Biochemistry

Unit 6 · Lipid, Amino Acid and Nucleotide Metabolism

Fats, proteins and nucleotides are broken down and built up by pathways that share a few hubs: acetyl-CoA, the TCA cycle, glutamate and PRPP. After this unit you can draw beta-oxidation, fatty acid synthesis, ketogenesis and the urea cycle step by step, count the ATP they make or cost, trace where each atom of a purine or pyrimidine comes from, explain classic inborn errors and predict what insulin and glucagon do to every one of these pathways.

Taught at 8 of 10 | Usually Sem 3 | 9 chapters

About this unit

Unit 5 followed glucose. This unit follows the other two fuels and the nitrogen they carry. Chapters 6.1 to 6.4 are about lipids: how fatty acids are burned (6.1) and made (6.2), how the liver turns surplus acetyl-CoA into ketone bodies during fasting (6.3), and how cells build fats, membrane lipids and cholesterol (6.4). Chapters 6.5 and 6.6 are about amino acids: how their nitrogen is removed (6.5) and turned into urea (6.6). Chapter 6.7 makes and breaks the nucleotides, which reuse amino acids for their atoms. Chapter 6.8 shows what happens when one enzyme of these pathways is missing, and Chapter 6.9 shows how hormones switch all of them on and off together.

Chapters

1. Fatty acid oxidation
2. Fatty acid biosynthesis, elongation and desaturation
3. Ketone bodies and ketogenesis
4. Synthesis of triacylglycerols, phospholipids and cholesterol ADVANCED
5. Amino acid catabolism: transamination and deamination
6. Urea cycle
7. Purine and pyrimidine biosynthesis and degradation
8. Inborn errors of metabolism ADVANCED
9. Overview of hormones and metabolic regulation

How to use this book

- Read the lead paragraph and the figure of each chapter first; they carry the one idea.

- Cover the solution of the worked example and do it yourself with a pencil, then compare step by step.
- Answer each "Check yourself" question in your head or on paper before you open it.

6.1 Fatty acid oxidation

Fatty acid oxidation is how a cell burns stored fat. A long fatty acid chain is cut two carbons at a time, inside the mitochondrion, into acetyl-CoA; every cut also hands electrons to NAD^+ and FAD, which feed the electron transport chain. The process is called **β-oxidation** because the carbon that gets oxidised is the β-carbon (C-3, counted from the carboxyl end). Heart muscle, resting skeletal muscle and the liver during fasting run mostly on it, and one molecule of palmitate gives about 106 ATP against about 32 for glucose.

> You need: fatty acid structure and numbering from **Unit 3, Fatty acids: saturated and unsaturated**, and the TCA cycle and P/O ratios from **Unit 5, Citric acid (TCA) cycle** and **Biological oxidation**.

Step 0: getting the fatty acid ready and into the mitochondrion

Fat stored in adipose tissue is triacylglycerol. When the body needs fuel, **hormone-sensitive lipase** (switched on by glucagon and adrenaline, Chapter 6.9) releases free fatty acids, which travel in blood bound to serum albumin.

1. **Activation** (outer mitochondrial membrane, cytosolic side). Acyl-CoA synthetase (thiokinase) joins the fatty acid to coenzyme A: fatty acid + CoA + ATP → fatty acyl-CoA + AMP + PPi. Pyrophosphatase then splits PPi into 2 Pi, so activation costs **two high-energy bonds**, counted as 2 ATP.
2. **Carnitine shuttle.** The inner membrane does not let acyl-CoA through. Carnitine palmitoyltransferase I (**CPT-I**, outer membrane) moves the acyl group onto carnitine, forming acyl-carnitine. A translocase carries acyl-carnitine across the inner membrane in exchange for free carnitine.
3. **Back to acyl-CoA.** CPT-II, on the matrix side, puts the acyl group back on CoA. The matrix now holds fatty acyl-CoA, ready for β-oxidation.

CPT-I is the control point. It is inhibited by **malonyl-CoA**, the first intermediate of fatty acid synthesis (Chapter 6.2). So when the cell is making fat, it cannot burn fat at the same time. Short and medium chains (up to about C10) enter without carnitine.

The four reactions of one round

Each round shortens the acyl-CoA by two carbons. The four reactions repeat the same chemistry you saw in the TCA cycle from succinate to oxaloacetate: oxidise, add water, oxidise, cut.

1. **Dehydrogenation.** Acyl-CoA dehydrogenase removes two hydrogens from C-2 and C-3, making a *trans*-Δ²-enoyl-CoA. FAD → $FADH_2$. There are separate dehydrogenases for very-long, long, medium and short chains.
2. **Hydration.** Enoyl-CoA hydratase adds water across the double bond, giving L-3-hydroxyacyl-CoA.
3. **Dehydrogenation.** L-3-hydroxyacyl-CoA dehydrogenase oxidises the hydroxyl to a keto group, giving 3-ketoacyl-CoA (β-ketoacyl-CoA). NAD^+ → NADH.
4. **Thiolysis.** β-Ketothiolase uses a fresh CoA to cut between C-2 and C-3. Products: one acetyl-CoA and an acyl-CoA two carbons shorter, which goes round again.

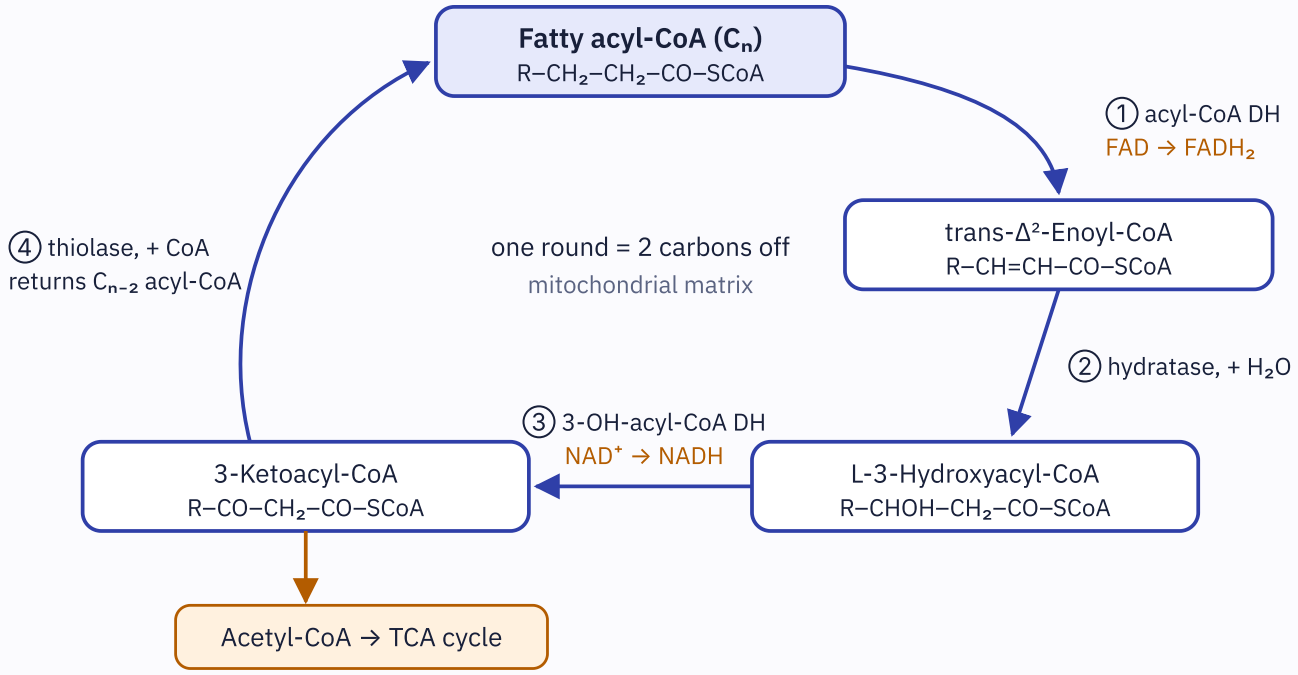


Figure 6.1.1 One round of β-oxidation. Reactions ① and ③ are oxidations that feed the electron transport chain; ④ releases acetyl-CoA and sends a chain two carbons shorter back to the top. Notice that the oxidations happen at C-3, the β-carbon.

Counting the ATP

For a saturated fatty acid with an even number of carbons, n:

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rounds of β-oxidation   = n/2 − 1
acetyl-CoA produced     = n/2
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FADH₂ and NADH          = n/2 − 1 each
ATP = (n/2)×10 + (n/2 − 1)×1.5 + (n/2 − 1)×2.5 − 2
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Here 10 is the ATP from one acetyl-CoA through the TCA cycle and oxidative phosphorylation (3 NADH, 1 $FADH_2$, 1 GTP), 2.5 and 1.5 are the P/O ratios of NADH and $FADH_2$, and the final −2 is the activation cost. Older Indian textbooks use P/O ratios of 3 and 2 and 12 ATP per acetyl-CoA; check which values your university's answer key uses and show your assumptions in the answer.

Odd chains, double bonds and other routes

- **Odd-chain fatty acids** end with the three-carbon **propionyl-CoA**. Propionyl-CoA carboxylase (needs biotin) makes D-methylmalonyl-CoA; an epimerase makes the L form; methylmalonyl-CoA mutase (needs vitamin B_{12}) turns it into succinyl-CoA, which enters the TCA cycle. This is why propionyl-CoA is the one part of fat that can become glucose.
- **Unsaturated fatty acids.** Natural double bonds are *cis* and sit at odd or even positions that the hydratase cannot use. **Enoyl-CoA isomerase** moves a *cis*-Δ^3 bond to *trans*-Δ^2; for polyunsaturated chains **2,4-dienoyl-CoA reductase** (uses NADPH) is also needed. Each double bond skips the FAD step, so it yields 1.5 ATP less.
- **Peroxisomal β-oxidation** shortens very-long-chain fatty acids (C22 and longer). Its first enzyme, acyl-CoA oxidase, passes electrons straight to O_2, making H_2O_2 and no ATP from that step.
- **α-oxidation** removes one carbon at a time from branched chains such as phytanic acid (from dairy and ruminant fat); its defect causes Refsum disease. **ω-oxidation** in the endoplasmic reticulum oxidises the far (methyl) end and is a minor route.

WORKED EXAMPLE

Question (8 marks). Calculate the net ATP produced when one molecule of palmitic acid (C16:0) is completely oxidised to CO_2 and H_2O. Show each step. Use P/O = 2.5 for NADH and 1.5 for $FADH_2$.

1. Carbons n = 16. Rounds of β-oxidation = 16/2 − 1 = **7**. Acetyl-CoA = 16/2 = **8**.
2. From β-oxidation: 7 $FADH_2$ × 1.5 = 10.5 ATP; 7 NADH × 2.5 = 17.5 ATP. Subtotal 28 ATP.
3. From 8 acetyl-CoA in the TCA cycle: 8 × 10 = 80 ATP.
4. Gross = 28 + 80 = 108 ATP. Activation uses 2 high-energy bonds (ATP → AMP + PPi).
5. **Net = 108 − 2 = 106 ATP per palmitate.** With the older values (3, 2, 12): 7×2 + 7×3 + 8×12 − 2 = 129 ATP.

Follow-up. Stearic acid (C18:0): 8 rounds, 9 acetyl-CoA → 8×1.5 + 8×2.5 + 9×10 − 2 = 12 + 20 + 90 − 2 = **120 ATP**. Oleic acid (C18:1, Δ^9) loses one $FADH_2$: 120 − 1.5 = **118.5 ATP**.

EXAM TRAPS

- Rounds are n/2 − 1, not n/2: the last round makes two acetyl-CoA at once.
- Activation costs 2 ATP equivalents even though only one ATP molecule is used, because it goes to AMP.
- Malonyl-CoA inhibits CPT-I, not acyl-CoA dehydrogenase. Examiners like "how is β-oxidation regulated?"
- β-oxidation happens in the mitochondrial matrix (and peroxisomes); fatty acid synthesis is in the cytosol. Do not swap them.
- Even-chain fatty acids give no net glucose: acetyl-CoA cannot become pyruvate in animals. Only the propionyl-CoA of odd chains can.

CHECK YOURSELF

– REMEMBER **Name the four enzymes of one round of β-oxidation in order.** 2 marks

Acyl-CoA dehydrogenase, enoyl-CoA hydratase, L-3-hydroxyacyl-CoA dehydrogenase, β-ketothiolase.

– REMEMBER **Which two vitamins are needed to oxidise propionyl-CoA?** 2 marks

Biotin (propionyl-CoA carboxylase) and vitamin B_{12} (methylmalonyl-CoA mutase).

– UNDERSTAND **Why is carnitine needed, and why does malonyl-CoA block fat oxidation?** 4 marks

The inner mitochondrial membrane is impermeable to long-chain acyl-CoA, so the acyl group crosses as acyl-carnitine (CPT-I, translocase, CPT-II). Malonyl-CoA, made when the cell is synthesising fat, inhibits CPT-I, so new fatty acids are not sent straight into β-oxidation.

– APPLY **Net ATP from lauric acid (C12:0), P/O 2.5 and 1.5?** 4 marks

5 rounds, 6 acetyl-CoA: 5×1.5 + 5×2.5 + 6×10 − 2 = 7.5 + 12.5 + 60 − 2 = **78 ATP**.

– APPLY **How many acetyl-CoA and propionyl-CoA come from a C17 saturated fatty acid?** 2 marks

17 − 3 = 14 carbons as acetyl-CoA = **7 acetyl-CoA**, plus **1 propionyl-CoA**, after 7 rounds.

– ANALYZE **Per carbon, why does fat give more ATP than glucose?** 4 marks

Fatty acid carbons are almost fully reduced (–CH_2–), while glucose carbons already carry oxygen (–CHOH–). More reduced carbon means more electrons to hand to NAD^+ and FAD. Palmitate: 106/16 ≈ 6.6 ATP per carbon; glucose: about 32/6 ≈ 5.3. Fat is also stored without water, so its yield per gram of stored fuel is far higher.

BEYOND THE SYLLABUS

Newborn screening by tandem mass spectrometry reads the acyl-carnitine pattern in a dried blood spot, and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is one of the disorders it picks up (see Chapter 6.8). Drug research targets CPT-I and malonyl-CoA levels for obesity, type 2 diabetes and heart failure. In biotechnology, engineered *E. coli* and yeast run β-oxidation in reverse to make fuels and chemicals. Search "reverse β-oxidation metabolic engineering" if this interests you. None of this is for the exam.

6.2 Fatty acid biosynthesis, elongation and desaturation

When you eat more carbohydrate than you can burn or store as glycogen, the liver and adipose tissue turn the surplus into fat. They build fatty acids two carbons at a time from acetyl-CoA, in the cytosol, using ATP and NADPH. The main product is **palmitate (C16:0)**. Other enzymes then lengthen it (**elongation**) or put double bonds into it (**desaturation**). It

looks like β-oxidation run backwards, but it uses a different place, different carriers and different enzymes, so that the cell can switch one on while the other is off.

> You need: 6.1 Fatty acid oxidation to compare, and **Unit 5, Pentose phosphate pathway** as the source of NADPH.

Getting acetyl-CoA out of the mitochondrion

Acetyl-CoA is made in the mitochondrion (from pyruvate) but used in the cytosol, and the inner membrane has no carrier for it. The **citrate shuttle** solves this:

1. In the matrix, acetyl-CoA + oxaloacetate → citrate (citrate synthase, as in the TCA cycle).
2. Citrate leaves through the citrate carrier.
3. In the cytosol, **ATP-citrate lyase** splits it back: citrate + CoA + ATP → acetyl-CoA + oxaloacetate + ADP + Pi.
4. Oxaloacetate is reduced to malate (malate dehydrogenase, NADH), and **malic enzyme** turns malate into pyruvate + CO_2, making **NADPH**. Pyruvate goes back into the mitochondrion.

So the shuttle also supplies some of the NADPH. The rest comes from the pentose phosphate pathway, which is very active in liver, adipose tissue and lactating mammary gland.

The committed step: acetyl-CoA carboxylase

Acetyl-CoA carboxylase (ACC) carboxylates acetyl-CoA to **malonyl-CoA**: acetyl-CoA + HCO_3^- + ATP → malonyl-CoA + ADP + Pi. It uses **biotin** as the CO_2 carrier. This is the rate-limiting, regulated step:

- **Allosteric:** citrate activates it (plenty of acetyl-CoA and ATP); palmitoyl-CoA inhibits it (product feedback).
- **Covalent:** phosphorylation by AMP-activated protein kinase (AMPK), triggered by low energy and by glucagon and adrenaline, inactivates ACC; insulin favours the active, dephosphorylated form.

Malonyl-CoA has a second job you met in 6.1: it inhibits CPT-I, so newly made fatty acids are not burned.

Fatty acid synthase: the four-step cycle

In animals, all the remaining steps are done by **fatty acid synthase (FAS)**, a large multifunctional protein (type I FAS) that works as a dimer. Bacteria and plant plastids use separate enzymes for

each step (type II). The growing chain is held on the **acyl carrier protein (ACP)** through a phosphopantetheine arm, the same reactive –SH group that CoA carries.

1. **Loading.** An acetyl group goes onto the cysteine –SH of the ketoacyl synthase domain; a malonyl group goes onto ACP.
2. **Condensation.** β-Ketoacyl-ACP synthase joins them and releases the CO_2 that ACC added, giving acetoacetyl-ACP (C4). Losing CO_2 is what drives this step forward.
3. **Reduction.** β-Ketoacyl-ACP reductase: keto → D-3-hydroxy, using **NADPH**.
4. **Dehydration.** β-Hydroxyacyl-ACP dehydratase removes water to make a *trans*-Δ^2 double bond.
5. **Reduction.** Enoyl-ACP reductase reduces the double bond, using **NADPH**. The saturated butyryl group (C4) moves to the synthase cysteine, and a new malonyl loads onto ACP.

After seven rounds the chain is palmitoyl (C16). The **thioesterase** domain releases free palmitate; the active site cannot hold longer chains, which is why FAS stops at C16. The overall equation from acetyl-CoA is:

$$8\ \text{acetyl-CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 14\ \text{H}^+ \rightarrow \text{palmitate} + 8\ \text{CoA} + 7\ \text{ADP} + 7\ \text{Pi} + 14\ \text{NADP}^+ + 6\ \text{H}_2\text{O}$$

One acetyl-CoA is the primer (it becomes C-15 and C-16, the methyl end); the other seven enter as malonyl-CoA, each costing one ATP at ACC; each round uses two NADPH.

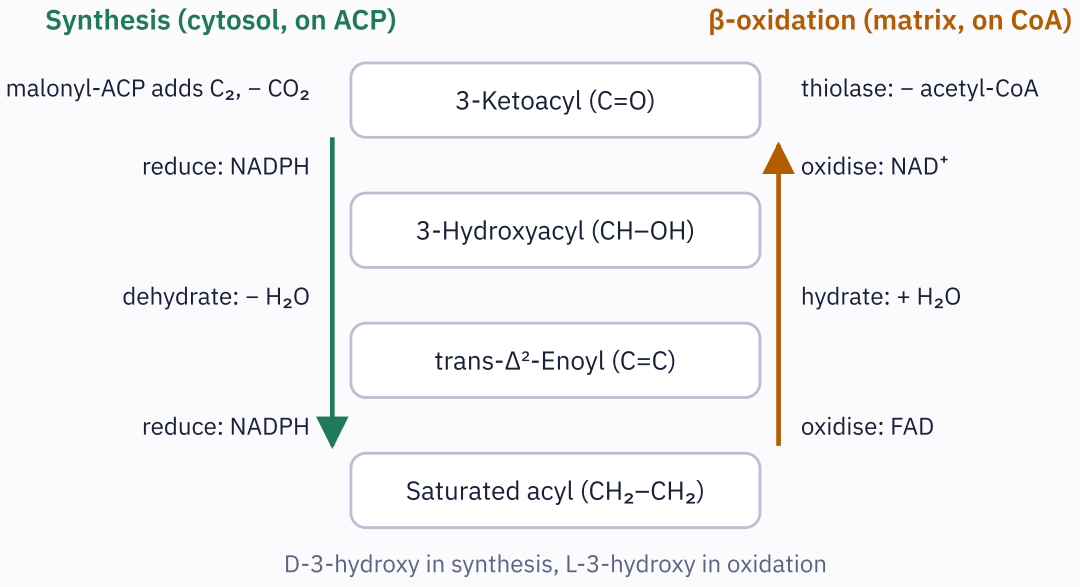


Figure 6.2.1 The same four chemical states, travelled in opposite directions. Read down the green side for synthesis and up the orange side for β-oxidation. The carriers (ACP or CoA), electron carriers (NADPH or FAD and NAD^+), compartment and hydroxy stereochemistry all differ.

Elongation and desaturation

Elongation beyond C16 happens mainly on the cytosolic face of the **endoplasmic reticulum (ER)**. Enzymes called elongases add two carbons from malonyl-CoA to an acyl-CoA, with the same four chemical steps (condense, reduce, dehydrate, reduce) and NADPH. Palmitate (C16) becomes stearate (C18), and so on, up to the very-long-chain fatty acids of sphingolipids in nerve myelin. Mitochondria have a minor elongation system that uses acetyl-CoA.

Desaturation is also in the ER. **Fatty acyl-CoA desaturases** are mixed-function oxidases: they use O_2 and electrons from NADH, passed through cytochrome b_5 reductase and cytochrome b_5. Stearoyl-CoA desaturase puts a *cis* double bond at Δ^9, turning stearate into oleate (C18:1 Δ^9). Mammals cannot put a double bond beyond C-9 (counting from the carboxyl end), so **linoleic acid** (18:2, $\Delta^{9,12}$; an ω-6 fatty acid) and **α-linolenic acid** (18:3, $\Delta^{9,12,15}$; ω-3) are **essential fatty acids** that must come from the diet, for example from sunflower, soybean or mustard oil. From linoleate the body makes arachidonate (20:4), the parent of the eicosanoids in Unit 3.

FEATURE	SYNTHESIS	B-OXIDATION
Location	Cytosol	Mitochondrial matrix
Acyl carrier	ACP	CoA
2-carbon unit	Malonyl-CoA (added)	Acetyl-CoA (removed)
Electron carrier	NADPH (used)	FAD, NAD^+ (reduced)
Key control	ACC (citrate +, palmitoyl-CoA –)	CPT-I (malonyl-CoA –)
Hormone that favours it	Insulin	Glucagon, adrenaline

WORKED EXAMPLE

Question (6 marks). Stearate (C18:0) is made in the liver from acetyl-CoA by fatty acid synthase followed by one round of ER elongation. How many acetyl-CoA, ATP and NADPH are used per stearate? Ignore the cost of the citrate shuttle.

1. Palmitate by FAS: 8 acetyl-CoA, of which 7 are first made into malonyl-CoA (7 ATP); 7 rounds × 2 NADPH = 14 NADPH.
2. Elongation C16 → C18: one more malonyl-CoA, from one more acetyl-CoA (1 ATP), and one round of two reductions (2 NADPH).
3. Total: **9 acetyl-CoA, 8 ATP, 16 NADPH** per stearate.
4. Check: 9 acetyl-CoA × 2 carbons = 18 carbons. ✓

Extension. If the citrate shuttle is included, ATP-citrate lyase spends one more ATP per acetyl-CoA exported, so 9 more ATP (17 in total).

EXAM TRAPS

- Only seven malonyl-CoA are used for palmitate, not eight: one acetyl-CoA is the primer.
- The CO_2 added by ACC is lost again at condensation, so no carbon from HCO_3^- ends up in the fatty acid.
- Synthesis uses NADPH, never NADH; desaturation uses NADH (via cytochrome b_5) and O_2.
- "Mammals cannot desaturate beyond C-9" is counted from the carboxyl end (Δ numbering). ω-3 and ω-6 are counted from the methyl end.
- FAS releases palmitate, not stearate; longer chains need elongases.

CHECK YOURSELF

– REMEMBER **Which enzyme catalyses the committed step of fatty acid synthesis, and which vitamin does it need?** 2 marks

Acetyl-CoA carboxylase; biotin.

– REMEMBER **Name two sources of cytosolic NADPH for fat synthesis.** 2 marks

The oxidative phase of the pentose phosphate pathway and malic enzyme.

– UNDERSTAND **Why does the cell use malonyl-CoA instead of adding acetyl-CoA directly?** 4 marks

Condensing two acetyl groups is thermodynamically unfavourable. The ATP spent at ACC puts a carboxyl on the acetyl group; its loss as CO_2 during condensation makes the step strongly favourable. Malonyl-CoA also serves as a signal that blocks CPT-I.

– UNDERSTAND **Why are linoleic and α-linolenic acids essential?** 3 marks

They have double bonds beyond C-9 (Δ^{12} and Δ^{15}); mammalian desaturases cannot introduce bonds there, so they must come from plant foods.

– APPLY **How many NADPH are needed to make myristate (C14:0) by FAS?** 2 marks

C14 needs 7 acetyl units: 1 primer + 6 malonyl, so 6 rounds × 2 = **12 NADPH** (and 6 ATP).

– ANALYZE **A drug activates AMPK in liver. Predict its effect on fat synthesis and oxidation.** 4 marks

AMPK phosphorylates and inactivates ACC, so malonyl-CoA falls. Synthesis slows, and with less malonyl-CoA, CPT-I is released, so β-oxidation rises. This is part of how metformin is thought to act on liver lipid metabolism.

BEYOND THE SYLLABUS

Many cancer cells switch on fatty acid synthase strongly, and FAS and ACC inhibitors are being tested as drugs. Bacterial type II FAS is a drug target too: isoniazid, the first-line TB drug in India's national programme, blocks the enoyl-ACP reductase (InhA) of *Mycobacterium tuberculosis*. Microalgae and oleaginous yeasts are engineered to overproduce fatty acids for biodiesel and omega-3 oils. Search "type II fatty acid synthase drug target" or "oleaginous yeast metabolic engineering". Not for the exam.

6.3 Ketone bodies and ketogenesis

Ketone bodies are small, water-soluble fuels that the liver makes from fatty acids when glucose is short: during an overnight fast, a long fast (such as some religious fasts), a very low-carbohydrate diet or uncontrolled type 1 diabetes. The three ketone bodies are **acetoacetate**, **β-hydroxybutyrate** (3-hydroxybutyrate) and **acetone**. The liver makes them

but cannot use them; heart, skeletal muscle, kidney and, after a few days of fasting, the brain burn them instead of glucose. Making them is **ketogenesis**; using them is **ketolysis**.

You need: 6.1 Fatty acid oxidation and **Unit 5, Gluconeogenesis**.

Why the liver makes them

In fasting, glucagon is high and insulin is low. Adipose tissue releases fatty acids, and liver β-oxidation produces a flood of acetyl-CoA. At the same time the liver is running gluconeogenesis, which pulls **oxaloacetate** out of the mitochondrion to make glucose. Without enough oxaloacetate, acetyl-CoA cannot enter the TCA cycle as citrate. The liver therefore packs the extra acetyl-CoA into ketone bodies and exports them. It also frees CoA, which β-oxidation needs to keep going.

Ketone bodies help the body save protein. The brain cannot burn fatty acids (they cross the blood–brain barrier poorly), and making glucose for it in prolonged fasting would break down muscle protein. Using ketone bodies for a large part of the brain's fuel reduces that need.

Ketogenesis: four steps in liver mitochondria

1. **Thiolase** joins two acetyl-CoA into acetoacetyl-CoA (the last step of β-oxidation running in reverse).
2. **HMG-CoA synthase** (mitochondrial) adds a third acetyl-CoA, giving β-hydroxy-β-methylglutaryl-CoA (**HMG-CoA**). This is the rate-limiting, regulated step; fasting and glucagon increase the amount of this enzyme.
3. **HMG-CoA lyase** splits HMG-CoA into **acetoacetate** and acetyl-CoA. Net result: two acetyl-CoA have become one acetoacetate.
4. **β-Hydroxybutyrate dehydrogenase** reduces much of the acetoacetate to **β-hydroxybutyrate** using NADH. In fasting, liver NADH is high, so β-hydroxybutyrate is usually the main ketone body in blood.

A small part of acetoacetate loses CO_2 without any enzyme, giving **acetone**. Acetone is not used as fuel; it is breathed out, which gives the fruity smell on the breath in diabetic ketoacidosis.

Do not confuse this with cholesterol synthesis (Chapter 6.4): it also makes HMG-CoA, but in the **cytosol**, with a cytosolic HMG-CoA synthase, and then reduces it to mevalonate.

Ketolysis: using ketone bodies in other tissues

1. β-Hydroxybutyrate is oxidised back to acetoacetate (β-hydroxybutyrate dehydrogenase), giving one NADH.
2. **Succinyl-CoA:acetoacetate CoA transferase** (also called thiophorase or SCOT) moves CoA from succinyl-CoA to acetoacetate, giving acetoacetyl-CoA and succinate. The succinyl-CoA is taken from the TCA cycle, so the GTP it would have made is lost.
3. **Thiolase** splits acetoacetyl-CoA into two acetyl-CoA, which enter the TCA cycle.

The liver has very little of the transferase, so it cannot use the ketone bodies it makes. This one missing enzyme is why ketone bodies are a liver export.

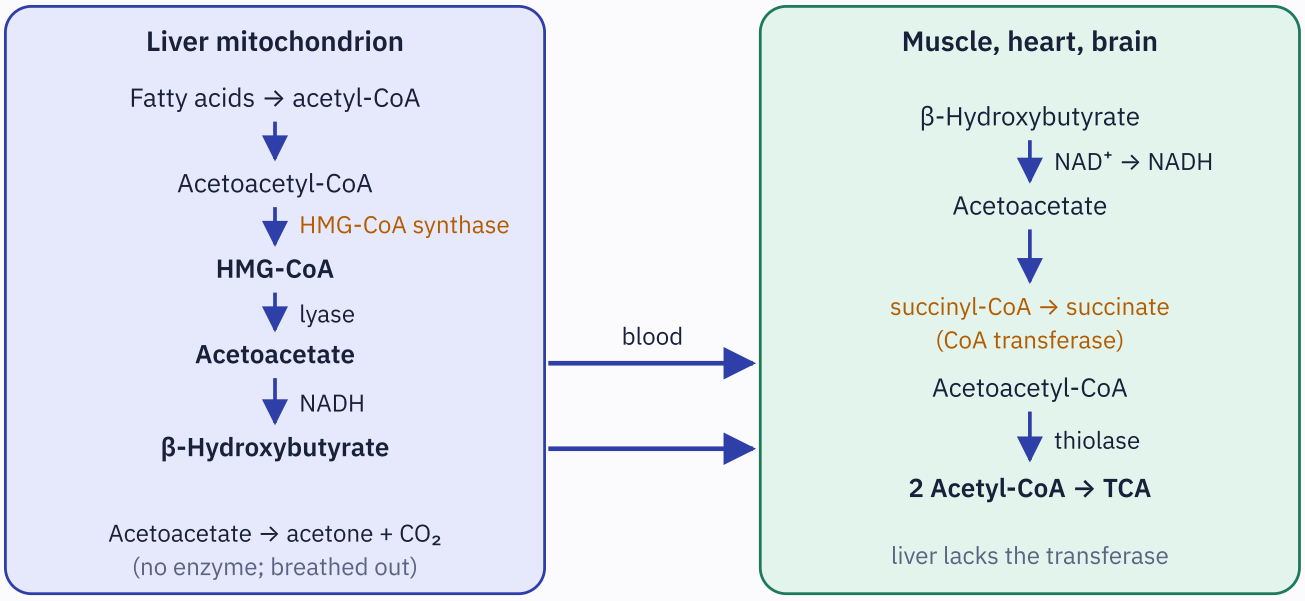


Figure 6.3.1 Ketogenesis in the liver (left) and ketolysis in other tissues (right). Notice the HMG-CoA synthase step that controls the rate, and the CoA transferase that only extrahepatic tissues have.

Ketosis and ketoacidosis

Ketosis is a raised level of ketone bodies in blood. It is normal in fasting and on a ketogenic diet. Acetoacetic acid and β-hydroxybutyric acid are acids, so at very high levels the blood's bicarbonate buffer is used up and pH falls: this is **ketoacidosis**. It is a medical emergency, seen mainly in uncontrolled type 1 diabetes (**diabetic ketoacidosis**, DKA). With no insulin, lipolysis in adipose tissue is unchecked, the liver receives far more fatty acid than it can oxidise fully, and glucagon keeps ketogenesis switched on. The patient shows ketonaemia, ketonuria, the acetone smell and deep rapid breathing (Kussmaul breathing) that blows off CO_2 to compensate for the acidosis.

In the laboratory, **Rothera's test** (sodium nitroprusside, purple colour) and urine dipsticks based on the same reaction detect acetoacetate, and acetone weakly, but **not β-hydroxybutyrate**. Because β-hydroxybutyrate is usually the main ketone body in DKA, a nitroprusside test can underestimate it; hospitals measure β-hydroxybutyrate in blood directly.

WORKED EXAMPLE

Question (6 marks). Calculate the ATP yield when one molecule of β-hydroxybutyrate is completely oxidised in skeletal muscle. Use 2.5 ATP per NADH and 10 ATP per acetyl-CoA.

1. β-Hydroxybutyrate → acetoacetate: 1 NADH = **+2.5 ATP**.
2. Acetoacetate + succinyl-CoA → acetoacetyl-CoA + succinate. That succinyl-CoA would normally have given one GTP at succinyl-CoA synthetase. So the cost is **−1 ATP equivalent**.
3. Acetoacetyl-CoA → 2 acetyl-CoA: 2 × 10 = **+20 ATP**.
4. Net = 2.5 − 1 + 20 = **21.5 ATP**. For acetoacetate itself the answer is 20 − 1 = **19 ATP**. With older values (3 and 12) the answers are 26 and 23.

EXAM TRAPS

- Acetone is a ketone body but not a fuel; it is excreted in breath.
- The liver makes ketone bodies but cannot use them, because it lacks the CoA transferase (thiophorase).
- Mitochondrial HMG-CoA synthase (ketogenesis) is different from cytosolic HMG-CoA synthase (cholesterol). Only cholesterol synthesis goes on to HMG-CoA reductase.
- β-Hydroxybutyrate has no keto group, but it is still called a ketone body.
- Rothera's test does not detect β-hydroxybutyrate.

CHECK YOURSELF

– REMEMBER **Name the three ketone bodies and the rate-limiting enzyme of ketogenesis.** 2 marks

Acetoacetate, β-hydroxybutyrate, acetone; mitochondrial HMG-CoA synthase.

– REMEMBER **Which organs use ketone bodies, and which cannot?** 2 marks

Heart, skeletal muscle, kidney cortex and brain (in prolonged fasting) use them. The liver cannot; red blood cells cannot either, because they have no mitochondria.

– UNDERSTAND **Explain why oxaloacetate shortage leads to ketogenesis in fasting.** 4 marks

Gluconeogenesis uses oxaloacetate, so less is available to condense with acetyl-CoA into citrate. Acetyl-CoA from β-oxidation then accumulates and is diverted to HMG-CoA and acetoacetate, which also frees CoA.

– UNDERSTAND **Why does uncontrolled type 1 diabetes cause ketoacidosis but type 2 diabetes rarely does?** 4 marks

In type 1 there is almost no insulin, so lipolysis and ketogenesis are unchecked. In most type 2 patients some insulin is still secreted, enough to restrain lipolysis in adipose tissue even though glucose control is poor.

– APPLY **How many acetyl-CoA are needed to make one acetoacetate, and how many are recovered when muscle uses it?** 2 marks

Three acetyl-CoA go in, one comes back out at the lyase step, so net 2 are used; muscle recovers 2.

– ANALYZE **A patient with DKA tests weakly positive by nitroprusside dipstick, yet blood β-hydroxybutyrate is very high. After insulin, the dipstick becomes more positive. Explain.** 4 marks

The dipstick detects acetoacetate, not β-hydroxybutyrate. In DKA the high NADH/NAD^+ ratio keeps most ketone body as β-hydroxybutyrate. As treatment restores metabolism, β-hydroxybutyrate is oxidised back to acetoacetate, so the dipstick reading rises even though total ketones are falling.

BEYOND THE SYLLABUS

Ketogenic diets are an established treatment for some drug-resistant childhood epilepsies, and β-hydroxybutyrate is studied as a signalling molecule (it inhibits some histone deacetylases), not only as fuel. Point-of-care blood ketone meters are now used in diabetes clinics. SGLT2-inhibitor drugs can cause "euglycaemic" DKA, where ketones are high but glucose is near normal. Search "euglycaemic diabetic ketoacidosis" and "ketogenic diet epilepsy" to read further. Not for the exam.

6.4 Synthesis of triacylglycerols, phospholipids and cholesterol

Fatty acids made in Chapter 6.2 are not stored free. Cells attach them to glycerol to make storage fat (**triacylglycerol**, TAG) and membrane lipids (**glycerophospholipids**), and the liver builds **cholesterol** from acetyl-CoA. Their structures are in **Unit 3, Phospholipids** and **Cholesterol and steroids**.

Triacylglycerol: the phosphatidic acid route

1. **Glycerol 3-phosphate** comes from dihydroxyacetone phosphate of glycolysis (glycerol 3-phosphate dehydrogenase, NADH) in adipose tissue and liver, or from free glycerol by **glycerol kinase**, which liver has and adipose tissue has very little of.
2. Two acyltransferases add two fatty acyl-CoA at C-1 and C-2, giving **phosphatidic acid** (phosphatidate), the branch point for TAG and phospholipids.
3. **Phosphatidate phosphatase** (lipin) removes the phosphate, giving 1,2-**diacylglycerol** (DAG).
4. **DAG acyltransferase** (DGAT) adds the third acyl-CoA, giving TAG.

The liver exports TAG in VLDL; adipose tissue stores it as fat droplets. In the intestine, TAG is rebuilt from 2-monoacylglycerol, a different route used after digestion.

Glycerophospholipids: two ways to attach the head group

Both ways use **CTP** to make an activated intermediate. Either the DAG part or the head group carries the CDP:

- **CDP-alcohol route** (the Kennedy pathway). Choline is phosphorylated, then activated as **CDP-choline**; CDP-choline + DAG → **phosphatidylcholine** (lecithin) + CMP. Ethanolamine follows the same route to phosphatidylethanolamine (a cephalin). In the liver, phosphatidylethanolamine can also be methylated three times by S-adenosylmethionine (SAM) to phosphatidylcholine.
- **CDP-diacylglycerol route.** Phosphatidate + CTP → **CDP-DAG**, which reacts with inositol to give phosphatidylinositol, or with glycerol 3-phosphate to give phosphatidylglycerol and then cardiolipin, the lipid of the inner mitochondrial membrane.

Phosphatidylserine is made in mammals by swapping the head group of an existing phospholipid for serine.

Cholesterol: from acetyl-CoA to a C27 sterol

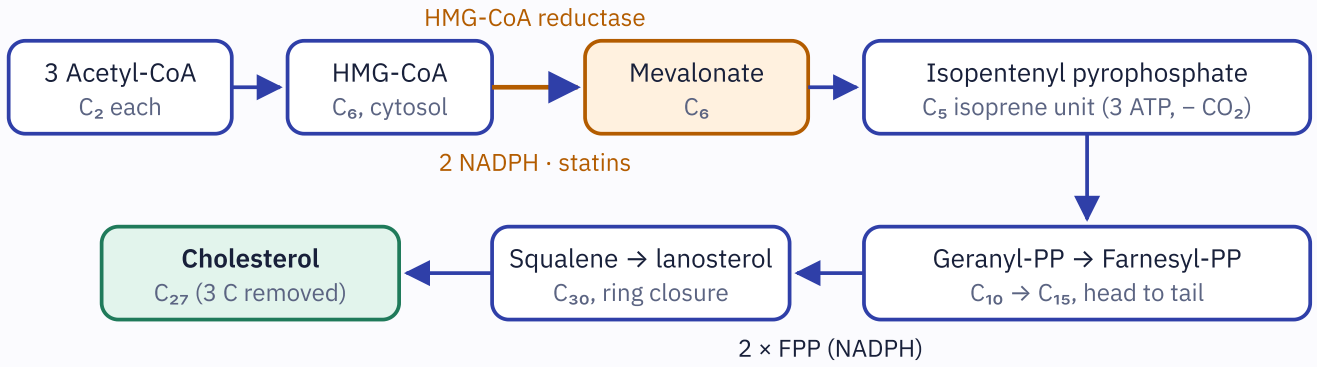


Figure 6.4.1 Cholesterol synthesis in four stages, with the carbon count at each stage. The orange step, HMG-CoA reductase, is the rate-limiting step and the target of statin drugs.

1. **Acetyl-CoA → mevalonate** (cytosol and ER). Two acetyl-CoA make acetoacetyl-CoA; cytosolic HMG-CoA synthase adds a third to give HMG-CoA. **HMG-CoA reductase**, an ER membrane enzyme, reduces it to mevalonate with 2 NADPH. This is the committed, rate-limiting step.
2. **Mevalonate → activated isoprene units.** Three ATP are used to phosphorylate and decarboxylate mevalonate to **isopentenyl pyrophosphate** (C5), which is isomerised to dimethylallyl pyrophosphate.
3. **Condensation.** C5 + C5 → geranyl pyrophosphate (C10); + C5 → **farnesyl pyrophosphate** (C15). Two farnesyl pyrophosphates join head to head, with NADPH, to make **squalene** (C30).
4. **Ring closure and trimming.** Squalene is epoxidised (O_2, NADPH) and cyclised to **lanosterol**, the first sterol. A series of about 20 further reactions remove three methyl carbons and move a double bond to give cholesterol (C27).

Regulation. HMG-CoA reductase is controlled at every level: the transcription factor **SREBP** raises its gene expression when cell sterol is low; high sterol speeds the enzyme's breakdown; AMPK phosphorylation inactivates it; insulin favours and glucagon opposes its activity. **Statins** (atorvastatin, rosuvastatin) are competitive inhibitors of HMG-CoA reductase, with a part that resembles mevalonate, and lower blood LDL cholesterol.

WORKED EXAMPLE

Question (4 marks). How many acetyl-CoA are needed to make one squalene, and how many carbons are lost on the way from squalene to cholesterol?

1. Each mevalonate uses 3 acetyl-CoA (6 carbons) and loses 1 CO_2 to give one C5 isoprene unit.
2. Squalene is C30 = 30 / 5 = **6 isoprene units** = 6 mevalonate = **18 acetyl-CoA**.
3. Cholesterol is C27, so **3 carbons** are removed after lanosterol.

EXAM TRAPS

- Phosphatidic acid is the shared precursor of TAG and glycerophospholipids; DAG is not phosphorylated to make TAG.
- Cholesterol needs NADPH, not NADH, and the rate-limiting enzyme is HMG-CoA reductase, not HMG-CoA synthase.
- Squalene is C30; cholesterol is C27.

CHECK YOURSELF

– REMEMBER **Name the activated intermediate that carries choline to DAG.** 1 mark

CDP-choline.

– UNDERSTAND **Why does adipose tissue depend on glycolysis to make TAG?** 3 marks

It has very little glycerol kinase, so it cannot reuse free glycerol; glycerol 3-phosphate must come from dihydroxyacetone phosphate of glycolysis.

– APPLY **How many NADPH are used from 3 acetyl-CoA to one isopentenyl pyrophosphate?** 2 marks

2 NADPH, at HMG-CoA reductase (plus 3 ATP for the phosphorylations).

– ANALYZE **Statins lower LDL in blood even though they act inside liver cells. Explain.** 4 marks

Lower cholesterol synthesis lowers liver sterol, which activates SREBP; SREBP raises LDL-receptor production, so the liver takes up more LDL from blood.

– REMEMBER **Which lipid is made from CDP-DAG and is found in the inner mitochondrial membrane?** 1 mark

Cardiolipin.

BEYOND THE SYLLABUS

The isoprenoid branch from farnesyl pyrophosphate also makes coenzyme Q, dolichol and the prenyl groups attached to Ras proteins. Plants and many bacteria make isopentenyl pyrophosphate by a different, non-mevalonate (MEP) route, which is the target of engineering for artemisinin and other terpenoid drugs. Search "MEP pathway terpenoid engineering". Not for the exam.

6.5 Amino acid catabolism: transamination and deamination

The body cannot store extra amino acids. Those not needed for proteins and other molecules are broken down, and breaking one down has two parts: remove the amino group, then burn or reuse the carbon skeleton. The amino group is removed in two moves. **Transamination** passes it to α-ketoglutarate, collecting nitrogen from most amino acids as glutamate. **Oxidative deamination** of glutamate then releases it as ammonium (NH_4^+), which the liver turns into urea (Chapter 6.6). The carbon skeletons end up as pyruvate or TCA cycle intermediates.

You need: amino acid structures from **Unit 2, Amino acids: structure and classification**, and the TCA cycle from **Unit 5**.

Transamination

An **aminotransferase** (transaminase) moves the α-amino group of an amino acid onto an α-keto acid. The amino acid becomes a keto acid and the keto acid becomes an amino acid:

```
amino acid₁ + α-ketoglutarate  ⇌  α-keto acid₁ + glutamate
```

- The coenzyme is **pyridoxal phosphate (PLP)**, from vitamin B_6. PLP accepts the amino group (becoming pyridoxamine phosphate) and then hands it to the keto acid, a **ping-pong** mechanism.

- The reaction is freely reversible ($\Delta G^{\circ\prime}$ close to zero), so the same enzymes also make non-essential amino acids.
- Most aminotransferases use α-ketoglutarate as the acceptor, so nitrogen from many amino acids is funnelled into one molecule, **glutamate**. Lysine and threonine are the usual exceptions that are not transaminated.

Two aminotransferases matter clinically:

- **Alanine aminotransferase (ALT**, older name SGPT): alanine + α-ketoglutarate ⇌ pyruvate + glutamate.
- **Aspartate aminotransferase (AST**, older name SGOT): aspartate + α-ketoglutarate ⇌ oxaloacetate + glutamate.

Both are inside cells, so high levels in serum mean cell damage. ALT is more specific for liver; AST is also found in heart and muscle. A "liver function test" report from any Indian lab lists both.

Oxidative deamination by glutamate dehydrogenase

In liver mitochondria, **glutamate dehydrogenase (GDH)** removes the amino group of glutamate as free ammonium:



GDH is unusual because it can use either NAD^+ or $NADP^+$. It is allosterically activated by ADP (low energy: burn amino acids) and inhibited by GTP. Transamination and GDH working together are called **transdeamination**: α-ketoglutarate is regenerated, ready to collect the next amino group.

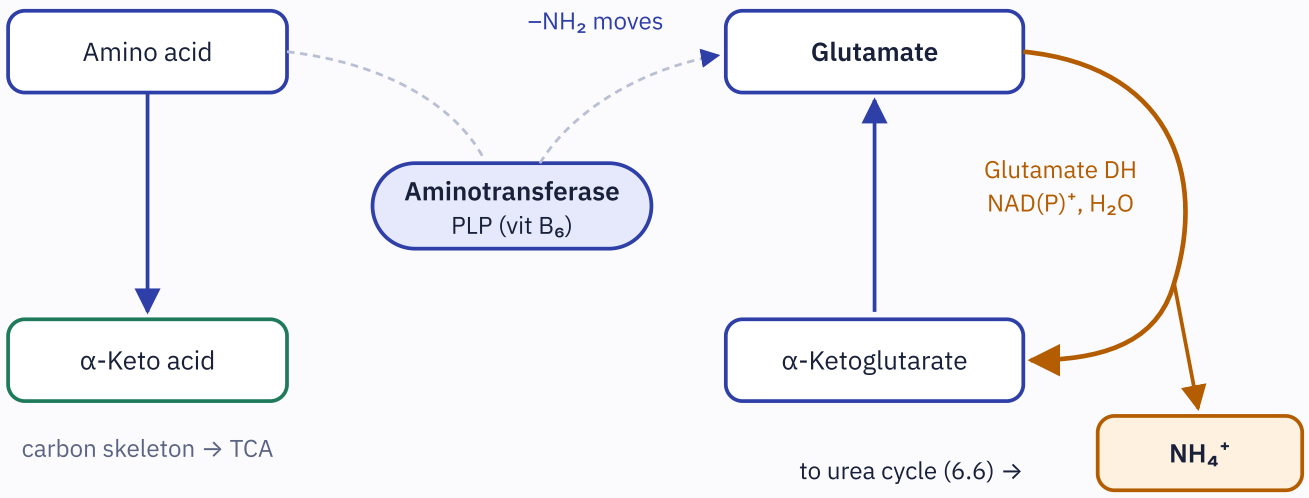


Figure 6.5.1 Transdeamination. The aminotransferase moves the amino group to α-ketoglutarate; glutamate dehydrogenase releases it as NH_4^+ and gives back α-ketoglutarate. Notice that α-ketoglutarate goes round in a loop and is not used up.

Other ways to remove or carry nitrogen

- **Non-oxidative deamination.** Serine and threonine are deaminated by PLP-dependent dehydratases (serine dehydratase gives pyruvate + NH_4^+).
- **Amino acid oxidases.** L- and D-amino acid oxidases (flavoproteins, mainly in liver and kidney peroxisomes) deaminate some amino acids, producing H_2O_2. D-amino acid oxidase removes D-amino acids from bacterial cell walls in food.
- **Carrying ammonia to the liver.** Free NH_4^+ is toxic, so tissues carry it safely. Most tissues add it to glutamate as **glutamine** (glutamine synthetase, uses ATP); liver and kidney release it again with glutaminase. Muscle uses the **glucose–alanine cycle**: pyruvate picks up nitrogen as alanine, alanine goes to the liver, gives its nitrogen for urea, and its pyruvate becomes glucose that returns to muscle.

Decarboxylation and the fate of carbon skeletons

Decarboxylation of amino acids by PLP-dependent decarboxylases removes the carboxyl as CO_2 and makes biologically active amines: histidine → **histamine**; glutamate → **GABA** (γ-aminobutyrate, a brain neurotransmitter); 5-hydroxytryptophan → **serotonin**; DOPA → **dopamine**, the precursor of noradrenaline and adrenaline.

After the amino group is gone, the carbon skeletons of the 20 amino acids enter metabolism at just seven molecules:

ENTRY POINT	AMINO ACIDS	TYPE
Pyruvate	Ala, Gly, Ser, Cys, Thr, Trp	Glucogenic

ENTRY POINT	AMINO ACIDS	TYPE
α-Ketoglutarate	Glu, Gln, Arg, His, Pro	Glucogenic
Succinyl-CoA	Ile, Met, Thr, Val	Glucogenic
Fumarate	Phe, Tyr (Asp via urea cycle)	Glucogenic
Oxaloacetate	Asp, Asn	Glucogenic
Acetyl-CoA, acetoacetyl-CoA	Leu, Lys, Phe, Tyr, Trp, Ile, Thr	Ketogenic

Glucogenic amino acids give pyruvate or a TCA intermediate and so can make glucose; **ketogenic** ones give acetyl-CoA or acetoacetyl-CoA and can make ketone bodies but not glucose. **Leucine and lysine** are the only purely ketogenic amino acids; isoleucine, phenylalanine, tryptophan, tyrosine and threonine are both.

WORKED EXAMPLE

Question (6 marks). Write the reactions by which the amino group of aspartate and of alanine can each reach free NH_4^+ in the liver. Name enzymes and coenzymes, and say what happens to each carbon skeleton.

1. Alanine + α-ketoglutarate → pyruvate + glutamate (ALT, PLP). Pyruvate can make glucose by gluconeogenesis.
2. Aspartate + α-ketoglutarate → oxaloacetate + glutamate (AST, PLP). Oxaloacetate enters gluconeogenesis or the TCA cycle.
3. Each glutamate + NAD^+ + H_2O → α-ketoglutarate + NH_4^+ + NADH (glutamate dehydrogenase, mitochondrial).
4. Net for one alanine: alanine + NAD^+ + H_2O → pyruvate + NH_4^+ + NADH. α-Ketoglutarate is regenerated.

Note. In the liver, aspartate also gives its amino group directly to the urea cycle (Chapter 6.6), so some of its nitrogen bypasses GDH.

EXAM TRAPS

- Transamination does not release free ammonia; it only moves the amino group. Deamination releases it.
- The coenzyme of transamination and decarboxylation is PLP (vitamin B_6), not NAD^+.
- Leucine and lysine are purely ketogenic; "leucine and lysine" is a favourite one-mark answer.
- SGPT is ALT and SGOT is AST. Do not swap them.
- Glutamate dehydrogenase uses both NAD^+ and $NADP^+$; most dehydrogenases use one only.

CHECK YOURSELF

– REMEMBER **What is the common amino-group acceptor in transamination?** 1 mark

α-Ketoglutarate, which becomes glutamate.

– REMEMBER **Name the amine formed by decarboxylating histidine and glutamate.** 2 marks

Histamine and GABA (γ-aminobutyrate).

– UNDERSTAND **Explain the term transdeamination.** 3 marks

Coupling of transamination (amino group to α-ketoglutarate, making glutamate) with oxidative deamination of glutamate by GDH (releasing NH_4^+ and regenerating α-ketoglutarate). Together they remove the amino group of many amino acids through one enzyme.

– UNDERSTAND **Why does muscle send nitrogen to the liver as alanine or glutamine and not as NH_4^+?** 3 marks

Free ammonia is toxic, especially to the brain. Alanine and glutamine are non-toxic carriers; alanine also brings pyruvate for gluconeogenesis.

– APPLY **Which keto acids are formed by transamination of alanine, aspartate and glutamate?** 3 marks

Pyruvate, oxaloacetate and α-ketoglutarate.

– ANALYZE **A patient's report shows ALT 400 U/L and AST 300 U/L (both far above normal). Which organ is most likely damaged, and why?** 4 marks

The liver. Both enzymes are released by damaged cells, and ALT is mainly a liver enzyme; ALT higher than AST is the typical pattern of acute liver-cell injury, as in viral hepatitis. Heart or muscle injury raises AST more than ALT.

BEYOND THE SYLLABUS

Aminotransferases are used industrially to make chiral amines for medicines; an engineered transaminase replaced a metal catalyst in the commercial synthesis of the diabetes drug sitagliptin. Amino acid oxidases are used in biosensors. The pattern of serum enzymes is a basic tool of clinical biochemistry, the subject of an elective in many biotechnology programmes. Search "biocatalytic transamination sitagliptin". Not for the exam.

6.6 Urea cycle

The urea cycle is how the liver turns toxic ammonia into **urea**, a neutral, highly water-soluble molecule that the kidneys excrete. Each urea carries two nitrogen atoms: one from free NH_4^+ and one from aspartate. Hans Krebs and Kurt Henseleit described it in 1932, the first metabolic cycle discovered. Humans and other land mammals are **ureotelic** (they excrete nitrogen mainly as urea); most bony fish are **ammonotelic** and birds and reptiles are **uricotelic** (uric acid).

You need: 6.5 Transamination and deamination, which delivers the NH_4^+ and the aspartate.

The five reactions

The cycle runs in two compartments of the liver cell: the first two steps in the mitochondrial matrix, the last three in the cytosol.

1. **Carbamoyl phosphate synthetase I (CPS-I)**, mitochondria: $NH_4^+ + HCO_3^- + 2$ ATP → carbamoyl phosphate + 2 ADP + Pi. This is the committed, rate-limiting step. CPS-I is absolutely dependent on its allosteric activator **N-acetylglutamate (NAG)**.
2. **Ornithine transcarbamoylase (OTC)**, mitochondria: carbamoyl phosphate + ornithine → **citrulline** + Pi. Citrulline leaves the mitochondrion in exchange for ornithine.

3. **Argininosuccinate synthetase**, cytosol: citrulline + **aspartate** + ATP → argininosuccinate + AMP + PPi. The second nitrogen enters here. Splitting ATP to AMP costs two high-energy bonds.
4. **Argininosuccinate lyase**, cytosol: argininosuccinate → **arginine** + **fumarate**.
5. **Arginase**, cytosol: arginine + H_2O → **urea** + ornithine. Ornithine goes back into the mitochondrion to start the next turn. Arginase is found in large amounts only in the liver, which is why only the liver makes much urea.

Overall:

```
NH4+ + HCO3− + aspartate + 3 ATP + H2O
      → urea + fumarate + 2 ADP + 2 Pi + AMP + PPi
```

Three ATP are used, but four high-energy phosphate bonds are spent (two at CPS-I, two at argininosuccinate synthetase because PPi is hydrolysed).

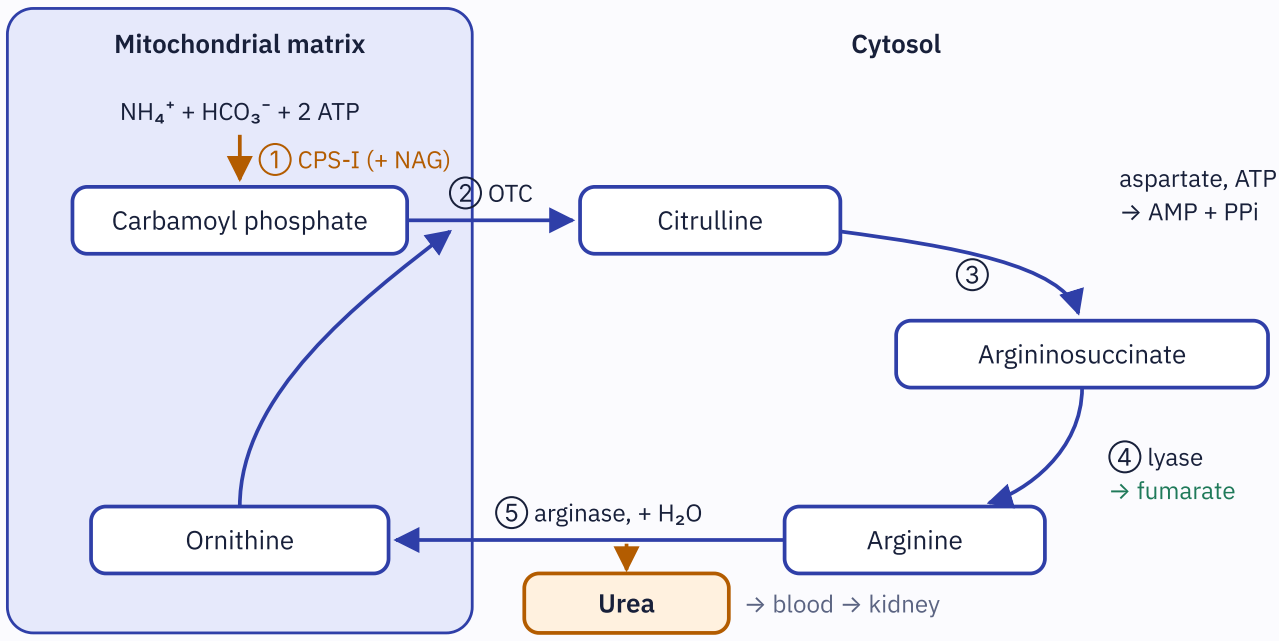


Figure 6.6.1 The urea cycle. Steps ① and ② are in the mitochondrion, ③ to ⑤ in the cytosol. One nitrogen enters as NH_4^+ at ①, the other from aspartate at ③; ornithine is regenerated and the carbon of aspartate leaves as fumarate.

Links to the TCA cycle and regulation

The fumarate made at step ④ is hydrated to malate and oxidised to oxaloacetate in the cytosol (giving NADH); oxaloacetate is transaminated back to aspartate, which feeds step ③ again. This link between the two cycles is called the **aspartate–argininosuccinate shunt** or the "Krebs

bicycle". The NADH it produces (worth about 2.5 ATP in the electron transport chain) pays back part of the cycle's cost.

Regulation works on two time scales:

- **Short term:** NAG activates CPS-I. NAG is made from acetyl-CoA and glutamate by NAG synthase, which arginine activates. When amino acid breakdown rises, glutamate and arginine rise, NAG rises and the cycle speeds up.
- **Long term:** a high-protein diet or starvation (when muscle protein is used for fuel) increases the amounts of all five enzymes.

Hyperammonaemia and urea cycle disorders

If the liver fails (cirrhosis, severe hepatitis) or a urea cycle enzyme is missing, blood ammonia rises. Ammonia is especially toxic to the brain: one proposed reason is that converting excess ammonia to glutamine drains α-ketoglutarate and glutamate from brain cells and makes astrocytes swell. Symptoms are vomiting, lethargy, tremor, slurred speech and, at high levels, coma.

A deficiency of each of the cycle enzymes is known. **OTC deficiency** is the most common and is X-linked; the others are autosomal recessive. In OTC deficiency, carbamoyl phosphate leaks into the cytosol and is used for pyrimidine synthesis, so **orotic acid** appears in urine (see Chapter 6.7). Treatment is a low-protein diet with essential amino acids, and drugs such as sodium benzoate and sodium phenylbutyrate, which combine with glycine and glutamine respectively and carry nitrogen out in urine, bypassing the cycle.

WORKED EXAMPLE

Question (6 marks). An adult excretes 30 g of urea in 24 hours. Calculate (a) the nitrogen excreted as urea and (b) the amount of protein this nitrogen represents. Urea is $CO(NH_2)_2$, molar mass 60 g/mol; protein is about 16% nitrogen.

1. Each urea has 2 N atoms: 2 × 14 = 28 g N per 60 g urea.
2. (a) Nitrogen = 30 × 28/60 = **14 g N per day**.
3. Protein is 16% N, so 1 g N comes from 100/16 = 6.25 g protein.
4. (b) Protein = 14 × 6.25 = **87.5 g protein per day**.
5. Check: an adult eating about 85–90 g protein a day and in nitrogen balance would excrete about this much urea N (urea is the major, but not the only, nitrogenous waste; the rest is ammonium, creatinine and uric acid).

EXAM TRAPS

- CPS-I (mitochondrial, uses NH_4^+, needs NAG, urea cycle) is not CPS-II (cytosolic, uses glutamine, no NAG, pyrimidine synthesis).
- Urea's two nitrogens come from NH_4^+ and aspartate. Its carbon comes from HCO_3^- (CO_2), not from ornithine.
- Three ATP are used but four high-energy bonds are spent.
- Ornithine and citrulline are amino acids that are not in proteins; arginine is made in the cycle but is semi-essential in children.
- Fumarate links the urea cycle to the TCA cycle; draw the link if the question says "relationship between".

CHECK YOURSELF

– REMEMBER **Name the two urea cycle enzymes in the mitochondrion.** 2 marks

Carbamoyl phosphate synthetase I and ornithine transcarbamoylase.

– REMEMBER **What is the allosteric activator of CPS-I?** 1 mark

N-acetylglutamate.

– UNDERSTAND **Explain the "Krebs bicycle".** 4 marks

Fumarate from argininosuccinate lyase becomes malate and oxaloacetate (giving NADH), and oxaloacetate is transaminated to aspartate, which re-enters the urea cycle. The two cycles are thus joined through fumarate and aspartate.

– UNDERSTAND **Why does orotic acid appear in urine in OTC deficiency?** 3 marks

Carbamoyl phosphate cannot be used to make citrulline, builds up, leaves the mitochondrion and enters the cytosolic pyrimidine pathway, producing excess orotate.

– APPLY **How many grams of nitrogen are in 45 g of urea?** 2 marks

45 × 28/60 = **21 g N**.

– ANALYZE **Why does a person on a very high-protein diet show higher urea cycle enzyme levels than a person on a normal diet?** 4 marks

Extra amino acids are not stored, so more are deaminated and more NH_4^+ must be disposed of. Rising glutamate and arginine raise NAG within hours; over days, the liver makes more of all five enzymes to match the higher nitrogen load.

BEYOND THE SYLLABUS

Urea cycle disorders are now treated with newer nitrogen scavengers (glycerol phenylbutyrate), liver transplantation, and in trials with gene therapy delivering the OTC gene to liver cells. Blood urea nitrogen (BUN) and creatinine are routine kidney function markers. Engineered probiotic bacteria that consume ammonia in the gut have been tested for hyperammonaemia. Search "urea cycle disorder gene therapy". Not for the exam.

6.7 Purine and pyrimidine biosynthesis and degradation

Every dividing cell needs nucleotides for DNA and RNA, and every cell needs ATP, GTP, NAD^+ and CoA, all of which contain a nucleotide. Cells make nucleotides in two ways: **de novo** (from small molecules: amino acids, CO_2, one-carbon units and ribose 5-phosphate) and by **salvage** (reusing free bases from broken-down nucleic acids). Purines and pyrimidines are built in opposite orders: a **purine** ring is assembled on the ribose, while a **pyrimidine** ring is made first and then attached to ribose. Degradation also differs: purines end as **uric acid**, which can crystallise (gout), while pyrimidines end as soluble products.

You need: base, nucleoside and nucleotide structures from **Unit 4, Purine and pyrimidine bases** and **Nucleosides and nucleotides**; the pentose phosphate pathway from **Unit 5**.

PRPP: the activated ribose

Both pathways and salvage use **5-phosphoribosyl-1-pyrophosphate (PRPP)**, made by PRPP synthetase: ribose 5-phosphate + ATP → PRPP + AMP. Ribose 5-phosphate comes from the pentose phosphate pathway.

Purine synthesis de novo

1. **Committed step.** Glutamine-PRPP amidotransferase replaces the pyrophosphate of PRPP with the amide nitrogen of glutamine, giving 5-phosphoribosylamine. The product's nitrogen becomes N-9 of the purine. This enzyme is inhibited by the end products AMP, GMP and IMP.
2. **Ring assembly on ribose.** In ten steps in all, glycine, a formyl group from N^{10}-formyl-tetrahydrofolate (THF, from folic acid), another glutamine nitrogen, CO_2 and aspartate are added, closing first the five-membered and then the six-membered ring.

3. The first complete purine nucleotide is **inosine monophosphate (IMP)**, whose base is hypoxanthine.
4. **IMP → AMP:** aspartate adds an amino group at C-6 (adenylosuccinate synthetase, uses **GTP**), then fumarate is removed (adenylosuccinate lyase).
5. **IMP → GMP:** IMP dehydrogenase oxidises IMP to XMP (NAD^+); GMP synthetase adds an amino group from glutamine (uses **ATP**).

The cross-use of GTP for AMP and ATP for GMP keeps the two in balance: plenty of one drives synthesis of the other.

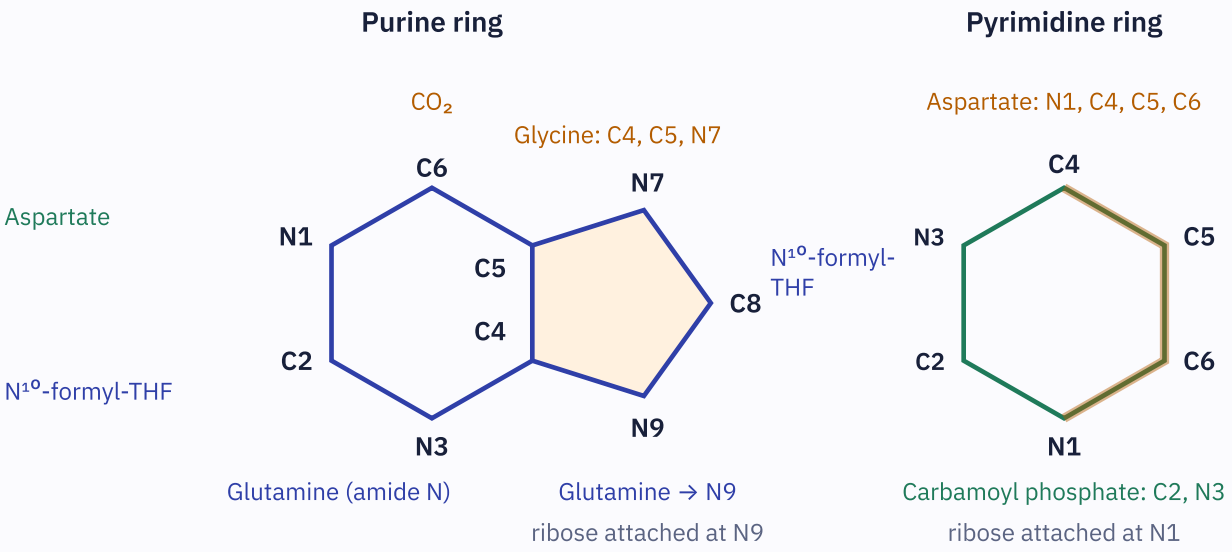


Figure 6.7.1 Where each ring atom comes from. Purine (left): glycine supplies the shaded C4, C5 and N7; two glutamines give N3 and N9; formyl-THF gives C2 and C8; aspartate gives N1; CO_2 gives C6. Pyrimidine (right): carbamoyl phosphate gives C2 and N3; aspartate gives the highlighted N1, C4, C5 and C6.

Pyrimidine synthesis de novo

1. **Carbamoyl phosphate synthetase II (CPS-II)**, cytosol: glutamine + HCO_3^- + 2 ATP → carbamoyl phosphate. Unlike CPS-I of the urea cycle, it uses glutamine and does not need NAG. In animals this is the regulated step (UTP inhibits, PRPP activates).
2. **Aspartate transcarbamoylase (ATCase)**: carbamoyl phosphate + aspartate → carbamoyl aspartate. In *E. coli* this is the regulated step and the textbook example of an allosteric enzyme: CTP inhibits, ATP activates.
3. **Dihydroorotase** closes the ring to dihydroorotate.
4. **Dihydroorotate dehydrogenase**, on the outer face of the inner mitochondrial membrane, oxidises it to **orotate**.

5. **Orotate phosphoribosyltransferase** attaches ribose phosphate from PRPP, giving OMP; **OMP decarboxylase** makes **UMP**.
6. UMP → UDP → UTP by kinases; **CTP synthetase** adds an amino group from glutamine to UTP, giving CTP.

In mammals the first three activities sit on one protein (called CAD) and the last two on another (UMP synthase), so intermediates pass straight from one active site to the next. A defect of UMP synthase causes **hereditary orotic aciduria** (Chapter 6.8).

Deoxyribonucleotides and thymidylate

Ribonucleotide reductase reduces the 2′-OH of ribonucleoside diphosphates (ADP, GDP, CDP, UDP) to give dNDPs, using electrons from NADPH through thioredoxin. **Thymidylate synthase** then methylates dUMP to dTMP, with N^5,N^{10}-methylene-THF as the one-carbon donor; this leaves dihydrofolate, which **dihydrofolate reductase (DHFR)** reduces back to THF. These enzymes are classic anticancer targets: **5-fluorouracil** inhibits thymidylate synthase, **methotrexate** inhibits DHFR and **hydroxyurea** inhibits ribonucleotide reductase.

Salvage and degradation

Salvage saves energy. Hypoxanthine-guanine phosphoribosyltransferase (**HGPRT**) makes IMP or GMP from the free base + PRPP; adenine phosphoribosyltransferase (APRT) does the same for adenine. Its deficiency causes Lesch–Nyhan syndrome (Chapter 6.8).

Purine degradation in humans:

1. AMP → IMP (AMP deaminase), or AMP → adenosine → inosine (**adenosine deaminase**, ADA). ADA deficiency kills developing lymphocytes and causes severe combined immunodeficiency (SCID).
2. Nucleosides lose their ribose to purine nucleoside phosphorylase, giving the free bases hypoxanthine and guanine.
3. Hypoxanthine → xanthine → **uric acid**, both steps by **xanthine oxidase**; guanine → xanthine by guanine deaminase.

Humans and other apes lack urate oxidase, so uric acid is the end product. It is poorly soluble; a high blood level (hyperuricaemia) can deposit sodium urate crystals in joints, which is **gout**. **Allopurinol**, a hypoxanthine analogue, inhibits xanthine oxidase and lowers uric acid.

Pyrimidine degradation opens the ring: cytosine and uracil give **β-alanine**, CO_2 and NH_4^+; thymine gives **β-aminoisobutyrate**. These are water-soluble, so pyrimidine breakdown causes no crystal disease.

WORKED EXAMPLE

Question (6 marks). A researcher grows liver cells with (a) [^{15}N]-glycine, (b) [^{14}C]-bicarbonate and (c) [^{15}N]-aspartate. Which ring atoms of newly made AMP and UMP will be labelled in each case?

1. (a) Glycine gives C4, C5 and N7 of the purine; only its nitrogen is ^{15}N, so **N7 of AMP** is labelled. Glycine is not used for pyrimidines: **UMP unlabelled**.
2. (b) CO_2 gives **C6 of the purine**. For UMP, the bicarbonate taken up by CPS-II becomes **C2**. The CO_2 released by OMP decarboxylase came from aspartate, not from bicarbonate, so no label is lost there.
3. (c) Aspartate gives **N1 of the purine ring** and, in AMP, also the **6-amino group** (from adenylosuccinate). In UMP, aspartate's nitrogen becomes **N1**.

EXAM TRAPS

- Purines: ring built on PRPP. Pyrimidines: ring first, then PRPP. A favourite "differentiate" question.
- CPS-II (pyrimidines, cytosol, glutamine) is not CPS-I (urea cycle, mitochondria, NH_4^+, NAG).
- The first purine formed is IMP, not AMP; the first pyrimidine nucleotide formed is OMP, then UMP.
- Uric acid is the end product of purines only. Pyrimidines give β-alanine and β-aminoisobutyrate.
- Ribonucleotide reductase acts on diphosphates (NDPs), not monophosphates.

CHECK YOURSELF

– REMEMBER **Name the committed step of purine synthesis and two of its inhibitors.** 2 marks

Glutamine-PRPP amidotransferase; inhibited by AMP, GMP and IMP.

– REMEMBER **Which enzyme does allopurinol inhibit?** 1 mark

Xanthine oxidase.

– UNDERSTAND **Why do methotrexate and 5-fluorouracil kill dividing cells faster than resting cells?** 4 marks

Dividing cells need large amounts of dTMP for DNA replication. 5-FU blocks thymidylate synthase directly; methotrexate blocks DHFR, so THF is not regenerated and thymidylate (and purine) synthesis stops. Resting cells make little DNA and are affected less.

– UNDERSTAND **How does the cell keep AMP and GMP in balance?** 3 marks

IMP → AMP uses GTP and IMP → GMP uses ATP; also AMP and GMP each inhibit the first enzyme of their own branch and, with IMP, the amidotransferase.

– APPLY **Which atom of UMP is labelled if cells are given [^{15}N]-amide glutamine?** 2 marks

N3, via carbamoyl phosphate made by CPS-II.

– ANALYZE **Compare the end products of purine and pyrimidine breakdown and explain why only one causes a crystal disease.** 4 marks

Purines are oxidised to uric acid, which humans cannot break down (no urate oxidase) and which is poorly soluble, so it can crystallise as sodium urate (gout, kidney stones). Pyrimidine rings are opened and reduced to β-amino acids, which are soluble and can be further metabolised.

BEYOND THE SYLLABUS

Nucleotide metabolism is a frontline of drug design: antivirals such as remdesivir and sofosbuvir are nucleotide analogues, mycophenolate (an IMP dehydrogenase inhibitor) suppresses lymphocytes after organ transplant, and ADA-SCID was among the first diseases treated by gene therapy. The newer urate-lowering drug febuxostat also inhibits xanthine oxidase. Search "nucleotide analogue antivirals mechanism". Not for the exam.

6.8 Inborn errors of metabolism

An **inborn error of metabolism** is an inherited disease in which one enzyme (or transporter) of a pathway is missing or faulty because of a gene mutation. The substrate before the block piles up, often spilling into abnormal side-pathways, and the product after the block runs short. The physician Archibald Garrod coined the term in 1908 from his study of alkaptonuria, an early clue to the "one gene, one enzyme" idea. Most are **autosomal**

recessive. Each is rare, but together they are an important cause of illness in newborns. The other universities meet the same diseases inside the pathway chapters.

Reading any inborn error

Every disorder can be explained in four lines, and examiners expect all four: **the block** (which enzyme), **what accumulates** (substrate and side products, often seen in urine), **what is deficient** (product), and **the treatment** (usually restricting the substrate in the diet or supplying the product).

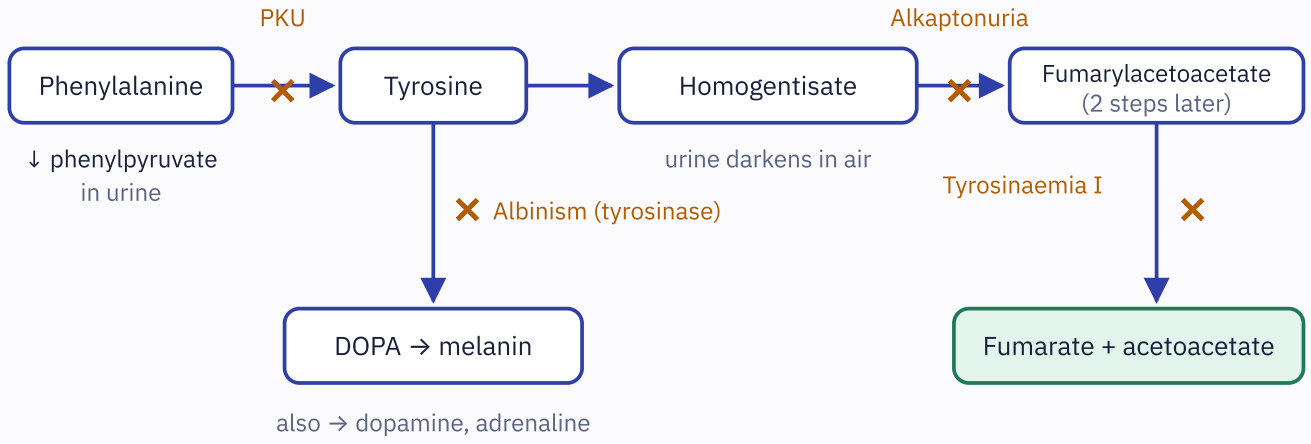


Figure 6.8.1 Four classic disorders on one pathway. Each ✕ is a missing enzyme. In PKU phenylalanine is diverted to phenylpyruvate; in alkaptonuria homogentisate builds up; in albinism melanin is not made. Tyrosine becomes an essential amino acid in PKU.

The classic disorders

DISORDER	ENZYME MISSING	ACCUMULATES OR IS LACKING	KEY POINT
Phenylketonuria (PKU)	Phenylalanine hydroxylase (or its cofactor tetrahydrobiopterin)	Phe, phenylpyruvate, phenyllactate, phenylacetate	Intellectual disability, mousy odour; low-Phe diet from birth
Alkaptonuria	Homogentisate oxidase	Homogentisic acid	Urine darkens on standing; dark connective tissue (ochronosis), arthritis
Albinism (oculocutaneous)	Tyrosinase	No melanin	Pale skin and hair, light sensitivity
Maple syrup urine disease	Branched-chain α-keto acid dehydrogenase	Leu, Ile, Val and their keto acids	Sweet-smelling urine; brain damage if

DISORDER	ENZYME MISSING	ACCUMULATES OR IS LACKING	KEY POINT
			untreated
Galactosaemia	Galactose 1-phosphate uridylyltransferase	Galactose 1-phosphate	Liver damage, cataract; remove milk sugar
Urea cycle defects (OTC most common)	A urea cycle enzyme (6.6)	NH_3; orotic acid in OTC deficiency	Hyperammonaemia; low-protein diet, nitrogen scavengers
Lesch–Nyhan syndrome	HGPRT (6.7), X-linked	Uric acid (salvage fails, PRPP rises)	Gout, self-injury, neurological signs
Hereditary orotic aciduria	UMP synthase (6.7)	Orotic acid; low pyrimidines	Anaemia, poor growth; treated with uridine
MCAD deficiency	Medium-chain acyl-CoA dehydrogenase (6.1)	Medium-chain acyl-carnitines	Low blood sugar without ketones in fasting; avoid fasting

WORKED EXAMPLE

Question (8 marks). A six-month-old infant has delayed development, fair skin and a musty smell in the urine. Plasma phenylalanine is very high and tyrosine is low. Identify the disorder and explain the biochemistry, diagnosis and treatment.

1. **Disorder:** phenylketonuria, a deficiency of phenylalanine hydroxylase (autosomal recessive).
2. **Block:** Phe + O_2 + tetrahydrobiopterin → Tyr cannot occur. Phe accumulates and is transaminated to **phenylpyruvate** (a "phenylketone", hence the name), then to phenyllactate and phenylacetate, which give the musty odour.
3. **Deficiency:** less tyrosine, so less melanin (fair skin) and possibly less dopamine and noradrenaline. High Phe also competes with other large neutral amino acids for transport into the brain; the damage is to the developing brain.
4. **Diagnosis:** newborn screening of a heel-prick blood spot, once by the Guthrie bacterial inhibition assay and now mostly by tandem mass spectrometry, confirmed by plasma Phe.
5. **Treatment:** a low-phenylalanine diet started in the first weeks of life, with tyrosine supplied (it is now essential), and avoidance of the sweetener aspartame, which contains phenylalanine.

EXAM TRAPS

- In PKU tyrosine becomes essential; do not write that tyrosine accumulates.
- Alkaptonuria is not "black urine at passing": the urine darkens on standing in air as homogentisate oxidises.
- Lesch–Nyhan and OTC deficiency are X-linked; most other inborn errors are autosomal recessive.

CHECK YOURSELF

– REMEMBER **Who coined "inborn errors of metabolism", and from which disease?** 1 mark

Archibald Garrod, from alkaptonuria.

– REMEMBER **Which enzyme is missing in maple syrup urine disease?** 1 mark

Branched-chain α-keto acid dehydrogenase complex.

– UNDERSTAND **Why do PKU patients often have fair skin?** 2 marks

Less tyrosine is made, so less melanin; high phenylalanine also inhibits tyrosinase.

– APPLY **A child has high blood ammonia and orotic acid in urine. Which enzyme is likely deficient?** 2 marks

Ornithine transcarbamoylase: carbamoyl phosphate builds up and enters pyrimidine synthesis.

– ANALYZE **Why does MCAD deficiency cause low blood glucose with low ketones during fasting?** 4 marks

Fatty acids cannot be fully β-oxidised, so the liver lacks acetyl-CoA for ketogenesis and lacks the ATP and NADH that drive gluconeogenesis. Both ketone bodies and glucose fall together.

BEYOND THE SYLLABUS

Expanded newborn screening by tandem mass spectrometry tests one blood spot for dozens of disorders, and some Indian states and hospitals now run such programmes. Enzyme replacement therapy treats lysosomal storage disorders such as Gaucher disease, and an enzyme (pegvaliase) that degrades phenylalanine is approved for adult PKU in some countries. Search "expanded newborn screening India". Not for the exam.

6.9 Overview of hormones and metabolic regulation

Each pathway in this unit and in Unit 5 has a pair: glycolysis and gluconeogenesis, fatty acid synthesis and β-oxidation, glycogen synthesis and breakdown. If both members of a pair ran at once, the cell would waste ATP in a **futile cycle**. Hormones prevent this by switching the whole body between two states: the **fed state** (store fuel) under **insulin**, and the **fasting or stress state** (release fuel) under **glucagon**, **adrenaline** (epinephrine) and **cortisol**.

You need: the regulated enzymes of each chapter in this unit, and **Unit 5, Glycolysis: steps, energetics and regulation**.

Four ways a cell controls a pathway

1. **Allosteric control** (seconds): a metabolite binds away from the active site. Examples: citrate activates acetyl-CoA carboxylase (6.2); malonyl-CoA inhibits CPT-I (6.1); N-acetylglutamate activates CPS-I (6.6).
2. **Covalent modification** (seconds to minutes): mostly phosphorylation by kinases and removal by phosphatases, set by hormones. Phosphorylation inactivates acetyl-CoA carboxylase and HMG-CoA reductase but activates glycogen phosphorylase and hormone-sensitive lipase.
3. **Enzyme amount** (hours to days): hormones change gene transcription. A high-protein diet raises urea cycle enzymes (6.6); fasting raises mitochondrial HMG-CoA synthase (6.3); SREBP raises HMG-CoA reductase (6.4).
4. **Compartmentation**: opposing pathways sit in different places, such as fatty acid synthesis in the cytosol and β-oxidation in mitochondria.

How hormones reach the enzymes

Hormones are grouped by chemistry, which decides where their receptor is:

- **Peptide and protein hormones** (insulin, glucagon) and **catecholamines** (adrenaline) are water-soluble. They bind receptors on the cell surface and act through second messengers within minutes.
- **Steroid hormones** (cortisol) and **thyroid hormones** are lipid-soluble and enter the cell. They bind intracellular (nuclear) receptors that act as transcription factors, so they act over hours by changing enzyme amounts.

Glucagon (on liver) and **adrenaline** (on liver, muscle and adipose tissue, through β-adrenergic receptors) use the same cascade: receptor → G-protein (Gs) → adenylyl cyclase → **cAMP** → **protein kinase A (PKA)** → phosphorylation of target enzymes. Each enzyme in the cascade activates many copies of the next, so a tiny amount of hormone produces a large response; this is **amplification**.

Insulin binds a **receptor tyrosine kinase**. The receptor phosphorylates itself and insulin receptor substrate (IRS) proteins, which switch on the PI3-kinase–Akt pathway. The results include moving **GLUT4** glucose transporters to the membrane of muscle and fat cells, activating phosphatases that dephosphorylate enzymes, and lowering cAMP by activating a phosphodiesterase. So insulin largely reverses the phosphorylations made by PKA.

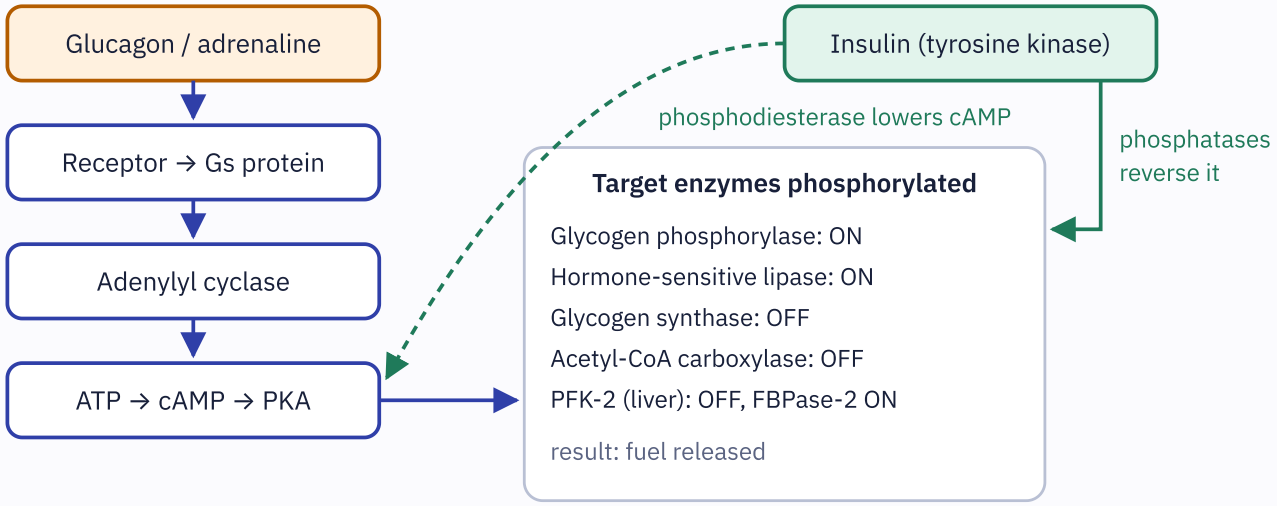


Figure 6.9.1 The cAMP cascade used by glucagon and adrenaline (left) and how insulin opposes it (green). Notice that one kinase, PKA, turns breakdown enzymes on and synthesis enzymes off at the same time, so the pairs never run together.

The hormones and what they do

HORMONE	SIGNAL FOR	MAIN METABOLIC EFFECTS
Insulin (β-cells of pancreas)	High blood glucose, fed state	Glucose uptake into muscle and fat; glycogen, fat and protein synthesis; inhibits gluconeogenesis, lipolysis and ketogenesis
Glucagon (α-cells)	Low blood glucose	Liver only: glycogen breakdown, gluconeogenesis, β-oxidation and ketogenesis; inhibits glycolysis and fat synthesis
Adrenaline (adrenal medulla)	Stress, exercise	Glycogen breakdown in liver and muscle; lipolysis in adipose tissue
Cortisol (adrenal cortex)	Long-term stress, prolonged fasting	Muscle protein breakdown; more gluconeogenic enzymes in liver

HORMONE	SIGNAL FOR	MAIN METABOLIC EFFECTS
Thyroid hormones (T_3, T_4)	Long-term metabolic rate	Raise basal metabolic rate and oxygen use in most tissues

Muscle has no glucagon receptors, so glucagon cannot break down muscle glycogen; only adrenaline can, and muscle keeps that glucose for itself. Inside every cell, **AMP-activated protein kinase (AMPK)** acts as a fuel gauge: when AMP rises it switches off fat and cholesterol synthesis and switches on fat oxidation.

WORKED EXAMPLE

Question (8 marks). A student skips dinner and breakfast and writes an exam 16 hours after the last meal. Predict the hormone levels and the state (on or off) of each pathway in the liver, with the enzyme that sets it.

1. **Hormones:** insulin low, glucagon high (cortisol slightly up). Liver cAMP and PKA activity are high.
2. **Glycogen:** liver glycogen is largely used up by now; phosphorylase was on, synthase off.
3. **Gluconeogenesis: ON.** Low fructose 2,6-bisphosphate releases fructose 1,6-bisphosphatase and slows PFK-1 (Unit 5). Alanine from muscle (6.5) and glycerol from adipose tissue are substrates.
4. **Fatty acid synthesis: OFF.** ACC is phosphorylated, so malonyl-CoA falls (6.2).
5. **β-Oxidation: ON.** With little malonyl-CoA, CPT-I is free; hormone-sensitive lipase in adipose tissue supplies fatty acids (6.1).
6. **Ketogenesis: ON.** Oxaloacetate is used for gluconeogenesis, and HMG-CoA synthase is induced (6.3).
7. **Urea cycle:** active, as amino acids from muscle protein are used for gluconeogenesis (6.6).

EXAM TRAPS

- Glucagon does not act on muscle; adrenaline does.
- Phosphorylation is not always activation: it turns phosphorylase and hormone-sensitive lipase on but glycogen synthase and ACC off.
- Steroid and thyroid hormones act on nuclear receptors (gene expression), not through cAMP.
- Insulin's receptor is a tyrosine kinase, not a G-protein-coupled receptor.

CHECK YOURSELF

– REMEMBER **Name the second messenger of glucagon and the kinase it activates.** 2 marks

cAMP; protein kinase A.

– REMEMBER **List four levels of metabolic regulation.** 2 marks

Allosteric control, covalent modification, change in enzyme amount, compartmentation.

– UNDERSTAND **What is a futile cycle and how is it avoided?** 3 marks

Two opposing pathways running at once, with net hydrolysis of ATP and no useful product. Reciprocal regulation (one hormone signal turning one pathway on and its opposite off) prevents it.

– UNDERSTAND **Why does a lipid-soluble hormone act more slowly than glucagon?** 3 marks

It works by changing gene transcription through a nuclear receptor, so new enzyme protein must be made (hours). Glucagon acts by phosphorylating existing enzymes (minutes).

– APPLY **State the effect of insulin on ACC, CPT-I activity and HMG-CoA reductase.** 3 marks

ACC activated (dephosphorylated); CPT-I inhibited indirectly through higher malonyl-CoA; HMG-CoA reductase activated.

– ANALYZE **In untreated type 1 diabetes, blood glucose and ketone bodies are both high. Explain using hormone actions.** 4 marks

No insulin: muscle and fat cannot take up glucose (no GLUT4 movement), and glucagon is unopposed, so liver gluconeogenesis and glycogen breakdown continue. Unchecked lipolysis floods the liver with fatty acids, and with low malonyl-CoA and induced HMG-CoA synthase, ketogenesis runs fast.

BEYOND THE SYLLABUS

Newer diabetes and obesity drugs act on these signals: GLP-1 receptor agonists such as semaglutide raise insulin release and reduce appetite, and dual GLP-1/GIP agonists go further. Researchers now model whole-body regulation with genome-scale metabolic models and measure pathway activity directly with isotope-tracer "fluxomics". Search "hormonal regulation of fuel metabolism integration" or "metabolic flux analysis". Not for the exam.

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
β-Oxidation yield	ATP = (n/2)×10 + (n/2 − 1)×4 − 2, for an even, saturated C_n acid; palmitate = 106	6.1
CPT-I	Carnitine shuttle gate; inhibited by malonyl-CoA	6.1
Acetyl-CoA carboxylase	Acetyl-CoA → malonyl-CoA (biotin, ATP); committed step of fat synthesis	6.2
Palmitate synthesis	8 acetyl-CoA + 7 ATP + 14 NADPH → palmitate	6.2
Essential fatty acids	Linoleic (ω-6) and α-linolenic (ω-3): bonds beyond Δ^9	6.2
Ketone bodies	Acetoacetate, β-hydroxybutyrate, acetone; via mitochondrial HMG-CoA synthase	6.3
HMG-CoA reductase	HMG-CoA → mevalonate (2 NADPH); rate-limiting for cholesterol; statin target	6.4
Transamination	Amino acid + α-ketoglutarate ⇌ keto acid + glutamate (PLP)	6.5
Glutamate dehydrogenase	Glutamate → α-ketoglutarate + NH_4^+ (NAD^+ or $NADP^+$)	6.5
Urea cycle	NH_4^+ + HCO_3^- + aspartate + 3 ATP → urea + fumarate; 4 high-energy bonds	6.6
Urea nitrogen	g N = g urea × 28/60; protein ≈ g N × 6.25	6.6
PRPP	Activated ribose for purine, pyrimidine and salvage synthesis	6.7
Xanthine oxidase	Hypoxanthine → xanthine → uric acid; allopurinol target	6.7
Inborn error	Inherited single-enzyme defect: block, build-up, deficiency, treatment	6.8
cAMP cascade	Glucagon/adrenaline → Gs → adenylyl cyclase → cAMP → PKA	6.9

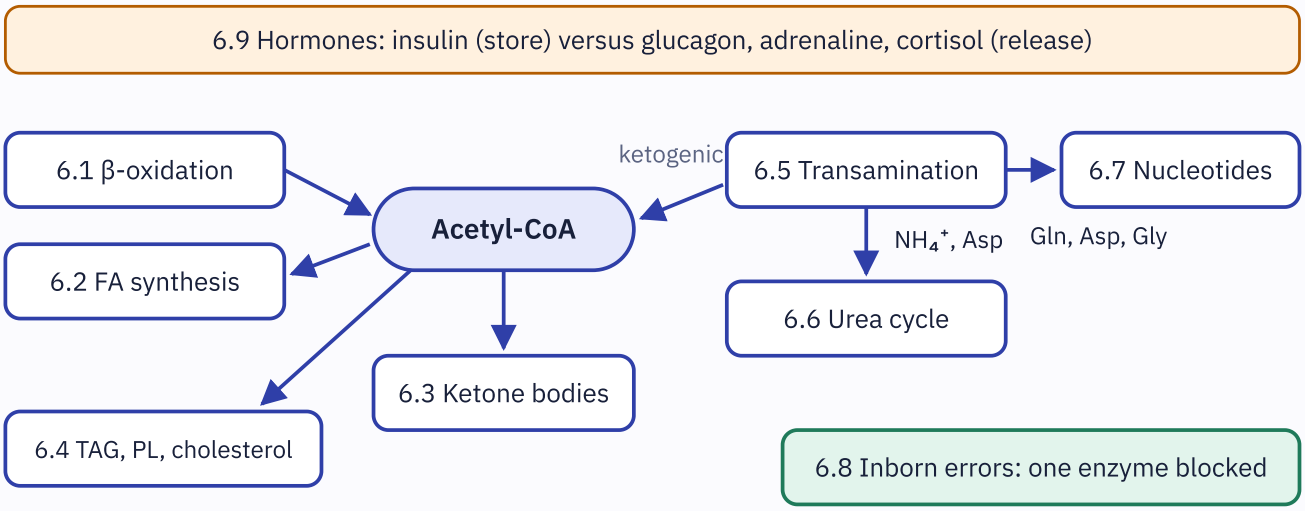


Figure 6.S.1 How the chapters connect. Lipid pathways meet at acetyl-CoA; amino acid nitrogen flows through glutamate to the urea cycle and, as glutamine, aspartate and glycine, into nucleotides; hormones (6.9) set the direction of every pathway, and inborn errors (6.8) are blocks on any of them.

1. Fatty acids are burned two carbons at a time in mitochondria (β-oxidation) and built two carbons at a time in the cytosol from malonyl-CoA (fatty acid synthase); malonyl-CoA blocks CPT-I so both never run together.
2. Palmitate gives 106 ATP (129 with older P/O values); always state your P/O ratios.
3. Mammals desaturate only up to Δ^9, so linoleic and α-linolenic acids are essential.
4. In fasting the liver turns surplus acetyl-CoA into ketone bodies, which it exports because it lacks the CoA transferase to use them.
5. Phosphatidic acid is the branch point for TAG and phospholipids; HMG-CoA reductase is the control point for cholesterol.
6. Amino groups are collected on glutamate by PLP-dependent transamination and released as NH_4^+ by glutamate dehydrogenase.
7. The urea cycle uses NH_4^+ and aspartate to make urea, spending four high-energy bonds, and is switched on by N-acetylglutamate.
8. Purine rings are built on PRPP and end as uric acid; pyrimidine rings are built first, then attached to PRPP, and end as soluble β-amino acids.
9. An inborn error is one missing enzyme: describe the block, the build-up, the deficiency and the treatment.
10. Insulin and the cAMP hormones (glucagon, adrenaline) set every pathway's direction through phosphorylation, allosteric effectors and enzyme amount.

Exam question bank

Remember and understand

▼ 1. Describe the β-oxidation of a saturated fatty acid, including activation and transport into mitochondria. `12 marks`

Outline: activation by acyl-CoA synthetase (ATP → AMP + PPi); carnitine shuttle (CPT-I, translocase, CPT-II); the four steps with enzymes and cofactors; diagram of the spiral; products per round; regulation by malonyl-CoA; mention of odd-chain and unsaturated fatty acids.

▼ 2. Explain the biosynthesis of palmitic acid. Add a note on elongation and desaturation. `12 marks`

Outline: citrate shuttle and NADPH sources; ACC reaction and regulation; FAS domains and ACP; the four-step cycle; overall equation; thioesterase; ER elongases; desaturases with cytochrome b_5; essential fatty acids.

▼ 3. What are ketone bodies? Describe their synthesis and utilisation, and explain ketosis in diabetes. `10 marks`

Outline: three ketone bodies; ketogenesis steps (thiolase, HMG-CoA synthase, lyase, dehydrogenase); acetone formation; ketolysis with CoA transferase; why the liver cannot use them; DKA mechanism and signs; Rothera's test limitations.

▼ 4. Describe the urea cycle with its regulation and its link to the TCA cycle. `12 marks`

Outline: five reactions with compartments; sources of the two nitrogens and the carbon; energetics (3 ATP, 4 bonds); NAG and CPS-I; long-term enzyme induction; aspartate–argininosuccinate shunt; hyperammonaemia and one disorder.

▼ 5. Write notes on transamination, oxidative deamination and decarboxylation of amino acids. `10 marks`

Outline: aminotransferases, PLP, ping-pong mechanism, ALT and AST with clinical use; GDH reaction and allosteric control; transdeamination; decarboxylases and their amine products (histamine, GABA, serotonin, dopamine).

Apply

▼ 6. Calculate the net ATP from complete oxidation of (a) stearic acid and (b) oleic acid. State your assumptions. `8 marks`

Outline: stearate 8 rounds, 9 acetyl-CoA → 12 + 20 + 90 − 2 = 120; oleate one $FADH_2$ fewer → 118.5 (with P/O 2.5/1.5). Older values: 146 and 144. Credit is for showing rounds, products and

activation cost.

▼ 7. Trace the fate of the amino group and carbon skeleton of alanine from muscle to urea and glucose in the liver. 10 marks

Outline: muscle transamination (pyruvate + glutamate → alanine); glucose–alanine cycle; liver ALT → pyruvate + glutamate; GDH → NH_4^+; urea cycle; pyruvate → glucose by gluconeogenesis; glucose returns to muscle. Spans 6.5, 6.6 and Unit 5.

▼ 8. Give the sources of each atom of the purine and pyrimidine rings and outline IMP to AMP and GMP. 10 marks

Outline: labelled ring diagrams; PRPP; committed step; IMP; AMP branch (aspartate, GTP); GMP branch (NAD^+, glutamine, ATP); reciprocal regulation; pyrimidine pathway through orotate to UMP and CTP.

▼ 9. A man excretes 24 g urea per day. Calculate the nitrogen excreted and the protein equivalent, and comment on his nitrogen balance if he eats 50 g protein daily. 8 marks

Outline: 24 × 28/60 = 11.2 g N; × 6.25 = 70 g protein. Intake 50 g protein = 8 g N, so output from urea alone exceeds intake: negative nitrogen balance (as in fasting, fever or injury), body protein is being lost.

Analyze and evaluate

▼ 10. Compare fatty acid synthesis and β-oxidation and explain how the cell prevents both running at once. 10 marks

Outline: comparison table (location, carrier, unit, electron carrier, stereochemistry, enzymes); reciprocal control: malonyl-CoA and CPT-I; ACC phosphorylation by AMPK/PKA; insulin versus glucagon. Spans 6.1, 6.2 and 6.9.

▼ 11. Explain the integration of carbohydrate, lipid and amino acid metabolism in the fed state and after 24 hours of fasting. 16 marks

Outline: hormone levels; fed state: glycogen and fat synthesis, protein synthesis; fasting: glycogenolysis then gluconeogenesis from alanine, lactate and glycerol; lipolysis and β-oxidation; ketogenesis; urea output; enzymes regulated at each step. Spans the whole unit and Unit 5.

▼ 12. Discuss any four inborn errors of amino acid or nucleotide metabolism with the enzyme defect, biochemical findings and treatment. 12 marks

Outline: for each (for example PKU, alkaptonuria, MSUD, Lesch–Nyhan or orotic aciduria): block, what accumulates, what is lacking, clinical signs, diagnosis, treatment; link each to its pathway

diagram.

▼ 13. Explain why statins, allopurinol and methotrexate are effective drugs, using the pathways they block. `10 marks`

Outline: statins inhibit HMG-CoA reductase → lower liver cholesterol → SREBP → more LDL receptors; allopurinol inhibits xanthine oxidase → less uric acid (gout); methotrexate inhibits DHFR → no THF → no dTMP or purines in dividing cells. Spans 6.4 and 6.7.

Glossary

Acyl carrier protein (ACP)	Protein domain whose phosphopantetheine arm holds the growing fatty acid in synthesis.
Acetyl-CoA carboxylase	Biotin enzyme that makes malonyl-CoA; committed step of fatty acid synthesis.
Aminotransferase	PLP enzyme that moves an amino group from an amino acid to a keto acid.
AMPK	AMP-activated protein kinase; energy sensor that stops synthesis and starts fuel oxidation.
β-Oxidation	Mitochondrial pathway that removes two-carbon acetyl-CoA units from fatty acyl-CoA.
Carnitine shuttle	CPT-I, translocase and CPT-II system that carries long-chain fatty acids into mitochondria.
Carbamoyl phosphate synthetase	CPS-I (mitochondria, NH_4^+, urea cycle) or CPS-II (cytosol, glutamine, pyrimidines).
Desaturase	ER enzyme that introduces a cis double bond using O_2 and NADH through cytochrome b_5.
Elongase	ER enzyme system that adds two carbons from malonyl-CoA to an acyl-CoA.
Essential fatty acid	Linoleic or α-linolenic acid; must come from the diet.
Futile cycle	Two opposing pathways running together, wasting ATP.
Glucogenic amino acid	One whose carbon skeleton can be made into glucose.
Glutamate dehydrogenase	Enzyme that releases NH_4^+ from glutamate, using NAD^+ or $NADP^+$.
HMG-CoA	β-Hydroxy-β-methylglutaryl-CoA; intermediate of both ketogenesis and cholesterol synthesis.

Inborn error of metabolism	Inherited deficiency of one enzyme or transporter of a pathway.
IMP	Inosine monophosphate, the first purine nucleotide made de novo.
Ketogenic amino acid	One whose skeleton gives acetyl-CoA or acetoacetyl-CoA; leucine and lysine are purely ketogenic.
Ketone bodies	Acetoacetate, β-hydroxybutyrate and acetone.
Ketoacidosis	Fall in blood pH caused by high ketone body levels, as in uncontrolled type 1 diabetes.
Malonyl-CoA	Three-carbon donor of fatty acid synthesis and inhibitor of CPT-I.
N-acetylglutamate	Obligate activator of carbamoyl phosphate synthetase I.
Phosphatidic acid	Diacylglycerol 3-phosphate; precursor of TAG and glycerophospholipids.
PLP	Pyridoxal phosphate, the vitamin B_6 coenzyme of transamination and decarboxylation.
PRPP	5-Phosphoribosyl-1-pyrophosphate, the activated ribose of nucleotide synthesis.
Salvage pathway	Reuse of free purine and pyrimidine bases to make nucleotides.
Transdeamination	Transamination to glutamate followed by oxidative deamination of glutamate.
Urea cycle	Liver pathway that converts NH_4^+ and aspartate nitrogen into urea.
Uric acid	End product of purine breakdown in humans; its crystals cause gout.

Sources and further reading

- D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, W. H. Freeman: chapters on fatty acid catabolism, amino acid oxidation and urea production, lipid biosynthesis, nucleotide biosynthesis and hormonal regulation (from memory; check chapter numbers in your edition).
- J. M. Berg, J. L. Tymoczko, G. J. Gatto and L. Stryer, *Biochemistry*, W. H. Freeman (from memory; check).
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- V. W. Rodwell and others, *Harper's Illustrated Biochemistry*, McGraw-Hill; clinical correlations and inborn errors (from memory; check).
- StatPearls, Physiology, Urea Cycle, NCBI Bookshelf (opened; confirms N-acetylglutamate as the obligate activator of CPS-I and OTC deficiency as the most common, X-linked urea cycle defect).
- StatPearls, Biochemistry, Ketogenesis, NCBI Bookshelf (opened; confirms that the liver lacks thiophorase and that urine dipsticks mainly measure acetoacetate).

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Built for the University free n easy YouTube channel from the universal syllabus of Biochemistry (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.