:

Citrate synthase

هذه condensation reaction.

Acetyl-CoA فيه 2 carbons.
Oxaloacetate فيه 4 carbons.
Citrate فيه 6 carbons.

Step 2

Citrate → Isocitrate

الإنزيم:

Aconitase

هذه isomerization.

يعني citrate يتعدل ترتيبه ليصبح isocitrate.

Step 3

Isocitrate → α-ketoglutarate

الإنزيم:

Isocitrate dehydrogenase

يحدث:

- NADH produced
- CO_2 released

هذه oxidative decarboxylation.

Isocitrate 6C → α-ketoglutarate 5C + CO_2

هذه واحدة من أهم regulatory steps في TCA.

Step 4

α-ketoglutarate → Succinyl-CoA

الإنزيم:

α-ketoglutarate dehydrogenase complex

يحدث:

- CO_2 released
- NADH produced

α-ketoglutarate 5C → Succinyl-CoA 4C + CO_2

هذا الإنزيم يشبه PDH complex ويحتاج نفس 5 coenzymes:

- TPP
- Lipoic acid
- CoA-SH
- FAD
- NAD^+

مهم جدًا:

PDH complex and α-ketoglutarate dehydrogenase complex require the same 5 coenzymes.

Step 5

Succinyl-CoA → Succinate

الإنزيم:

Succinate thiokinase
ويُسمى أيضًا succinyl-CoA synthetase.

هنا high-energy thioester bond ينكسر، وتُستخدم الطاقة لصناعة:

GDP + Pi → GTP

GTP يعادل ATP.

هذه تسمى:

Substrate-level phosphorylation

لأن الطاقة تكونت مباشرة من substrate عالي الطاقة، وليس من ETC.

Slide 29 — Steps of TCA: Steps 6–8

Step 6

Succinate → Fumarate

الإنزيم:

Succinate dehydrogenase

ينتج:

$FADH_2$

مهم:
Succinate dehydrogenase هو إنزيم في TCA وأيضًا جزء من electron transport chain
Complex II.

Step 7

Fumarate → Malate

الإنزيم:

Fumarase

هذه hydration reaction.

يعني إضافة H_2O.

Step 8

Malate → Oxaloacetate

الإنزيم:

Malate dehydrogenase

ينتج:

NADH

ثم oxaloacetate يبدأ cycle من جديد.

مهم:
Oxaloacetate لا يُستهلك نهائيًا؛ يتجدد في نهاية الدورة.

حفظ كل وحدة كم تعطي ATP + شو التوتال

Energy gain in Kreb's cycle

Isocitrate DH	1 NADH	2.5 ATP
α-ketoglutarate	1 NADH	2.5 ATP
Succinate thiokinase	1 GTP	1 ATP
Succinate DH	1 FADH2	1.5 ATP
Malate DH	1 NADH	2.5 ATP
Net gain		**10** ATP

The overall energy gain of glucose oxidation

- Glycolysis --------- 7 ATP + 2 pyruvate
- 2 pyruvate -------- 2 acetyl-coA + 2 NADH ----- 5 ATP
- 2 acetyl CoA --------- 20 ATP
- The net ATP produced by the oxidation of 1 mol of glucose = 32 ATP

Defects in Glycolysis

- **Pyruvate dehydrogenase deficiency:** leads to congenital lactic acidosis.

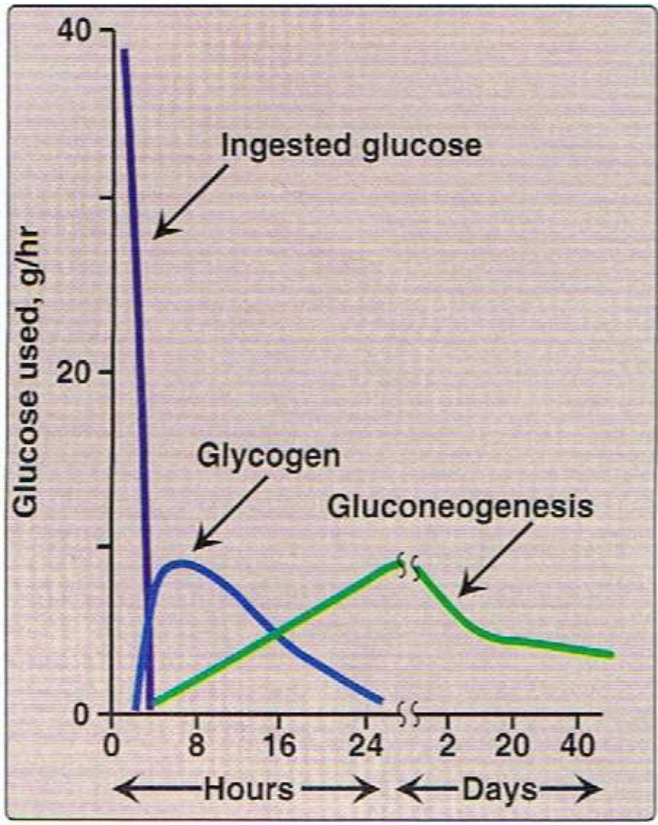
- This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactic acid via lactate dehydrogenase.

- This causes particular problems for the brain, which relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis.

Gluconeogenesis

When and where does it occur

- During prolonged fast and depletion of hepatic glycogen
- During overnight fast, liver is responsible for the majority of gluconeogenesis (90%) and the rest in the kidney
- During prolonged fast, kidney produces about 40% of glucose production.
- Glucose is formed from precursors as lactate, pyruvate, glycerol and ketoacids



Substrates for gluconeogenesis

- Those include all the intermediates of glycolysis and the citric acid cycle.

1. Glycerol: released during the hydrolysis of triglycerols in adipose tissue and delivered to the liver. Glycerol is phosphorylated by glycerol kinase to glycerol 3-phosphate, which is oxidized by glycerol 3-phosphate dehydrogenase to dihydroxyacetone phosphate which is an intermediate of glycolysis.

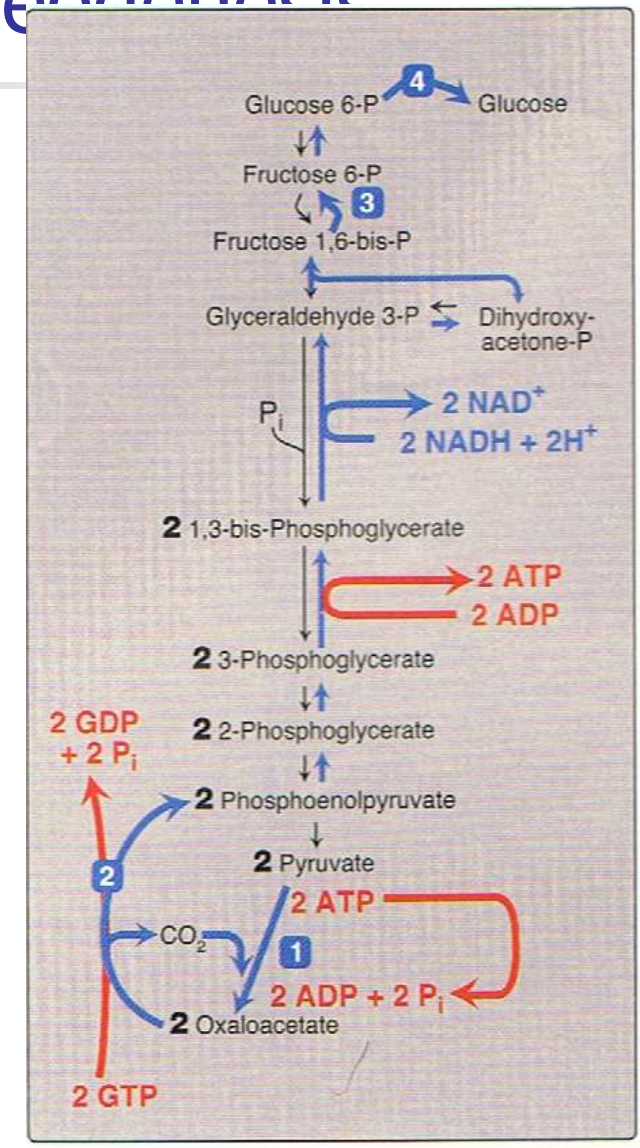
2. Lactate: released by exercising muscles and RBC's. This is transferred to the liver and reconverted to glucose.

3. Amino acids: hydrolysis of tissue proteins are the major source of glucose. α-ketoacids (oxaloacetate and α-ketoglutarate are derived from the metabolism of glucogenic aa which can enter the TCA

Reactions unique to gluconeogenesis

- Seven of the glycolysis reactions are reversible and are used for gluconeogensis while three of them are irreversible (Pyruvate kinase, phosphofructokinase and hexokinase)

1. Pyruvate carboxylase: Pyruvate is converted to phosphoenolpyruvate (PEP) by pyruvate carboxylase and PEP caboxykinase
   - Biotin: covalently bound to the N of lysine in the pyruvate carboxylase, requires CO_2 and ATP for the convertion of pyruvate to oxaloacetate. It occurs in mitochondria of liver and kidney. Muscles contain also pyruvate carboxylase for the use of OAA in TCA.
   - Allosteric regulation: it is allosterically activated by Acetyl coA.



Reactions unique to gluconeogenesis

2. Transport of oxaloacetate to the cytosol: oxaloacetate can't cross the mitochondrial membrane so it is reduced to malate by malate dehydrogenase that can cross. In cytosol malate is reoxidized to oxaloacetate by cytosolic malate dehydrogenase.

3. Oxaloacetate is decarboxylated and phosphorylated in the cytosol by PEP carboxykinase which utilize 1 GTP. PEP will continue in the reverse of glycolysis until reach fructose 1,6- biphosphate.

4. Dephosphorylation of frucose 1,6- biphosphate by frucose 1,6-biphosphatase to produce fructose 6-phosphate will bypass the irreversible PFK reaction.

The enzyme is inhibited by high levels of AMP and fructose 2,6-biphosphate, while high level of ATP and low AMP stimulate gluconeogenesis

Reactions unique to gluconeogenesis

5. Dephosphorylation of glucose 6-phosphate: occurs by glucose 6-phosphatase. This occurs only in liver and kidney. Two enzymes are required (glucose 6-phosphate translocase to transfer glucose 6-phosphate to ER and glucose 6-phosphatase)

Type 1a glycogen storage disease results from inherited diffeciency of one of them which has the following symptoms:

- Hypoglycemia
- Hepatomegaly and liver problems
- Lactic acidosis
- Growth failure

Pyruvate carboxylase

Pyruvate carboxylase

Reaction:

Pyruvate + CO_2 + ATP → Oxaloacetate + ADP + Pi

المكان

Mitochondria

يحتاج

Biotin

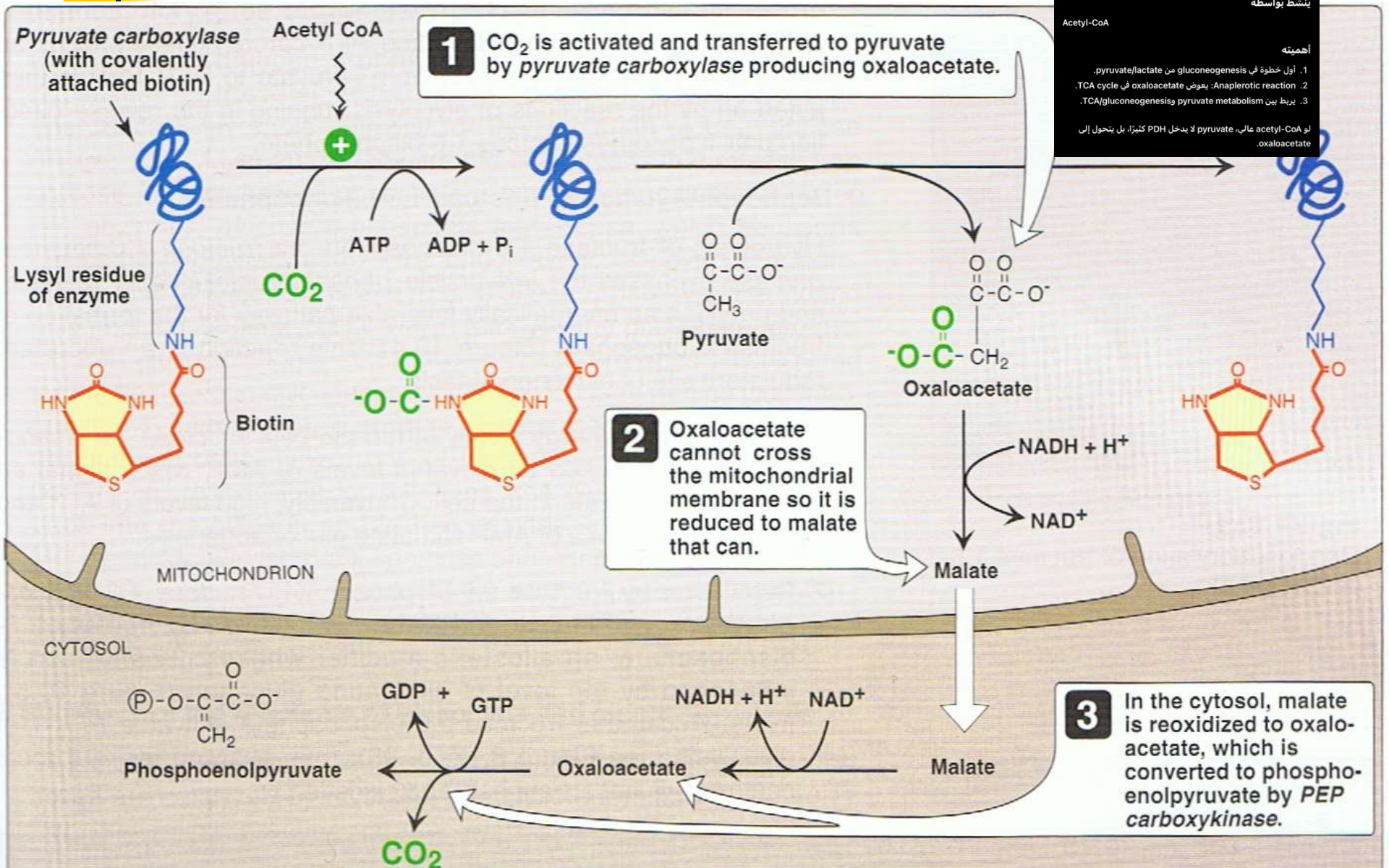
ينشط بواسطة

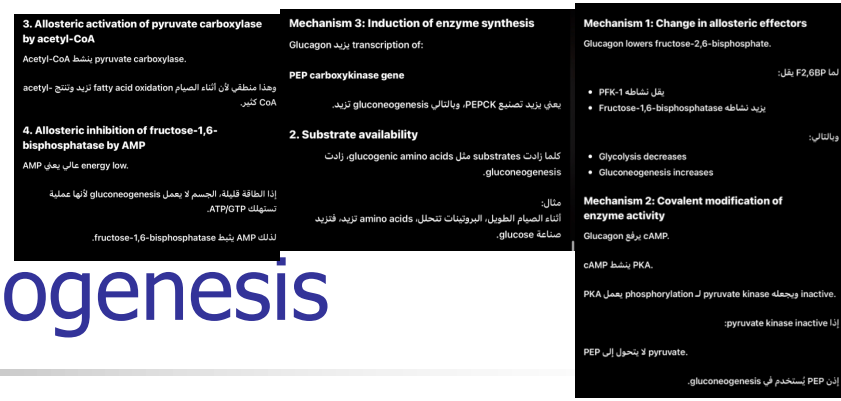
Acetyl-CoA

أهميته

1. أول خطوة في gluconeogenesis من pyruvate/lactate.
2. Anaplerotic reaction: يعوض oxaloacetate في TCA cycle.
3. يربط بين pyruvate metabolism وTCA/gluconeogenesis.

لو acetyl-CoA عالي، pyruvate لا يدخل PDH كثيرًا، بل يتحول إلى oxaloacetate.





Regulation of gluconeogenesis

1. Glucagon: stimulates gluconeogenesis in three mechanisms:
    1. **Change in allosteric effectors:** it lowers level of fructose 2,6-biphosphate leading to activation of fructose 1,6-biphosphatase and inhibition of phosphofructokinase.
    2. **Covalent modification of enzyme activity:** it elevate cAMP leading to activation of cAMP-dependent protein kinase activity which will phosphorylate pyruvate kinase to its inactive form.
    3. **Induction of enzyme synthesis:** it increases the transcription of PEP carboxykinase gene.
2. Substrate availability: like glucogenic amino acids
3. Allosteric activation of pyruvate carboxylase by acetyl coA.
4. Allosteric inhibition of fructose 1,6-bisphosphatase by AMP

Note: ATP and NADH are produced in large quantities during fasts from fatty acid oxidation is required for gluconeogenesis.