BIOCHEMISTRY

VEIN BATCH

Lecture : 10

Done by : Mohammad Alomari

Biochem lecture 10 Integration of metabolism questions

Ahmed Salem, MD, MSc, PhD, FRCR

تفريغ : محمد العمري

*** شوية notes قبل ما تبلشوا بالfile..

- عدد السلايدات كبير بس فعليا كلها عبارة عن إعادة و مراجعة لسلايدات سابقة, ومعظمها الدكتور ما قرأ منه اشي
- عدد الاسئلة 10 (مع الإنتباه إنه 9 عبارة عن فرعين), وإجابة كل سؤال بتكون بالسلايد اللي بعده
- السلايدات اللي فيها معلومات مهمة هي (15 / 17 / 19 / 22 / 35)

الأسئلة هاي عبارة عن تجميع معلومات, و زي ما عوّدنا الدكتور الفصل الماضي ف مش مستبعد نشوف بعض الخيارات اللي ما عمرها مرّت علينا من قبل وما عنّا أي معلومة عنها, لكن دايما رح يكون الجواب الصحيح اشي بنعرفه و مبني على ما تم شرحه, ف ما في داعي للتوتر لو شفنا هيك خيارات, المهم ندور عالصح

1. Which of the following is <u>not</u> an effect of insulin on glucose metabolism?

A. Increased number of glucose transporters on the muscle and fat cell membranes

B. Increased glucose uptake by muscles and adipose tissue cells

C. <u>Enhanced</u> glycolysis & glycogenesis and **<u>inhibited</u>** glycogenolysis & gluconeogenesis

(inhibition)

D. Suppression of HMP pathway

اللهم إني أسالك التوفيق والنجاح, في الدنيا والآخرة

Regulation of HMP pathway:

الinsulin بتم إفرازه بالfed state, وهو بعمل على تخليص الblood من الglucose ونقله للخلايا لاستخدامه أو تخزينه, وبعمل stimulation للglycolysis وبحوّل الglucose لglycogen..
ف بصفّي ** Q1 الجواب هو D**

- **Oxidative phase**

- Is controlled by the level of NADP+

- The first reaction (catalyzed by G6PD) is a **rate limiting step** and is **inhibited by ↑ NADPH**

  - **Induction:**

- CHO feeding → ↑ insulin → induction of synthesis of both dehydrogenases leading to **<u>activation</u>** of HMP Shunt

- Fasting → ↓ insulin → repression of synthesis of both dehydrogenases, so HMP is **<u>inhibited</u>**

Regulation of glycogen metabolism

- Covalent modification
  - Glycogen synthase: active in ***dephosphorylated*** form
  - Glycogen phosphorylase: active in ***phosphorylated*** form
  - Covalent modification of these enzymes is through hormones that act through the 2nd messenger cAMP
  - Glycogen synthase exists in 2 forms (interconverted by specific enzyme):
    - Less active phosphorylated form (glycogen synthase b)
    - More active ***dephosphorylated*** form (glycogen synthase a)
  - Glycogen phosphorylase exists in 2 forms:
    - Less active ***dephosphorylated*** form (phosphorylase b)
    - More active phosphorylated form (phosphorylase a)

During fed state

- Insulin dephosphorylates both glycogen synthase and glycogen phosphorylase via 2 mechanisms:
    - Activation of phosphodiesterase enzyme → inactivates cAMP to AMP → inhibits protein kinase
    - Activation of phosphatase enzyme which removes P:
        - » Dephosphorylation of glycogen synthase → its activation → GLYCOGENESIS
        - » Dephosphorylation of glycogen phosphorylase → its inactivation → inhibition of glycogenolysis

2. Which of the following is **not** an effect of glucagon on metabolism?

A. Enhanced glycogenolysis and gluconeogenesis in the liver

B. Enhanced lipolysis

C. Activated glycogenolysis in muscles

D. Inhibited glycolysis and glycogenesis

يا حيّ يا قيوم برحمتك أستغيث, أصلح لي شأني كله, ولا تَكِلْني إلى نفسي طرفة عين

من المعلومات المهمة واللي ركزنا عليها بإحدى المحاضرات سابقا إنه ال glucagon ما بأثّر عال muscles, ** Q2 الجواب C**

During fasting state

- Glucagon (in liver) & epinephrine (in liver & muscle):
  - Activate a membrane receptor (G protein) → activates adenyl cyclase (a membrane linked enzyme) → converts ATP to cAMP
  - cAMP activates cAMP dependent protein kinase → phosphorylates (inactivates) glycogen synthase → inhibits glycogensis
    - Activation of protein kinase → activate phosphorylase kinase enzyme → stimulation of glycogen phosphorylase → glycogenolysis

Regulation of Glycolysis:

Regulation of the 3 irreversible reactions

a) Glucokinase (GK) or (Hexokinase, HK)

b)Phosphofructokinase (PFK) which is the rate limiting Enzyme & most important regulatory site of glcolysis.

c) Pyruvate kinase (PK)

Regulation of glycolysis according to the feeding status

Regulation of the 3 irreversible reactions

- **Induction and Repression of the key enzymes:** Insulin induces (increases) the synthesis of these enzymes, while glucagon and adrenaline inhibit their synthesis

- **Allosteric regulation:**

- ***GK (Glucokinase)***: No regulation
- Hexokinase is allosterically inhibited by G-6-P.

N.B. hexokinase is present in all cells except liver and pancreatic islets/ glucokinase is present only in liver and pancreatic islets.

- ***PFK (Phosphofructokinase):***

- Allosterically activated by fructose-2,6-bis-phosphate, AMP& ADP
- Allosterically inhibited by ATP & Citrate and low pH

Fructose-2, 6-Bisphosphate: [F-2, 6–BP] is formed by phosphorylation of F-6-P by the enzyme phosphofructokinase–2 (PFK-2)

- ***PK (Pyruvate kinase):***

-Allosterically activated by Fructose-1,6- bis-phosphate, AMP

-Allosterically inhibited by ATP

A possible explanation for the acid- induced protein catabolism and increased amino acid oxidation is that impairment of glycolysis by low pH restricts the pyruvate supply to mitochondria, leading to catabolism of amino acids from protein as an alternative metabolic fuel

Covalent modification:

The pyruvate kinase (PK) is regulated by covalent modification (phosphorylation / dephosphorylation)

- Phosphorylated pyruvate kinase is inactive and **inhibits glycolysis**
- **Insulin** ↑ its' activity by dephosphorylation
  - Dephosphorylated pyruvate kinase is active leading to stimulation of glycolysis
- **Glucagon** ↓ its' activity by phosphorylation through action of cAMP

Regulation of glycolysis according to the feeding status

- **Carbohydrates feeding:**

  Intake of carbohydrates stimulates insulin secretion which leads to:

  - Increase glucose uptake by tissues
    - Glucose transporter-4 (GluT4) transports glucose from the extracellular fluid to muscle cells and adipocytes
  - Increase synthesis of GK, PFK & PK.
  - Increase PK by dephosphorylation. So, **carbohydrates feeding stimulate Glycolysis.**

- **Fasting (starvation): It leads to:**

1Decrease insulin and decrease glucose uptake by tissues

2-Increase glucagon and adrenaline leads to:

  - Decrease synthesis of GK, PFK &PK.
  - Decrease PK by phosphorylation. So, **fasting inhibits glycolysis.**

Table 9.3. Regulatory enzymes of glycolysis

Enzyme	Activation	Inhibition
HK		G-6-P
GK	Insulin	Glucagon
PFK	Insulin, AMP F-6-P, PFK-2 F2,6-BP	Glucagon, ATP Citrate, Low pH Cyclic AMP
PK	Insulin, F1,6-BP	Glucagon, ATP Cyclic AMP
PDH	CoA , NAD	Acetyl CoA, NADH

3. Which of the following pathways is <u>least</u> expected to be active during fasting?

A. TCA cycle

B. Lipolysis

C. Gluconeogensis

D. HMP pathway

اللهم إنك عفوّ تحب العفو فاعفُ عنا

ال HMP pathway هو anabolic/ synthetic pathway و بنطلع منه pentose phosphate و NADPH (اللي بنستخدمه للlipid synthesis), ما يعني إنه ** Q3 الجواب D**

بس ليش الجواب مش A ؟ هسا خلال الfasting رح يصير في shunting (تحويل) للKrebs و رح يكون في إعتماد على الgluconeogenesis بشكل أكبر, لكن الجسم لا زال بحاجة للطاقة, فالKrebs رح تضل شغالة لكن بشكل أقل نسبيا, أما الHMP و أي اشي anabolic رح يتسكر during fasting

4. In the fed state, all tissues produce energy by oxidation of?

A. Glucose

B. Fatty acids

C. Ketone bodies

D. Lactic acid

اللهم إني أسألك الهدى والتقى والعفاف والغنى

** Q4, الجواب A **

الجدول جدا مهم

TABLE 8.3: Major fuels in different organs

	Brain	*Skeletal muscle*	*Cardiac muscle*	*Adipose tissue*
After a meal	Glucose	Glucose, Fatty acids	Glucose, pyruvate	Fatty acids; glucose
Fasting (short-term)	Glucose	Fatty acids	Fatty acids	Fatty acids
Fasting (long-term)	Glucose; ketone bodies	Ketone bodies; Branched chain aa	Ketone bodies	Fatty acids; ketone bodies
Exercise		Glycogen	Fatty acids	

(بنلاحظ إنه الكل بستخدم الglucose)

**note : الRBCs ما بتستخدم إلا glucose في كل الحالات, الآن بالرغم من إنه الbrain بفضّل الglucose بشكل كبير, إلا إنه في الprolonged fasting بصير يعتمد عالketone bodies, والسبب عشان يترك الكمية اللي ضلت من glucose للRBCs, يعني باختصار.. أثناء الprolonged fasting الإحتياج الرئيسي للglucose بكون للRBCs, عشان هيك الorgans الثانية بتبطل تستخدمه

5. In the fasting state, the liver uses less glucose than in the fed state thanks to

A. Lack of insulin facilitation of glucose uptake

B. High km of glucokinase

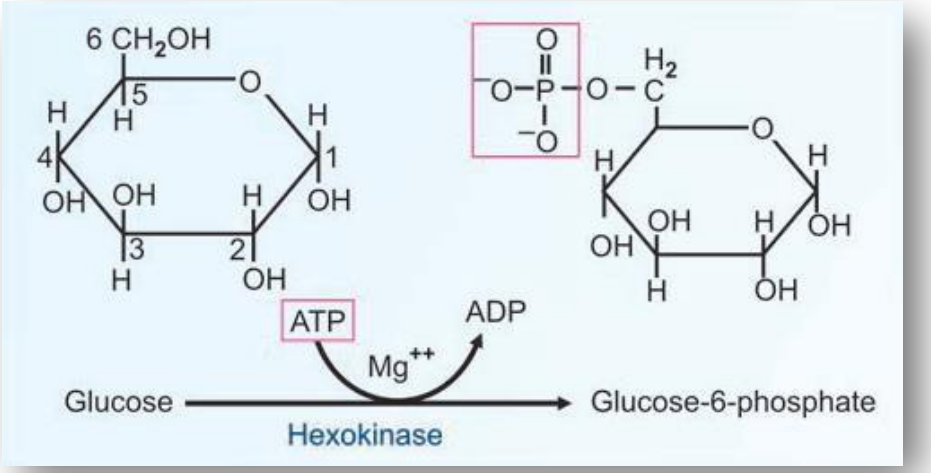
C. Increased formation of acetyl coA

D. Inhibition of TCA cycle

زي ما ذكرنا سابقا, الفرق الوحيد والأهم بين الglucokinaseوالhexokinase هو الKm و الaffinity, و الglucokinase موجود فقط في الliver, وعنده high Km (low affinity), ولولاها كان الliver رح يتنافس مع الorgans الثانية عالglucose في ظرف زي الfasting, **Q5 الجواب B**

Step 1: Phosphorylation of glucose

- Initiates glycolysis & **intracellular trapping** of intermediates
- Reaction is **irreversible**
- Catalyzed by hexokinase (present in all cells) or glucokinase (in liver)*
- Requires Mg2+ as true substrate of enzyme is Mg2+-ATP complex



*Differ in catalytic and regulatory properties

	Hexokinase	Glucokinase
Occurrence	In all tissues	Only in liver
Km value	10^{-2} mmol/L	20 mmol/L
Affinity to substrate	High	Low
Specificity	Acts on glucose, fructose and mannose	Acts only on glucose
Induction	Not induced	Induced by insulin and glucose
Function	Even when blood sugar level is low, glucose is utilized by body cells	Acts only when blood glucose level is more than 100 mg/dl; then Glucose is taken up by liver cells for glycogen synthesis

6. Excess acetyl coA from fatty acid oxidation in the liver cells during fasting

A. Is converted to ketone bodies

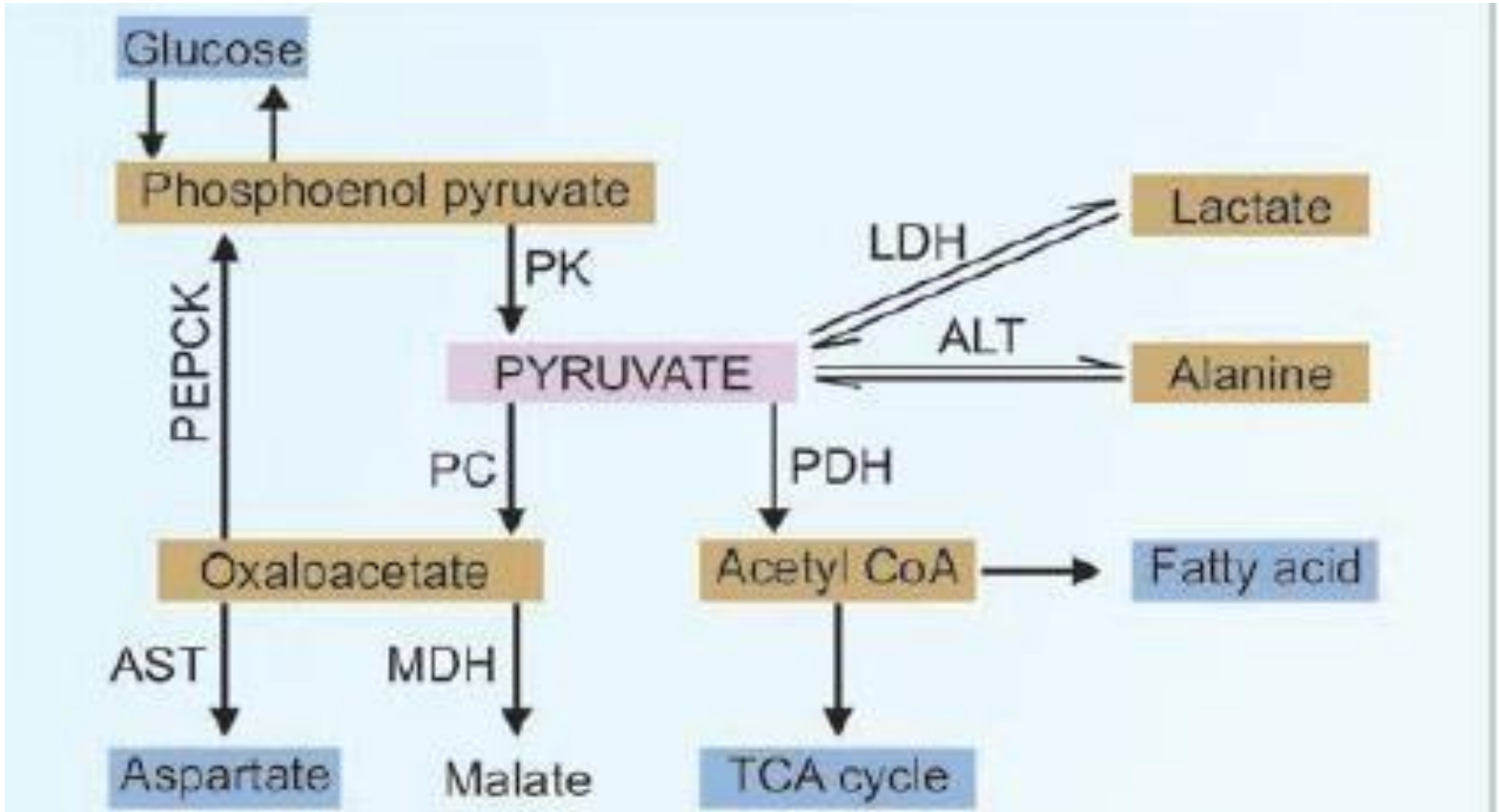
B. Activates pyruvate carboxylase, thus activating gluconeogenesis

C. Inhibits glycolysis

D. All of the above

اللهم إني أستودعك أموري كلها, فوفّقني لما تحبه وترضاه

الFA oxidation خلال الfasting رح يحوّل الacetyl CoA لketone bodies اللي الجسم فعليا بكون محتاجها, الآن بالنسبة للخيار B, ف زيادة تركيز الacetyl CoA رح تعمل inhibition للpyruvate dehydrogenase (PDH), وبالتالي بتحفز الpyruvate carboxylase (PC), وينتج عنا oxaloacetate, وبالتالي ينشّط الgluconeogenesis, وما ننسى إنه بعمل inhibition للglycolysis, يعني ** Q6 الجواب D **



PK = pyruvate kinase; LDH = lactate dehydrogenase; ALT = alanine amino transferase; PDH = pyruvate dehydrogenase; PC = pyruvate carboxylase; MDH = malate dehydrogenase; AST = aspartate amino transferase; PEPCK = phosphoenol pyruvate carboxy kinase

Table 9.3. Regulatory enzymes of glycolysis

Enzyme	Activation	Inhibition
HK		G-6-P
GK	Insulin	Glucagon
PFK	Insulin, AMP F-6-P, PFK-2 F2,6-BP	Glucagon, ATP Citrate, Low pH Cyclic AMP
PK	Insulin, F1,6-BP	Glucagon, ATP Cyclic AMP
PDH	CoA , NAD	Acetyl CoA, NADH

Third Stage: Lipolysis

The prevailing state of high glucagon-insulin ratio stimulates cAMP-mediated lipolysis by increasing the activity of hormone sensitive lipase. Then skeletal muscle, heart and kidney will shut down their glucose utilization; and will depend mainly on fatty acids for energy needs (glucose fatty acid cycle). Inactivation of pyruvate dehydrogenase by phosphorylation is the basis of this change. The stimulation of the activity of CAT by glucagon favors increased rate of beta oxidation. The increased rate of lipolysis and beta oxidation provides an alternate source of fuel as acetyl CoA and subsequently **ketone bodies**. Ketone bodies provide fuel for tissues like heart muscle, skeletal muscle and to some extent the brain.

Regulation of gluconeogenesis

- Gluconeogenesis and glycolysis are reciprocally regulated
    - Inhibition of glycolysis → stimulation of gluconeogenesis

- 4 key enzymes of gluconeogenesis:
    - Pyruvate carboxylase (PC)
    - Phosphoenolpyruvate carboxykinase (PEPCK)
    - **Fructose 1, 6 –Bisphosphatase (F-1, 6-BPtase; the key enzyme)**
    - Glucose-6-phosphatase (G-6-Ptase)

- Types of regulation
    - Allosteric regulation
    - Hormonal regulation

Allosteric regulation: Pyruvate carboxylase

- Allosterically activated by acetyl coA according to different conditions:
  - Low energy status of cell → important to replenish oxaloacetate for directing TCA to provide ATP
  - Hypoglycaemia → acetyl coA produced from lipolysis & β oxidation of FAs:
    - Promotes oxaloacetate synthesis (gluconeogenesis)
    - Inhibits pyruvate dehydrogenase → blocks consumption of pyruvate
  - High energy status of cells (excess CHO) → ↑ acetyl coA → ↑ in oxaloacetate (via stimulation of pyruvate carboxylase) → to form citrate → enables FA synthesis

Allosteric regulation: fructose 1,6 biphosphatase

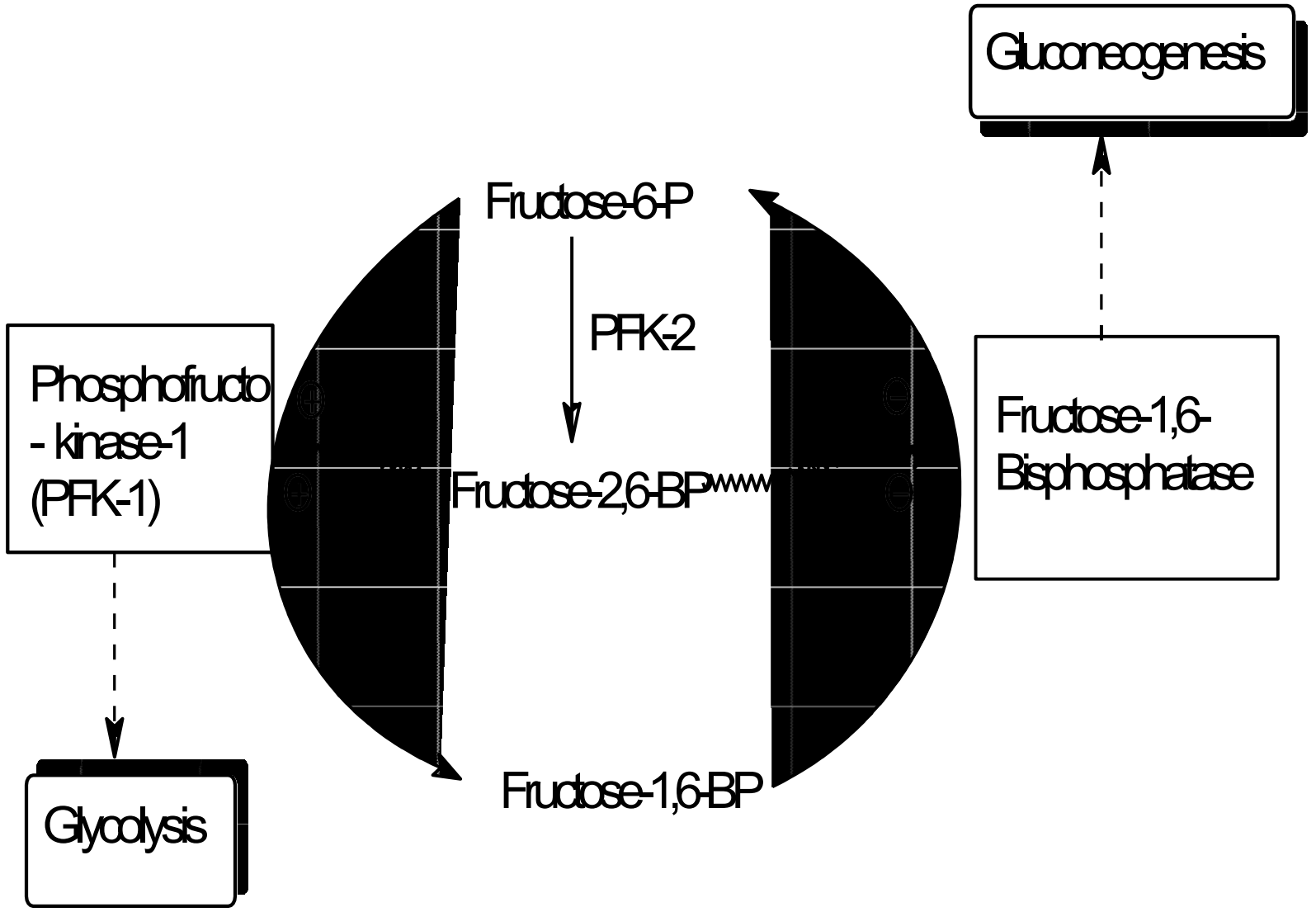
- The key enzyme of gluconeogenesis: F-1, 6-Bisphosphatase which is allosterically inhibited by F-2, 6–BP

- Fructose-2, 6-Bisphosphate: [F-2, 6–BP] is formed by phosphorylation of F-6-P by the enzyme phosphofructokinase–2 (PFK-2)

- **Fructose-2, 6-Bisphosphate** plays an important role in regulation of glycolysis and gluconeogenesis.

- CHO feeding $\rightarrow\uparrow\uparrow$ F-2, 6-BP $\rightarrow$
  - it allosterically stimulates PFK-1 and
  - inhibits F-1, 6-BPtase $\rightarrow$ stimulates Glycolysis and inhibits Gluconeogenesis

  - **So, glycolysis and Gluconeogenesis can't occur at the same time.**


Gluconeogenesis
Fructose-6-P
PFK-2
Phosphofructo
- kinase-1
(PFK-1)
Fructose-2,6-BP
Fructose-1,6-
Bisphosphatase
Fructose-1,6-BP
Glycolysis

Hormonal regulation

- Glucagon, epinephrine & glucocorticoids ↑ gluconeogenesis:
  - Induce synthesis of 4 key gluconeogenic enzymes
    - Pyruvate carboxylase, PEPC, G-6- phosphatase, F 1,6 bisphophatase
  - Repression/ inhibition of 3 key glycolytic enzymes (pyruvate kinase, PFK-1, glucokinase)
  - Promote lipolysis → ↑ fee FA → ↑ acetyl coA → activates pyruvate carboxylase
  - Release of glycerol → gives glucose in liver
  - **Glucocorticoids:**
    - promotes proteolysis of muscle protein → release of free AA → oxidation of AA → intermediates for gluconeogenesis
    - Induces transaminases
- Insulin → inhibits gluconeogenesis
  - Repressor of gluconeogenic enzymes
  - Inducer of glycolytic enzymes

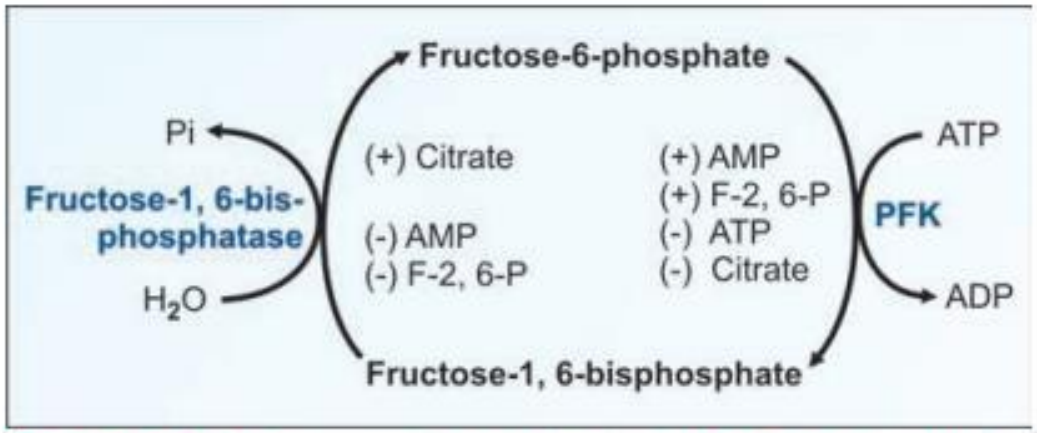


Fig. 9.32. Reciprocal regulation of PFK (glycolytic enzyme) and Fructose-1,6-bisphosphatase (gluconeogenic enzyme)

Table 9.8. Regulatory enzymes of gluconeogenesis (compare with Table 9.3)

Enzyme	*Activation*	*Inhibition*
PC	*Cortisol, Glucagon Adrenalin, Acetyl CoA*	*Insulin, ADP*
PEPCK	*do*	*Insulin*
F-1,6-bis-1 phosphatase	*do*	*F-1,6-BP, AMP F-2,6-BP*
G-6-phos-phatase	*do*	*Insulin*

7. During fasting, fat cells oxidise

A. Fatty acids

B. Glycerol

C. Glucose

D. All of the above

اللهم أعنا على شكرك وذكرك وحسن عبادتك

** Q7, الجواب A **

نفس الجدول المهم جدا

TABLE 8.3: Major fuels in different organs

	Brain	*Skeletal muscle*	*Cardiac muscle*	*Adipose tissue*
After a meal	Glucose	Glucose, Fatty acids	Glucose, pyruvate	Fatty acids; glucose
Fasting (short-term)	Glucose	Fatty acids	Fatty acids	Fatty acids
Fasting (long-term)	Glucose; ketone bodies	Ketone bodies; Branched chain aa	Ketone bodies	Fatty acids; ketone bodies
Exercise		Glycogen	Fatty acids	

8. During long-term fasting, the rate of gluconeogenesis is controlled by

A. cAMP in liver cells

B. Insulin : glucagon ratio

C. Availability of alanine in the blood

D. Availability of free fatty acids

اللهم إني أعوذ بك من الهم والحزن, وأعوذ بك من العجز والكسل

بالنهاية الalanine رح يكون هو الmain source للgluconeogenesis, ف * Q8 الجواب C *

معلومة مهمة للامتحان

الbranched chain amino acids مبدأهم الأساسي إنه الliver ما بقدر يعمللهم metabolism بكفاءة,
ف بصَفّي تكسيرهم بروح بشكل أساسي عشان يفيد الmuscles, ويعتبروا مهمات جدا للmuscles حقيقةً

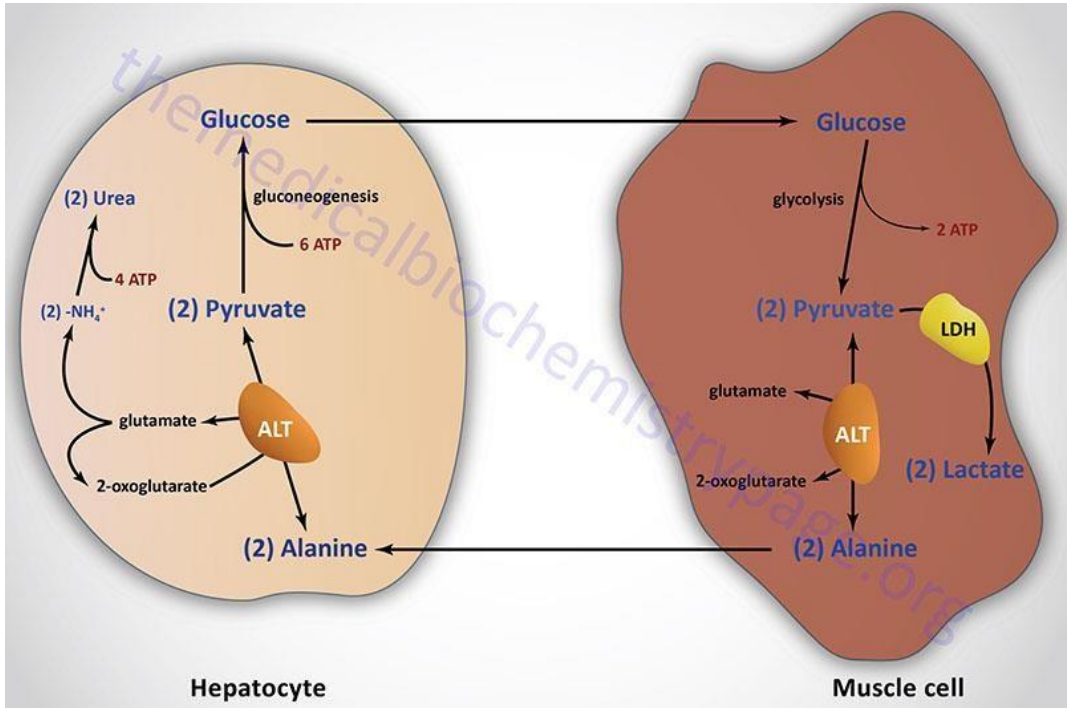
Gluconeogenesis: Definition

- Metabolic process by which glucose in synthesised from non-carbohydrate precursors:
  - Lactate
  - **Glucogenic amino acids (major source of glucose after glycogen is depleted)**
  - Glycerol (part of TAG)
  - Odd chain fatty acids (rare); Propionyl coA (minor source)

The main source of glucose carbons for gluconeogenesis is alanine derived from breakdown of muscle proteins

Glucose-Alanine cycle

- Alanine is transported from muscle to liver, transaminated → pyruvate → glucose
- Glucose can enter glycolytic pathway to form pyruvate which is transaminated → alanine
- Glucose-alanine cycle is of primary importance in conditions of starvation
- **Importance**
  - Transfer if 3C of pyruvate to the liver to give glucose
  - Transfer of NH3 in non-toxic form from muscle to liver to be converted to urea
  - Related to Cori cycle



Second Stage: Gluconeogenesis

Even before the glycogen stores are depleted, gluconeogenesis is accelerated (Figs 8.3 and 8.4). The amino acids released from muscle form the major substrate for gluconeogenesis. The amino nitrogen is transferred from other amino acids to pyruvate to form alanine. Thus the amino group reaches the liver as alanine where it is transaminated to give pyruvate for gluconeogenesis. This glucose alanine cycle (see Fig. 9.30) serves to transport the amino nitrogen of other amino acids to liver in a harmless form. Glutamic acid also serves as an important mode of transport of amino acids to liver (see Chapter 15).

The **branched chain amino acids** liberated by muscle protein catabolism especially leucine and isoleucine are utilized by the muscle to give energy. Brain can preferentially take up the glucogenic valine from the bloodstream. The plasma level of branched chain amino acids reaches a peak by 5th day of starvation.

9a. All of the following tissuesare capable of using ketone bodies except

A. Brain

B. Heart

C. RBCs

D. Skeletal muscle

اللهم لا تجعلنا من الذين ضل سعيهم في الحياة الدنيا

* Q9.a الجواب C *

9b. Which of the following can use glucose, fatty acids and ketone bodies as fuel?

A. Brain

B. Liver

C. RBCs

D. Muscle

سبحان الله وبحمده, سبحان الله العظيم

* Q9.b الجواب D *

نفس الجدول المهم جدا

TABLE 8.3: Major fuels in different organs

	Brain	*Skeletal muscle*	*Cardiac muscle*	*Adipose tissue*
After a meal	Glucose	Glucose, Fatty acids	Glucose, pyruvate	Fatty acids; glucose
Fasting (short-term)	Glucose	Fatty acids	Fatty acids	Fatty acids
Fasting (long-term)	Glucose; ketone bodies	Ketone bodies; Branched chain aa	Ketone bodies	Fatty acids; ketone bodies
Exercise		Glycogen	Fatty acids	

10. The rate of muscle proteolysis decreases by long term fasting due to

A. Utilization of ketone bodies by the brain and decreased need for gluconeogenesis

B. Saturation of the urea cycle

C. Inability to get rid of glutamine

D. Availability of fatty acids as an energy source

اللهم صرّف قلوبنا على طاعتك

* Q10 الجواب A *

vi. **Brain and starvation:** During starvation, a significant part (60–70%) of the energy requirement of the brain is met by ketone bodies (Fig. 8.1).

In long-term starvation, a major site of glutamine metabolism is the kidney, since the excretion of ketone bodies requires as a counterion formed from ammonia produced by glutaminase. The resulting glutamate is utilized for renal gluconeogenesis, the activity of which is quantitatively equivalent to that of liver.

اللهم إني أستودعك ما درست وقرأت وحفظت وفهمت.. فرُدَّه لي عند حاجتي إليه