Biochemistry

Unit 5 · Lesson 3 · Electron transport and gluconeogenesis

The notes for Lesson 3 of Unit 5: chapters 5.5 to 5.6 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 3 of 4 | 2 chapters | Chapters 5.5 to 5.6

About these notes

These are the notes for **Lesson 3 · Electron transport and gluconeogenesis** of the University free n easy video lessons on Biochemistry Unit 5, Bioenergetics and Carbohydrate Metabolism. They are chapters 5.5 to 5.6 of the unit book, unchanged, so a page here is the same page in the full book.

5.5 Biological oxidation: electron transport and oxidative phosphorylation

NADH and $FADH_2$ from glycolysis, the link reaction and the TCA cycle hand their electrons to a chain of carriers in the inner mitochondrial membrane. As electrons fall from NADH to oxygen, the chain pumps protons out of the matrix; the protons flow back through ATP synthase, which makes ATP. This coupling of electron flow to ATP synthesis is oxidative phosphorylation, and it gives about 90 percent of the ATP from glucose.

You need: $\Delta G^{\circ\prime}$ from chapter 5.2; the NADH and $FADH_2$ counts from chapter 5.3 and chapter 5.4.

Redox potential and free energy

Oxidation is loss of electrons; reduction is gain. Each redox pair (oxidised form/reduced form) has a **standard reduction potential** $E^{\circ\prime}$, measured in volts at pH 7 against the hydrogen electrode. A more negative $E^{\circ\prime}$ means a stronger tendency to give electrons away; electrons flow spontaneously from a pair of lower $E^{\circ\prime}$ to one of higher $E^{\circ\prime}$.

REDOX PAIR	E°′ (V)
NAD^+ / NADH	−0.320
Pyruvate / lactate	−0.185
Fumarate / succinate	+0.031
Ubiquinone / ubiquinol	+0.045
Cytochrome c (Fe^{3+} / Fe^{2+})	+0.254
½ O_2 / H_2O	+0.816

The free-energy change of electron transfer is $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$, where n is the number of electrons transferred, F is the Faraday constant (96.5 kJ V^{-1} mol^{-1}) and $\Delta E^{\circ\prime} = E^{\circ\prime}$(acceptor) − $E^{\circ\prime}$(donor). Actual potentials follow the Nernst equation, $E = E^{\circ\prime} + (RT/nF)\ \ln([\text{oxidised}]/[\text{reduced}])$.

The electron transport chain

The chain has four complexes and two mobile carriers, arranged in order of rising E°′ (Figure 5.5.1).

1. **Complex I** (NADH:ubiquinone oxidoreductase, NADH dehydrogenase) takes 2 e^- from NADH through FMN and iron–sulphur centres and passes them to ubiquinone (Q). It pumps 4 H^+ out per pair of electrons.
2. **Complex II** (succinate dehydrogenase, the TCA enzyme) passes electrons from succinate through FAD and Fe–S centres to Q. It pumps no protons, which is why $FADH_2$ yields less ATP.
3. **Ubiquinone** (coenzyme Q) is a small lipid-soluble carrier that moves within the membrane and collects electrons from Complexes I and II (and from the glycerol 3-phosphate shuttle).
4. **Complex III** (cytochrome bc_1 complex) passes electrons from ubiquinol (QH_2) to cytochrome c through the Q cycle, releasing 4 H^+ into the intermembrane space per pair.
5. **Cytochrome c** is a small haem protein on the outer face of the inner membrane that carries one electron at a time to Complex IV.
6. **Complex IV** (cytochrome c oxidase; haems a and a_3, copper centres CuA and CuB) gives electrons to O_2: $4\ e^- + 4\ H^+ + O_2 \rightarrow 2\ H_2O$. It pumps 2 H^+ per pair of electrons.

Totals: **10 H^+** pumped per NADH (4 + 4 + 2) and **6 H^+** per $FADH_2$ entering through Complex II (0 + 4 + 2).

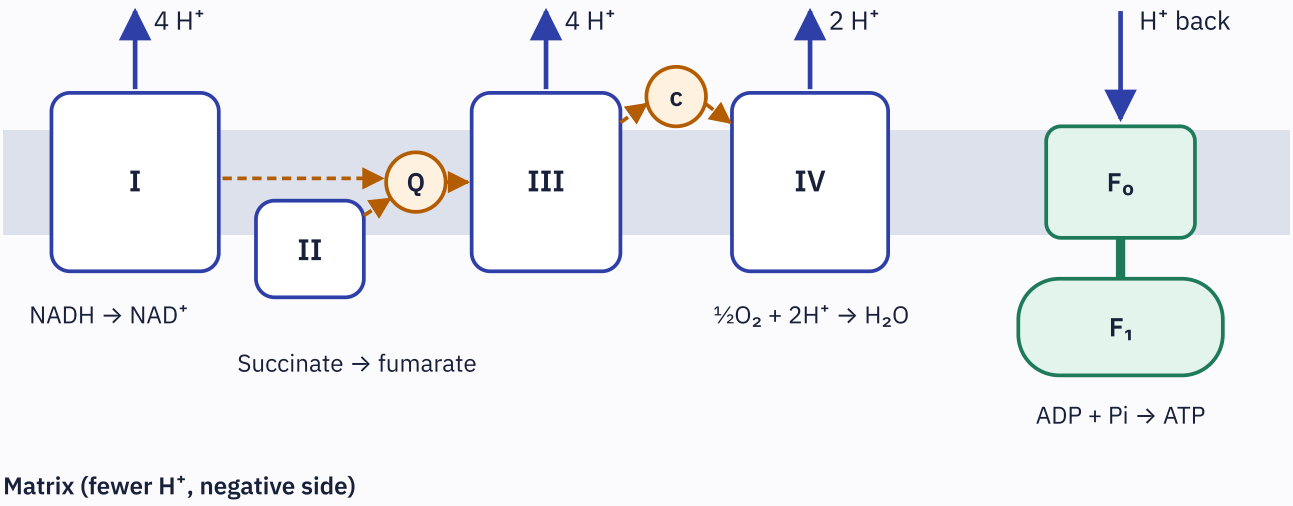


Figure 5.5.1 The respiratory chain and ATP synthase in the inner mitochondrial membrane. Dashed orange arrows trace electrons; blue arrows show protons pumped out by Complexes I, III and IV and returning through F_o to drive ATP synthesis in F_1. Notice that Complex II feeds Q but pumps no protons.

Chemiosmosis and ATP synthase

Peter Mitchell's **chemiosmotic theory** explains the coupling. Pumped protons create a **proton-motive force** with two parts: an electrical potential (the matrix side is negative) and a pH gradient (the matrix is more alkaline). The inner membrane is impermeable to H^+, so the protons can return only through ATP synthase.

ATP synthase (Complex V, F_oF_1-ATPase) has two parts. F_o sits in the membrane and contains a ring of c subunits; protons passing through turn the ring. The ring turns the central γ stalk inside F_1, a knob in the matrix made of three α and three β subunits. In Paul Boyer's **binding change mechanism**, each β subunit cycles through three states: open (O, releases ATP and takes in ADP + Pi), loose (L, holds them) and tight (T, joins them into ATP). One full turn of γ makes 3 ATP. The rotation has been watched directly in single molecules.

P/O ratio. The P/O ratio is the ATP made per pair of electrons (per oxygen atom reduced). About 4 H^+ are needed per ATP: roughly 3 to turn the motor and 1 to bring Pi and ADP into the matrix (the ATP leaves by the ADP–ATP translocase). So NADH gives 10/4 = **2.5 ATP** and $FADH_2$ gives 6/4 = **1.5 ATP**. Older books use 3 and 2.

Cytosolic NADH cannot cross the inner membrane. In skeletal muscle and brain the **glycerol 3-phosphate shuttle** delivers its electrons to Q as $FADH_2$ (1.5 ATP). In liver, heart and kidney the **malate–aspartate shuttle** delivers them to mitochondrial NAD^+ (2.5 ATP).

Inhibitors and uncouplers. Rotenone and amytal block Complex I; antimycin A blocks Complex III; cyanide, azide and carbon monoxide block Complex IV; oligomycin blocks the F_o channel of ATP synthase; atractyloside blocks the ADP–ATP translocase. **Uncouplers** such as 2,4-dinitrophenol carry protons back across the membrane, so electrons flow and oxygen is used but no ATP is made; the energy is lost as heat. Brown fat does this on purpose with the protein thermogenin (UCP1) to keep newborns warm. Normally the rate of respiration is set by ADP supply, called **respiratory control**.

WORKED EXAMPLE 1: ENERGY RELEASED BY NADH OXIDATION

Question. Calculate $\Delta G^{\circ\prime}$ for the transfer of two electrons from NADH to O_2, and the efficiency of capture if 2.5 ATP are made ($\Delta G^{\circ\prime}$ of ATP synthesis = +30.5 kJ/mol).

1. $\Delta E^{\circ\prime} = E^{\circ\prime}(\text{acceptor}) - E^{\circ\prime}(\text{donor}) = 0.816 - (-0.320) = 1.136$ V.
2. $\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime} = -2 \times 96.5$ kJ V^{-1} $mol^{-1} \times 1.136$ V $= -219$ kJ/mol.
3. Energy kept in ATP = 2.5 × 30.5 = 76.3 kJ/mol.
4. Efficiency = 76.3 / 219 × 100 = 35 percent under standard conditions.

Answer. $\Delta G^{\circ\prime} \approx -219$ kJ/mol; about 35 percent is captured as ATP under standard conditions (more under cell conditions, where ATP synthesis costs about 50 kJ/mol).

WORKED EXAMPLE 2: ATP YIELD FROM ONE GLUCOSE

Question. Tabulate the ATP yield from complete oxidation of one glucose to CO_2 and H_2O in (a) liver and (b) skeletal muscle.

STAGE	DIRECT	CARRIERS	ATP (MODERN)	ATP (OLDER)
Glycolysis	2 ATP	2 NADH (cytosol)	2 + 5 or 3	2 + 6 or 4
Pyruvate dehydrogenase (×2)	–	2 NADH	5	6
TCA cycle (×2)	2 GTP	6 NADH, 2 $FADH_2$	2 + 15 + 3	2 + 18 + 4
Total			**32 (liver) / 30 (muscle)**	**38 / 36**

Answer. (a) Liver, malate–aspartate shuttle: 32 ATP. (b) Muscle, glycerol 3-phosphate shuttle: 30 ATP. With older P/O values, 38 and 36.

EXAM TRAPS

- $\Delta E^{\circ\prime}$ is acceptor minus donor; reversing it gives the wrong sign of $\Delta G^{\circ\prime}$.
- Uncouplers increase oxygen use; inhibitors of the chain stop it. Oligomycin stops both ATP synthesis and, because of respiratory control, oxygen use.
- Cytochrome c and ubiquinone are mobile carriers, not complexes.
- State which P/O values you use: 2.5/1.5 gives 30–32 ATP per glucose; 3/2 gives 36–38. Mixing them is the most common error.
- F_1 is in the matrix and makes ATP; F_o is in the membrane and carries protons. The "o" is for oligomycin, not zero.

CHECK YOURSELF

– REMEMBER **Name one inhibitor each for Complexes I, III and IV and for ATP synthase.** 4 marks

Rotenone (I), antimycin A (III), cyanide or CO (IV), oligomycin (ATP synthase).

– REMEMBER **What are the two components of the proton-motive force?** 2 marks

The membrane potential (charge difference) and the pH gradient across the inner membrane.

– UNDERSTAND **Why does $FADH_2$ give fewer ATP than NADH?** 3 marks

Its electrons enter at Q through Complex II, bypassing Complex I, so only 6 H^+ are pumped instead of 10: 1.5 ATP instead of 2.5.

– UNDERSTAND **Explain the binding change mechanism in four lines.** 4 marks

Proton flow turns the c ring and the γ stalk. γ turns inside the $\alpha_3\beta_3$ head, forcing each β into O, L and T states in turn. ADP + Pi bind in L, form ATP in T, and ATP is released in O. One turn gives 3 ATP.

– APPLY **Calculate $\Delta G^{\circ\prime}$ for electron transfer from succinate to O_2 (2 e^-).** 3 marks

$\Delta E^{\circ\prime} = 0.816 - 0.031 = 0.785$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 0.785 = -151.5$ kJ/mol.

– APPLY **Antimycin A is added to mitochondria oxidising succinate. Which carriers become fully reduced and which oxidised?** 3 marks

Carriers before the block (Complex II, Q) become reduced; those after it (cytochrome c, Complex IV) become oxidised.

– ANALYZE **2,4-dinitrophenol was once sold as a slimming drug. Explain why it causes weight loss and why it is dangerous.** 4 marks

It carries protons across the inner membrane, dissipating the gradient. Fuel is oxidised without making ATP, so the body burns more fat to meet its ATP needs and loses the energy as heat. The heat and ATP shortage can cause fatal hyperthermia.

BEYOND THE SYLLABUS

Cryo-electron microscopy has now shown Complexes I to IV and ATP synthase at near-atomic detail, and has found them grouped into "respirasomes" in the membrane. Defects in mitochondrial DNA genes for these complexes cause mitochondrial diseases that pass down from the mother. Bacteria such as *Mycobacterium tuberculosis* depend on their own ATP synthase, which is the target of the TB drug bedaquiline. Search "respirasome cryo-EM" and "bedaquiline ATP synthase". This is not for the exam.

5.6 Gluconeogenesis

Gluconeogenesis makes new glucose from non-carbohydrate precursors: lactate, glycerol and most amino acids. The brain and red blood cells need glucose every minute, and liver glycogen lasts less than a day of fasting, so after that the blood glucose depends on this pathway. It runs mainly in the liver, with the kidney cortex contributing more in long fasting. It uses seven reversible steps of glycolysis backwards and goes around the three irreversible ones by four different enzymes.

You need: the ten steps and the three irreversible enzymes from chapter 5.3; pyruvate carboxylase from chapter 5.4.

Precursors

- **Lactate** from working muscle and red cells is oxidised to pyruvate by lactate dehydrogenase in the liver (the Cori cycle).
- **Alanine** and other **glucogenic amino acids** (from muscle protein in fasting) give pyruvate or TCA cycle intermediates, which become oxaloacetate. Amino-group removal is in Unit 6, Amino acid catabolism.

- **Glycerol** from fat breakdown is phosphorylated by glycerol kinase and oxidised to dihydroxyacetone phosphate, which enters at the triose level.
- **Propionate**, from odd-chain fatty acids and from rumen fermentation in cattle, enters as succinyl-CoA.

Even-chain fatty acids cannot give net glucose in animals. They yield only acetyl-CoA; the pyruvate dehydrogenase step is irreversible, and the two carbons of acetyl-CoA that enter the TCA cycle are lost as two CO_2, so no net oxaloacetate is made. Plants and bacteria get around this with the glyoxylate cycle (chapter 5.7).

The three bypasses

1. **Pyruvate → phosphoenolpyruvate** (bypasses pyruvate kinase), in two steps. In the mitochondrion, **pyruvate carboxylase** (biotin coenzyme, activated by acetyl-CoA) makes oxaloacetate: pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + Pi. Oxaloacetate cannot cross the inner membrane, so it is reduced to malate, which leaves and is re-oxidised to oxaloacetate in the cytosol (this also carries reducing power out as NADH). Then **phosphoenolpyruvate carboxykinase (PEPCK)** decarboxylates and phosphorylates it: oxaloacetate + GTP → PEP + CO_2 + GDP. The CO_2 added in the first step is removed in the second; carboxylation followed by decarboxylation is how the cell pays to climb back to the high-energy PEP.
2. **Fructose 1,6-bisphosphate → fructose 6-phosphate** (bypasses PFK-1): **fructose 1,6-bisphosphatase-1 (FBPase-1)** removes the phosphate by hydrolysis, no ATP made.
3. **Glucose 6-phosphate → glucose** (bypasses hexokinase): **glucose 6-phosphatase**, on the lumen side of the endoplasmic reticulum in liver and kidney. Muscle and brain lack it, so muscle cannot release free glucose into the blood.

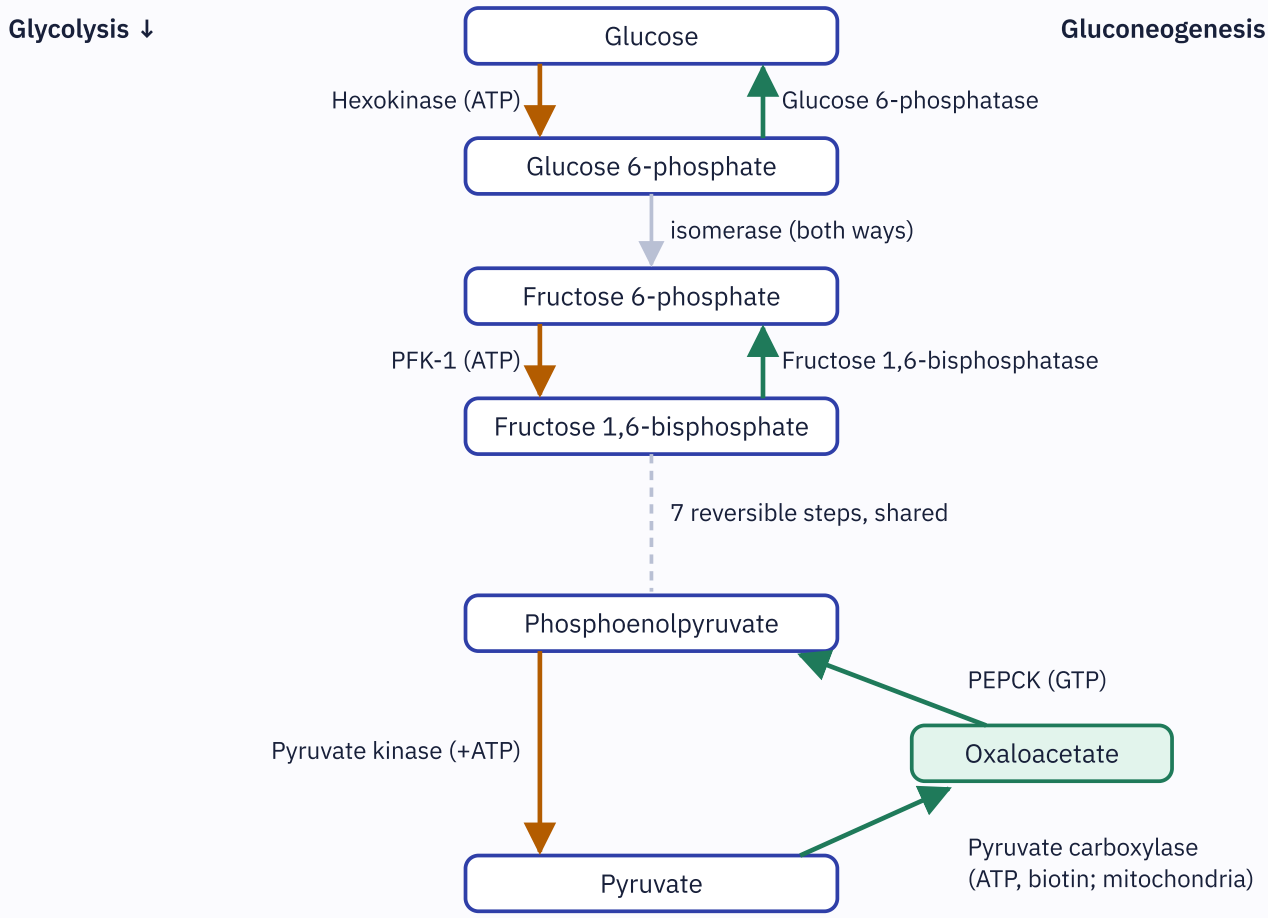


Figure 5.6.1 Glycolysis (orange, down) and gluconeogenesis (green, up) share seven reversible steps but use different enzymes at the three irreversible ones. Notice that the pyruvate kinase step needs a two-enzyme detour through oxaloacetate, and that pyruvate carboxylase works inside the mitochondrion.

Cost and regulation

$$2\ \text{Pyruvate} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ \text{H}^+ + 4\ \text{H}_2\text{O} \rightarrow \text{glucose} + 4\ \text{ADP} + 2\ \text{GDP} + 6\ \text{Pi} + 2\ \text{NAD}^+$$

Making one glucose from two pyruvate costs six high-energy phosphate bonds: 2 ATP at pyruvate carboxylase, 2 GTP at PEPCK and 2 ATP at phosphoglycerate kinase (run backwards). Glycolysis gives back only 2 ATP. The extra cost is what makes gluconeogenesis irreversible and strongly favourable in the cell.

Because glycolysis and gluconeogenesis run in the same cytosol, running both at once would be a **futile cycle** that only burns ATP. The cell prevents this by **reciprocal regulation**: the signals that switch one on switch the other off.

- **Acetyl-CoA** activates pyruvate carboxylase and inhibits pyruvate dehydrogenase, so in fasting (when fat breakdown makes acetyl-CoA) pyruvate goes to glucose, not to the TCA cycle.

- **AMP** inhibits FBPase-1 and activates PFK-1.
- **Fructose 2,6-bisphosphate** activates PFK-1 and inhibits FBPase-1. It is made and destroyed by one bifunctional enzyme, PFK-2/FBPase-2. In fasting, **glucagon** raises cAMP; protein kinase A phosphorylates the enzyme, which turns on its FBPase-2 activity, so fructose 2,6-bisphosphate falls and gluconeogenesis runs. After a meal **insulin** reverses this.
- Glucagon and cortisol also raise the amount of PEPCK by gene expression (slow, hours); insulin lowers it.

Cori cycle: lactate from muscle goes to the liver, becomes glucose, and returns to muscle. **Glucose–alanine cycle**: muscle transfers amino groups to pyruvate to make alanine, which goes to the liver and becomes glucose, while its nitrogen goes to urea (Unit 6, Urea cycle).

WORKED EXAMPLE

Question. During a 400 m sprint, muscle converts glucose to lactate, and the liver turns the lactate back to glucose. Calculate the net ATP cost of one turn of this Cori cycle per glucose, and say who pays.

1. Muscle, anaerobic glycolysis: glucose → 2 lactate gives **+2 ATP** (chapter 5.3).
2. Liver: 2 lactate → 2 pyruvate by lactate dehydrogenase, producing 2 NADH, which are exactly the 2 NADH needed at the GAPDH step run backwards. No net NADH.
3. Liver, 2 pyruvate → glucose: 2 ATP (pyruvate carboxylase) + 2 GTP (PEPCK) + 2 ATP (phosphoglycerate kinase) = **6 ATP equivalents**.
4. Net for the cycle = 2 − 6 = **−4 ATP** per glucose.

Answer. Each turn costs 4 ATP. The liver pays, using ATP from fatty acid oxidation, which shifts the metabolic burden away from the working muscle. This is part of why breathing stays fast after a sprint (the "oxygen debt").

EXAM TRAPS

- Gluconeogenesis is not glycolysis in reverse: four enzymes differ (pyruvate carboxylase, PEPCK, FBPase-1, glucose 6-phosphatase).
- Fatty acids (even-chain) cannot make net glucose in animals; glycerol from fat can.
- Muscle can make glucose 6-phosphate but not free glucose, because it lacks glucose 6-phosphatase.
- Count the cost correctly: 4 ATP + 2 GTP per glucose (6 high-energy bonds), not 2.
- Pyruvate carboxylase is mitochondrial and needs biotin; PEPCK uses GTP, not ATP, in animals.

CHECK YOURSELF

– REMEMBER **Name the four enzymes unique to gluconeogenesis.** 2 marks

Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase-1, glucose 6-phosphatase.

– REMEMBER **Which organs carry out most gluconeogenesis?** 1 mark

Liver mainly; kidney cortex, especially in prolonged fasting.

– UNDERSTAND **Why can glycerol but not palmitate be used to make glucose in humans?** 3 marks

Glycerol becomes dihydroxyacetone phosphate, a glycolytic intermediate. Palmitate gives only acetyl-CoA; PDH is irreversible and the TCA cycle loses two CO_2 per acetyl-CoA, so no net oxaloacetate forms.

– UNDERSTAND **Explain how fructose 2,6-bisphosphate prevents a futile cycle between PFK-1 and FBPase-1.** 4 marks

It activates PFK-1 and inhibits FBPase-1, so only one direction runs. Its level is high after a meal (insulin) and low in fasting (glucagon, via PKA phosphorylation of PFK-2/FBPase-2).

– APPLY **How many high-energy phosphate bonds are needed to make one glucose from two molecules of alanine (ignore urea cost)?** 2 marks

Alanine gives pyruvate by transamination, so the cost is the same as from pyruvate: 6 (4 ATP + 2 GTP).

– APPLY **Starting from glycerol, how many ATP are needed per glucose?** 3 marks

Two glycerol → two glycerol 3-phosphate (2 ATP) → two DHAP (makes 2 NADH) → one F1,6BP → glucose. Total 2 ATP; the costly pyruvate steps are skipped.

– ANALYZE **A child with deficiency of glucose 6-phosphatase becomes hypoglycaemic within hours of a meal. Explain, and predict what happens to liver glycogen.** 4 marks

Both gluconeogenesis and glycogen breakdown end in glucose 6-phosphate; without the phosphatase no free glucose leaves the liver, so blood glucose falls fast. G6P accumulates and is stored as glycogen, which builds up and enlarges the liver (von Gierke disease; Unit 6, Inborn errors of metabolism).

BEYOND THE SYLLABUS

In type 2 diabetes the liver keeps making glucose even when insulin is present, and too much gluconeogenesis is a major cause of the high fasting blood sugar. Metformin, the usual first-line drug for type 2 diabetes, lowers hepatic glucose output, though its exact molecular target is still debated. Researchers measure gluconeogenesis in people with stable-isotope tracers such as deuterated water. Search "hepatic glucose production metformin mechanism". This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Redox free energy	$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$, $\Delta E^{\circ\prime} = E^{\circ\prime}$(acceptor) − $E^{\circ\prime}$(donor)	5.5
P/O ratios	NADH 2.5, $FADH_2$ 1.5 (older: 3 and 2)	5.5
ATP per glucose	30–32 (older 36–38)	5.5
Gluconeogenesis cost	4 ATP + 2 GTP + 2 NADH per glucose from 2 pyruvate	5.6
Fructose 2,6-bisphosphate	Activates PFK-1, inhibits FBPase-1	5.3, 5.6

Glossary (terms used in this lesson)

ATP synthase	The F_oF_1 enzyme of the inner mitochondrial membrane that makes ATP using proton flow.
Binding change mechanism	Boyer's model: each β subunit of F_1 cycles through open, loose and tight states as γ rotates.
Chemiosmotic theory	Mitchell's idea that a proton gradient couples electron transport to ATP synthesis.
Cori cycle	Lactate from muscle becomes glucose in liver and returns to muscle.
Fructose 2,6-bisphosphate	Regulator that activates PFK-1 and inhibits FBPase-1.
Futile cycle	Two opposing reactions running together, wasting ATP as heat.
Malate–aspartate shuttle	Carries cytosolic NADH electrons into mitochondrial NAD^+ (liver, heart).
P/O ratio	ATP made per pair of electrons passed to one oxygen atom.
Proton-motive force	The energy of the proton gradient: membrane potential plus pH difference.
Uncoupler	A compound that lets protons leak back, so oxidation continues without ATP synthesis.

The full unit book

Every chapter of Unit 5, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 5 · Bioenergetics and Carbohydrate Metabolism (one file, works offline).

Sources and further reading

- D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 8th edition (2021): chapters on bioenergetics, glycolysis and gluconeogenesis, the citric acid cycle, oxidative phosphorylation, and carbohydrate biosynthesis in plants and bacteria (from memory; check).
- J. M. Berg, J. L. Tymoczko, G. J. Gatto and L. Stryer, *Biochemistry* (W. H. Freeman); the 5th edition is on the NCBI Bookshelf (from memory; check the edition you use).
- D. Voet and J. G. Voet, *Biochemistry* (Wiley) (from memory; check).
- U. Satyanarayana and U. Chakrapani, *Biochemistry* (Elsevier India), widely used in Indian colleges (from memory; check).

- J. L. Jain, S. Jain and N. Jain, *Fundamentals of Biochemistry* (S. Chand) (from memory; check).
- ExplorEnz, The Enzyme Database news item on the new EC 7 translocase class (August 2018): enzyme-database.org/news.php?id=5
- U.S. National Library of Medicine technical bulletin on adding Berg, Tymoczko and Stryer's *Biochemistry* (5th edition) to the Bookshelf: nlm.nih.gov technical bulletin

Standard ΔG°' and E°' values in this unit are the commonly tabulated textbook values (Lehninger tables; from memory; check against your prescribed book).

Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

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