Biochemistry

Unit 6 · Lesson 1 · Fatty acids and ketone bodies

The notes for Lesson 1 of Unit 6: chapters 6.1 to 6.3 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 1 of 3 | 3 chapters | Chapters 6.1 to 6.3

About these notes

These are the notes for **Lesson 1 · Fatty acids and ketone bodies** of the University free n easy video lessons on Biochemistry Unit 6, Lipid, Amino Acid and Nucleotide Metabolism. They are chapters 6.1 to 6.3 of the unit book, unchanged, so a page here is the same page in the full book.

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*-Δ^2-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.

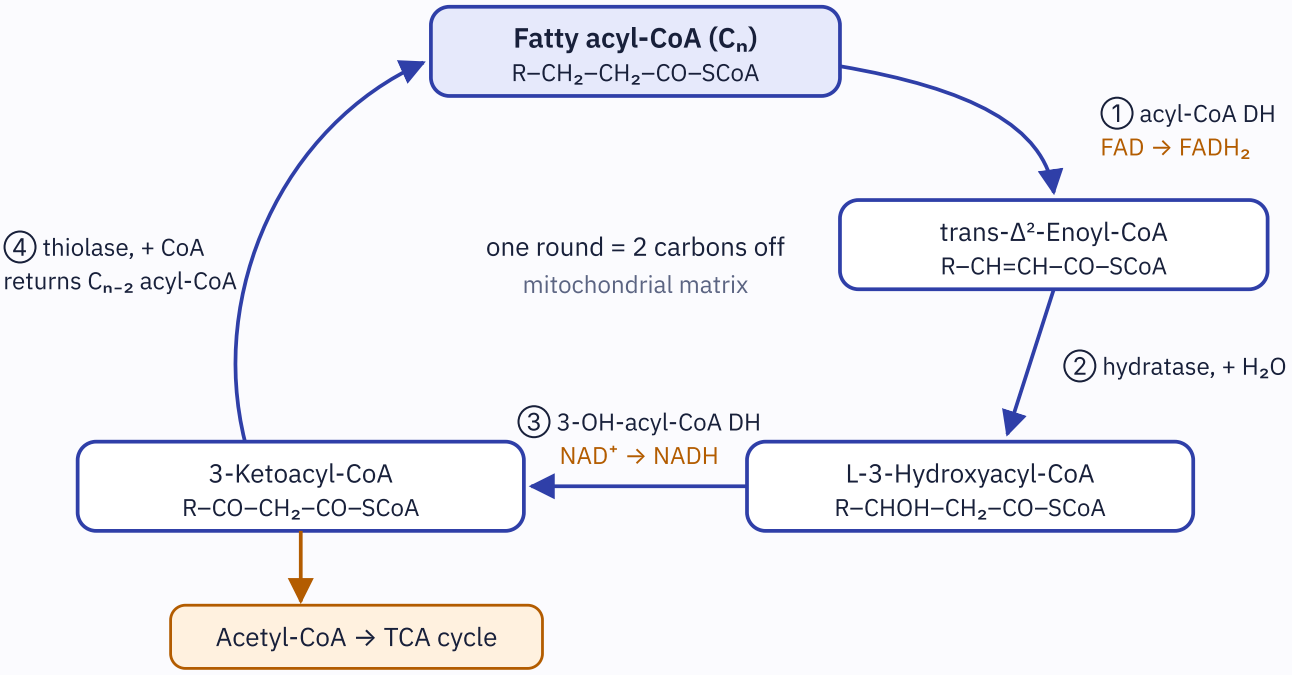


Figure 6.1.1 One round of β-oxidation. Reactions ① and ③ are oxidations that feed the electron transport chain; ④ releases acetyl-CoA and sends a chain two carbons shorter back to the top. Notice that the oxidations happen at C-3, the β-carbon.

Counting the ATP

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

```
rounds of β-oxidation   = n/2 − 1
acetyl-CoA produced     = n/2
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*-Δ³ bond to *trans*-Δ²; 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.

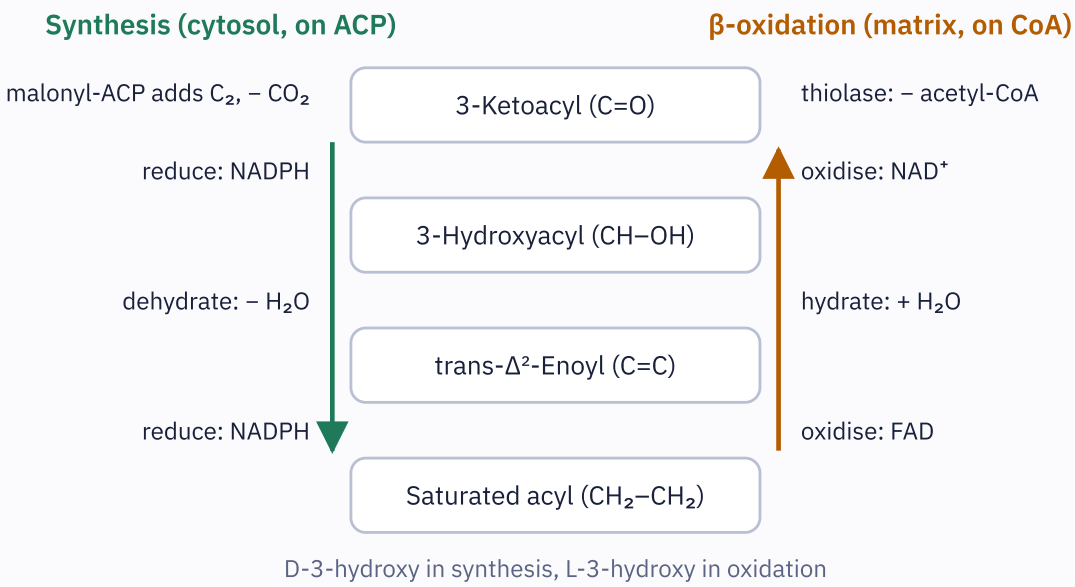


Figure 6.2.1 The same four chemical states, travelled in opposite directions. Read down the green side for synthesis and up the orange side for β-oxidation. The carriers (ACP or CoA), electron carriers (NADPH or FAD and NAD^+), compartment and hydroxy stereochemistry all differ.

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)

FEATURE	SYNTHESIS	B-OXIDATION
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.

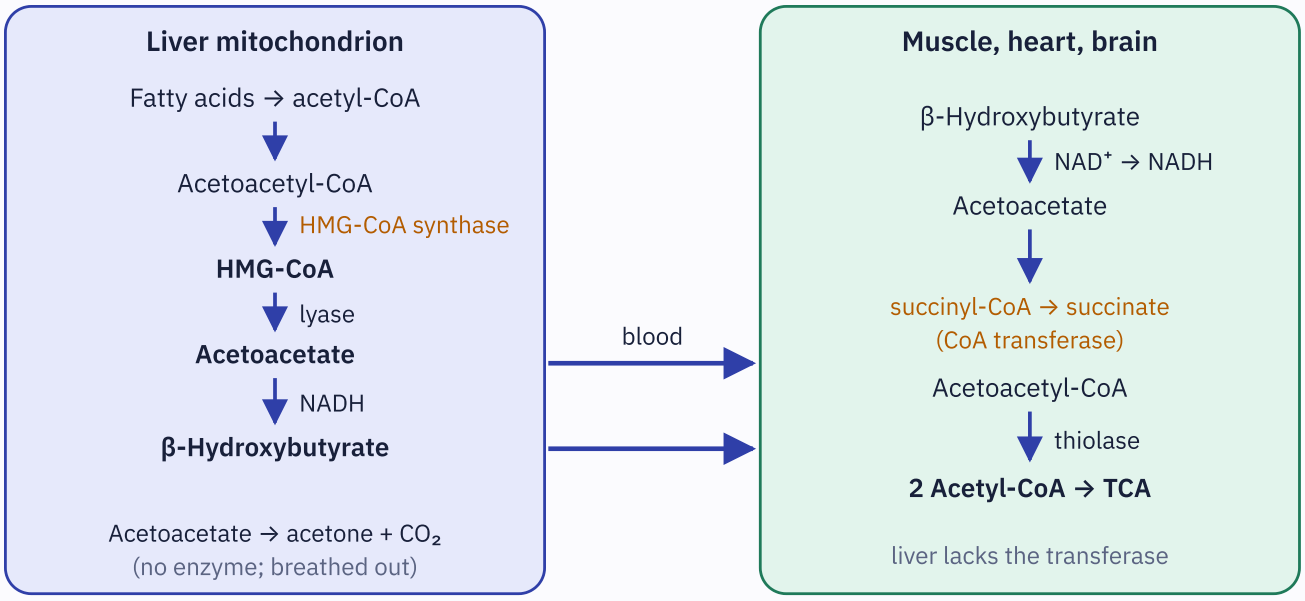


Figure 6.3.1 Ketogenesis in the liver (left) and ketolysis in other tissues (right). Notice the HMG-CoA synthase step that controls the rate, and the CoA transferase that only extrahepatic tissues have.

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.

Key terms of this lesson

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

Glossary (terms used in this lesson)

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.

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.

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.

HMG-CoA	β-Hydroxy-β-methylglutaryl-CoA; intermediate of both ketogenesis and cholesterol synthesis.
IMP	Inosine monophosphate, the first purine nucleotide made de novo.
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.
Uric acid	End product of purine breakdown in humans; its crystals cause gout.

The full unit book

Every chapter of Unit 6, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 6 · Lipid, Amino Acid and Nucleotide Metabolism (one file, works offline).

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).
- D. Voet and J. G. Voet, *Biochemistry*, Wiley (from memory; check).
- U. Satyanarayana and U. Chakrapani, *Biochemistry*, Elsevier India; widely prescribed in Indian universities, uses the older P/O values of 3 and 2 in ATP calculations (from memory; check).
- 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).

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

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