Biochemistry (with molecular biology)

MBBS Recap: high-yield notes for revision

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Revision notes for the MD/MS entrance (MEC PG): the facts an examiner asks in biochemistry and molecular biology, set out for quick recall after a wrong answer.

Enzymes and kinetics

Michaelis–Menten kinetics

- **Km is the substrate concentration at which velocity is half of Vmax.** Velocity reaches Vmax only at saturating substrate, never at Km.
- **Low Km means high apparent affinity** for substrate; high Km means low affinity. Km is a property of the enzyme and substrate, independent of enzyme amount; Vmax rises with more enzyme.
- Michaelis–Menten plot (velocity against substrate) is a **hyperbola**. The Lineweaver–Burk (double reciprocal) plot is a straight line: y-intercept = 1/Vmax, x-intercept = −1/Km, slope = Km/Vmax.
- Kcat (turnover number) = Vmax / total enzyme. Kcat/Km measures catalytic efficiency.

Inhibition patterns

Type	Binds	Km	Vmax	Overcome by more substrate?	Lineweaver–Burk
Competitive	Active site (free enzyme)	**Increases**	**Unchanged**	Yes	Lines cross on the y-axis
Noncompetitive	Allosteric site, either free enzyme or ES	Unchanged	**Decreases**	No	Lines cross on the x-axis
Uncompetitive	ES complex only	Decreases	Decreases	No	Parallel lines

- Competitive examples: **statins** (HMG-CoA reductase), methotrexate (DHFR), allopurinol (xanthine oxidase, also an irreversible "suicide" action through oxypurinol), malonate (succinate dehydrogenase), ethanol for methanol and ethylene glycol poisoning (alcohol dehydrogenase).
- Irreversible or suicide inhibitors: aspirin (cyclooxygenase), disulfiram, organophosphates (acetylcholinesterase), heavy metals binding SH groups.
- Allosteric enzymes give a **sigmoid** curve (cooperativity) and not a hyperbola; the regulated rate-limiting steps are usually allosteric or covalently modified (phosphorylation).

Rate-limiting enzymes to know

Pathway	Rate-limiting enzyme
Glycolysis	**Phosphofructokinase-1**
Gluconeogenesis	Fructose-1,6-bisphosphatase
TCA cycle	Isocitrate dehydrogenase
Glycogen synthesis	Glycogen synthase
Glycogenolysis	Glycogen phosphorylase
HMP shunt	**Glucose-6-phosphate dehydrogenase**
Urea cycle	**Carbamoyl phosphate synthetase I** (activator N-acetylglutamate)
Fatty acid synthesis	Acetyl-CoA carboxylase
Fatty acid oxidation	**Carnitine palmitoyltransferase I**
Cholesterol synthesis	**HMG-CoA reductase**
Ketogenesis	HMG-CoA synthase (mitochondrial)
Purine synthesis	PRPP amidotransferase
Pyrimidine synthesis (human)	CPS-II
Haem synthesis	ALA synthase
Catecholamine synthesis	Tyrosine hydroxylase
Serotonin synthesis	Tryptophan hydroxylase

- **HMG-CoA reductase** makes mevalonate and is the controlled step of cholesterol synthesis; HMG-CoA synthase (cytosolic isoform) makes HMG-CoA for cholesterol and the mitochondrial isoform for ketones; HMG-CoA lyase belongs to ketogenesis only. It is inhibited by statins, by cholesterol and by phosphorylation (AMPK), and induced by insulin.
- Insulin activates lipoprotein lipase; glucagon and catecholamines activate hormone-sensitive lipase.

Isoenzymes and diagnostic enzymes

- LDH is a tetramer of H and M chains. **LDH-1 (H4) predominates in heart and red cells**; LDH-5 (M4) in liver and skeletal muscle; LDH-3 in lung. In myocardial infarction or haemolysis the usual LDH-2 > LDH-1 pattern **flips (LDH-1 > LDH-2)**.
- CK-MB (heart), CK-MM (skeletal muscle), CK-BB (brain). Troponin I or T is the modern standard for infarction. **Exam answer for re-infarction: CK-MB**, because it returns to normal in 2–3 days while troponin stays raised for 7–14 days; **current practice** (Fourth Universal Definition of MI, ESC/ACC/AHA/WHF 2018) uses troponin for re-infarction too, a rise of more than 20% on repeat samples.
- Alkaline phosphatase: bone and cholestasis. Acid phosphatase (prostatic tartrate-labile): prostate cancer (PSA now preferred). Amylase and lipase: pancreatitis (lipase more specific). GGT: alcohol and cholestasis. 5′-nucleotidase: cholestasis; rises with ALP when the source is liver, not bone.
- Enzyme classes (EC numbers): 1 oxidoreductase, 2 transferase, 3 hydrolase, 4 lyase, 5 isomerase, 6 ligase (synthetase, needs ATP); **the exam list stops at six, but IUBMB added class 7, translocases** (enzymes that move ions

or molecules across membranes, such as the Na^+/K^+-ATPase), in 2018.

- Zymogens are activated by proteolysis (trypsinogen by enteropeptidase). Coenzymes carry groups and are often vitamins; cofactors are metal ions.
- **Ribozymes** are catalytic RNAs (peptidyl transferase of the ribosome, spliceosome).

Link with pharmacology

- A competitive antagonist shifts the dose–response curve to the right without lowering the maximum, the same pattern as a rise in Km with unchanged Vmax.

Plasma enzymes and enzyme use

- **Functional plasma enzymes** work in blood, most of them made by the liver (clotting factors, caeruloplasmin, pseudocholinesterase; lipoprotein lipase, made by adipose tissue and muscle and bound to capillary endothelium, is usually listed with them); **non-functional** ones leak from damaged cells (AST, ALT, CK, LDH, amylase) and their rise marks tissue injury. Pseudocholinesterase is low in liver disease, organophosphate poisoning and the atypical variant, giving prolonged suxamethonium apnoea.
- **Enzyme assays** measure activity from the rate of change of NADH at 340 nm (the coupled-assay principle); the reaction is run at zero-order (saturating substrate) so rate is proportional to enzyme amount.
- **Enzymes used as drugs:** streptokinase, alteplase and tenecteplase (thrombolysis), asparaginase (acute lymphoblastic leukaemia), rasburicase (urate oxidase, tumour lysis syndrome), pancreatic enzyme supplements, and enzyme replacement therapy in lysosomal diseases (imiglucerase in Gaucher, agalsidase in Fabry, alglucosidase alfa in Pompe).
- Enzymes by organelle: **cytosol** glycolysis, HMP shunt, fatty acid synthesis, part of the urea cycle; **mitochondria** TCA cycle, β-oxidation, ketogenesis, haem synthesis start, urea cycle start; **lysosome** acid hydrolases (Mannose-6-phosphate tags them; I-cell disease is the failure to add it); **peroxisome** very-long-chain fatty acid oxidation, catalase; **ER** cytochrome P450, glucose-6-phosphatase; **Golgi** glycosylation.
- **Temperature and pH:** rate rises with temperature up to an optimum (about 37 °C for human enzymes), then falls with denaturation; each enzyme has its pH optimum (pepsin 1.5–2, trypsin about 8). **Phosphorylation cascades and the clotting cascade amplify** a small signal because each activated enzyme activates many of the next.

Carbohydrate metabolism

Glycolysis

- Cytosolic; glucose to 2 pyruvate. **Rate-limiting: phosphofructokinase-1**, activated by AMP and fructose-2,6-bisphosphate (made by PFK-2 under insulin) and inhibited by ATP and citrate.
- Anaerobic glycolysis nets **2 ATP**; lactate dehydrogenase regenerates NAD^+ from NADH. Red cells have no mitochondria and so always make only 2 ATP; the Rapoport–Luebering shunt makes 2,3-BPG at the cost of those 2 ATP.
- Aerobic glycolysis: about 7 ATP (NADH via the malate–aspartate shuttle 2.5 each, $FADH_2$ 1.5, glycerol phosphate shuttle in muscle gives less); the older textbook figure is 8. Complete oxidation of glucose: about 30–32 ATP (older figure 36–38).
- Substrate-level phosphorylation: 1,3-BPG kinase (phosphoglycerate kinase) and pyruvate kinase.
- **Fluoride inhibits enolase**, so **blood for glucose is collected in sodium fluoride (with oxalate)** to stop cells consuming glucose. Iodoacetate inhibits glyceraldehyde-3-phosphate dehydrogenase; **arsenate replaces inorganic phosphate at that step, forms an unstable arseno-compound and so bypasses phosphoglycerate kinase: glycolysis continues but its net ATP is zero.** Arsenic poisoning: **acute**, garlic odour of the breath, rice-water diarrhoea, QT prolongation; **chronic** (groundwater arsenic, as in Nepal's Terai), raindrop pigmentation, palmoplantar hyperkeratosis, Mees lines, peripheral neuropathy, and cancers of skin, lung and bladder.
- **Hexokinase vs glucokinase:**

	Hexokinase	Glucokinase (hexokinase IV)
Site	Most tissues	Liver, pancreatic beta cells
Km	**Low (high affinity)**	**High (about 10 mmol/L)**
Vmax	Low	High
Inhibited by glucose-6-phosphate	**Yes**	**No** (regulated by the glucokinase regulatory protein, fructose-6-phosphate)
Role	Uptake at low glucose	Glucose sensor; works after meals

- MODY-2 is a glucokinase mutation (raised threshold of glucose sensing).
- **GLUT4** (insulin-dependent, muscle and adipose) vs GLUT2 (liver, beta cell, kidney; high Km, bidirectional), GLUT1 (red cells, brain barrier), GLUT3 (neurons), GLUT5 (fructose, small intestine), SGLT1 (intestinal glucose and galactose), SGLT2 (renal; target of gliflozins).
- Pyruvate dehydrogenase needs five cofactors (**thiamine pyrophosphate, lipoate, CoA, FAD, NAD^+**); it is inhibited by acetyl-CoA, NADH, ATP, and activated by insulin and ADP. Arsenic inhibits lipoate. PDH deficiency gives lactic acidosis; in thiamine deficiency pyruvate and lactate rise.

Gluconeogenesis

- Substrates: lactate (Cori cycle), glucogenic amino acids (alanine), glycerol; not even-chain fatty acids.
- Four bypass enzymes: pyruvate carboxylase (needs **biotin**; activated by acetyl-CoA), PEP carboxykinase, fructose-1,6-bisphosphatase, **glucose-6-phosphatase** (liver and kidney only; not in muscle).
- Ethanol metabolism raises NADH, diverting pyruvate to lactate and oxaloacetate to malate, so fasting alcoholics become hypoglycaemic.

HMP shunt (pentose phosphate pathway)

- **Rate-limiting: glucose-6-phosphate dehydrogenase**, inhibited by NADPH, induced by insulin. Oxidative phase gives NADPH; non-oxidative phase (transketolase needs thiamine; transaldolase) is reversible and gives ribose-5-phosphate.
- NADPH uses: fatty acid and steroid synthesis, glutathione reduction, respiratory burst (NADPH oxidase), nitric oxide synthase, cytochrome P450.
- G6PD deficiency (X-linked): haemolysis with oxidant drugs (primaquine, dapsone, sulfonamides, nitrofurantoin), fava beans, infection; **Heinz bodies, bite cells**. Chronic granulomatous disease = NADPH oxidase defect.

Glycogen storage diseases

Type	Enzyme	Key features
I von Gierke	**Glucose-6-phosphatase**	Fasting hypoglycaemia, hepatomegaly, **lactic acidosis, hyperuricaemia, hypertriglyceridaemia**, doll-like face; **no glucose rise with glucagon**
II Pompe	**Lysosomal acid α-1,4-glucosidase (acid maltase)**	Cardiomegaly, hypotonia, early death; no hypoglycaemia; alglucosidase alfa enzyme therapy
III Cori/ Forbes	Debranching enzyme	Limit dextrin; milder; gluconeogenesis intact, lactate not raised; glucagon response in fed state
IV Andersen	Branching enzyme	Abnormal amylopectin-like glycogen, cirrhosis
V **McArdle**	**Muscle glycogen phosphorylase**	Exercise cramps, myoglobinuria, **no lactate rise, "second wind"**
VI Hers	Liver phosphorylase	Mild hepatomegaly and hypoglycaemia
VII Tarui	Muscle PFK-1	Like McArdle, no second wind, worse after glucose load, haemolysis

- Muscle is involved in types II, V and VII, and in type III as well (type IIIa, the common form, affects liver and muscle, with raised CK and sometimes cardiomyopathy; IIIb liver only). Types I and VI are hepatic only. Von Gierke is the most common hepatic GSD; all are autosomal recessive except the X-linked liver phosphorylase kinase deficiency (type IXa) and Danon disease (LAMP2).

Fructose and galactose

- **Hereditary fructose intolerance (aldolase B):** fructose-1-phosphate accumulates, trapping phosphate and blocking glycogenolysis and gluconeogenesis, so **hypoglycaemia, vomiting and liver damage after fructose or sucrose**. Treatment: remove fructose, sucrose and sorbitol. Fructokinase deficiency (essential fructosuria) is benign.
- **Classic galactosaemia (galactose-1-phosphate uridyltransferase):** jaundice, hepatomegaly, *E. coli* sepsis, cataract, intellectual disability; **cataract is from galactitol made by aldose reductase** (same polyol mechanism as diabetic cataract, using sorbitol). Galactose-1-phosphate damages liver and kidney. Galactokinase deficiency: cataract only. Diagnose with urine reducing substances (Clinitest positive, glucose oxidase negative); treat by removing lactose.
- Sorbitol pathway: glucose to sorbitol (aldose reductase) to fructose (sorbitol dehydrogenase); lens, nerves and retina lack sorbitol dehydrogenase.

Lactose intolerance

- **Lactase at the brush border** splits lactose to glucose and galactose. Commonest form: **primary adult-type lactase non-persistence** (falls after weaning); **secondary** after gastroenteritis, coeliac disease or other mucosal injury; congenital deficiency is rare.
- Unabsorbed lactose is osmotically active and is fermented by colonic bacteria: bloating, cramps, watery diarrhoea after milk, **low stool pH (acid), stool reducing substances positive, raised breath hydrogen after a lactose load**. The **mucosal biopsy is normal** (in primary form), unlike coeliac disease.

Oxidative phosphorylation and energy

- Complex I NADH dehydrogenase (rotenone, amobarbital), II succinate dehydrogenase, III cytochrome b–c1 (antimycin A), IV cytochrome oxidase (**cyanide, CO, azide, H_2S**), V ATP synthase.
- **Oligomycin blocks the F0 proton channel of ATP synthase**: the proton gradient builds up and electron transport stops, so oxygen consumption falls. **Uncouplers** (2,4-dinitrophenol, thermogenin/UCP1, aspirin overdose) collapse the gradient and raise oxygen use and heat. Atractyloside blocks the adenine nucleotide translocase.
- P/O ratio: NADH 2.5, $FADH_2$ 1.5.
- **One turn of the TCA cycle (one acetyl-CoA) gives 3 NADH (isocitrate, α-ketoglutarate and malate dehydrogenases), 1 $FADH_2$ (succinate dehydrogenase), 1 GTP (succinyl-CoA synthetase) and 2 CO_2: about 10 ATP** (2.5 and 1.5 per NADH and $FADH_2$; older texts, using 3 and 2, give 12).

Fuel use by state

State	Muscle	Liver	Brain	Heart
Well fed	Glucose	Glucose	Glucose	**Fatty acids**
Fasting	Fatty acids	Fatty acids	Glucose	Fatty acids
Starvation	Fatty acids/ ketones	Amino acids/fatty acids	**Ketones**	Ketones

- The heart's main fuel at rest is **fatty acids (about 60–70% of ATP)**; it can use lactate in exercise. Red cells use only glucose.
- **Even-chain fatty acids cannot give net glucose**: acetyl-CoA's two carbons are lost as CO_2 in the TCA cycle, and the PDH reaction is irreversible. Only glycerol and the propionyl-CoA of odd-chain fatty acids contribute.
- Glucose–alanine (Cahill) cycle is under the amino acid section. Cori cycle: muscle lactate to liver glucose, costing 6 ATP in the liver for 2 made in muscle.

Glycated haemoglobin and other diabetes biochemistry

- **HbA1c** is haemoglobin whose β-chain N-terminal valine carries glucose non-enzymatically; it reflects **mean glucose of the last 8–12 weeks** (red cell life 120 days), weighted to the latest month. **Fructosamine** (glycated albumin and other proteins) reflects **2–3 weeks**; 1,5-anhydroglucitol reflects the last 1–2 weeks. Fructosamine is used when HbA1c is unreliable (haemoglobinopathy, haemolysis, recent transfusion, pregnancy changes).
- Chronic hyperglycaemia causes complications by the **polyol pathway** (sorbitol in lens, nerve, retina), non-enzymatic glycation (advanced glycation end-products), oxidative stress and protein kinase C activation.
- **Hyperosmolar hyperglycaemic state** (mainly older people with type 2 diabetes; glucose usually above 600 mg/dL, **effective osmolality above 320 mOsm/kg** in the classic criteria, little or no ketosis because the small amount of insulin present suppresses lipolysis). Current practice (ADA/EASD/JBDS/AACE/DTS consensus 2024): glucose ≥600 mg/dL with effective osmolality above 300 or total osmolality above 320 mOsm/kg, ketones below 3.0 mmol/L, pH 7.30 or more and bicarbonate 15 mmol/L or more. Diabetic ketoacidosis is insulin lack with ketones and acidosis (mostly type 1, but also type 2 and with SGLT2 inhibitors, where glucose may be near normal: euglycaemic DKA).
- **Investigating hypoglycaemia** (Whipple triad: low glucose, symptoms, relief with glucose): take the critical sample during the episode for glucose, insulin, C-peptide, 3-hydroxybutyrate and sulfonylurea screen. High insulin with high C-peptide:

insulinoma or sulfonylurea; high insulin with low C-peptide: exogenous insulin. Low insulin with low ketones in a child: fatty acid oxidation defect; low insulin with high ketones: ketotic hypoglycaemia or glycogen disorders. Alcohol causes fasting hypoglycaemia (raised NADH blocks gluconeogenesis).

- **Alcohol with barbiturates:** both are metabolised by microsomal enzymes and both depress the CNS, so acute alcohol delays barbiturate clearance and the effects add up; chronic drinkers induce P450 and clear barbiturates faster.
- **Pyruvate kinase deficiency** (autosomal recessive; the second commonest red cell enzyme defect after G6PD): red cells make less ATP, so cells lose shape and are destroyed in the spleen (chronic haemolytic anaemia, raised 2,3-BPG, rightward shift of the oxygen curve). Neonatal jaundice and splenomegaly are typical.
- **Glucuronic acid pathway:** glucose-6-phosphate to UDP-glucose to **UDP-glucuronate**, used to conjugate bilirubin, steroids and drugs and to make glycosaminoglycans. Humans lack gulonolactone oxidase and cannot make vitamin C. **Essential pentosuria** (L-xylulose reductase deficiency) is benign and gives a positive Benedict test with a negative glucose oxidase result.
- **Pyruvate dehydrogenase (PDH) deficiency:** the commonest form is the X-linked E1α defect. Pyruvate cannot enter the TCA cycle, so it is turned into **lactate and alanine, causing lactic acidosis**, with neurodegeneration (Leigh-like picture). Brain and other tissues depend on glucose, so carbohydrate worsens it. **A high-fat, low-carbohydrate ketogenic diet** gives acetyl-CoA from ketones and fatty acids without the enzyme, and a thiamine trial is given because the complex needs thiamine pyrophosphate.
- **Anaplerotic reactions** refill TCA intermediates: pyruvate carboxylase (pyruvate to oxaloacetate, biotin, activated by acetyl-CoA), glutamate to α-ketoglutarate, aspartate to oxaloacetate, propionyl-CoA to succinyl-CoA. **Anabolic roles of the TCA cycle:** citrate for fatty acid synthesis, oxaloacetate for gluconeogenesis, succinyl-CoA for haem, α-ketoglutarate for glutamate. TCA regulation: isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are inhibited by NADH and ATP and activated by ADP and calcium.
- **Mitochondrial myopathies and Luft disease:** Luft disease is a rare hypermetabolic myopathy with loosely coupled mitochondria (poor respiratory control: high oxygen use and heat, high thyroid-like metabolism with normal thyroid tests). **Cyanide** binds cytochrome oxidase (complex IV) and halts the electron transport chain; treatment is hydroxocobalamin (current first choice), or nitrites (which make methaemoglobin that binds cyanide) followed by **thiosulphate** (rhodanese converts cyanide to thiocyanate).

Lipid metabolism and lipoproteins

Fatty acid oxidation and synthesis

- Activation to acyl-CoA in the cytosol; long-chain entry to mitochondria by the carnitine shuttle. **Carnitine palmitoyltransferase I (outer membrane) is rate-limiting and is inhibited by malonyl-CoA**, which prevents synthesis and oxidation running together. CPT-II sits on the inner membrane. Carnitine deficiency and CPT-II deficiency cause muscle weakness and myoglobinuria with fasting or exercise.
- β-Oxidation: dehydrogenation (FAD), hydration, dehydrogenation (NAD^+), thiolysis. Palmitate (16C) gives net 106 ATP with the current P/O ratios (the older figure is 129).
- Very-long-chain fatty acids: peroxisomal β-oxidation. **Zellweger syndrome** (absent peroxisomes, PEX genes) and **X-linked adrenoleukodystrophy** (ABCD1) accumulate VLCFA.
- **Branched-chain phytanic acid (dairy, ruminant fat, chlorophyll) needs peroxisomal α-oxidation** (phytanoyl-CoA hydroxylase) because its β-methyl group blocks β-oxidation. **Refsum disease**: retinitis pigmentosa, anosmia, ataxia, ichthyosis, cardiomyopathy, peripheral neuropathy; treat by avoiding phytanic acid and plasmapheresis.
- ω-Oxidation is minor (microsomal). Odd-chain fatty acids yield propionyl-CoA, converted to succinyl-CoA by propionyl-CoA carboxylase (biotin) and methylmalonyl-CoA mutase (B12).
- **MCAD deficiency** (commonest oxidation defect): fasting hypoketotic hypoglycaemia, dicarboxylic aciduria, vomiting, lethargy. Without β-oxidation there is no acetyl-CoA for ketones, and no ATP and NADH to drive gluconeogenesis, so **glucose falls with absent ketones**. Treat with frequent feeds and avoidance of fasting. Jamaican vomiting sickness (hypoglycin) blocks acyl-CoA dehydrogenases.
- Synthesis: cytosolic, **acetyl-CoA carboxylase (biotin; activated by insulin and citrate, inhibited by AMP-kinase phosphorylation and palmitoyl-CoA)** then fatty acid synthase (needs NADPH, pantothenate). Palmitate is the end product. Citrate carries acetyl-CoA out of mitochondria.
- Ketone bodies: acetoacetate, 3-hydroxybutyrate (most abundant), acetone. Liver makes but cannot use them (no thiophorase, succinyl-CoA:acetoacetate CoA-transferase). **Red cells (no mitochondria) cannot use ketone bodies**; they depend on glucose throughout starvation. Nitroprusside tests detect acetoacetate (and acetone weakly), not 3-hydroxybutyrate; DKA may be underestimated by urine strips, so **current practice measures blood 3-hydroxybutyrate** (ADA consensus on hyperglycaemic crises 2024; JBDS UK).
- **Fuel of the heart:** at rest and well fed, the myocardium oxidises **fatty acids for about 60–70% of its ATP**; glucose, lactate (in exercise) and ketones (in prolonged fasting) supply the rest.
- Essential fatty acids: linoleic and α-linolenic acid. Eicosanoids from arachidonic acid.

Cholesterol

- Synthesised in the cytosol from acetyl-CoA; **HMG-CoA reductase** rate-limiting; excreted as bile acids (cholic and chenodeoxycholic acid, via 7α-hydroxylase, the rate-limiting step of bile acid synthesis) conjugated with glycine or taurine.
- ACAT in cells and **LCAT in plasma** esterify cholesterol.

Lipoproteins

Particle	Main apolipoproteins	Origin and role
Chylomicron	**B-48**, C-II, E	Gut; dietary triglyceride
VLDL	B-100, C-II, E	Liver; endogenous triglyceride
IDL	B-100, E	Remnant
LDL	**B-100**	Delivers cholesterol; the ligand for the LDL receptor
HDL	**A-I**, C-II, E	Reverse cholesterol transport

- **ApoC-II activates lipoprotein lipase** (LPL is induced by insulin and sits on capillary endothelium); apoC-III inhibits it. **ApoA-I activates LCAT.** ApoB-100 binds the LDL receptor; apoE binds remnant receptors and LRP.

- **ApoB-48 is made in the enterocyte by RNA editing (C to U) of the apoB mRNA** and is needed for chylomicron assembly; its absence (abetalipoproteinaemia: MTP defect; chylomicron retention disease) gives fat malabsorption, acanthocytes, fat-soluble vitamin deficiency and retinitis.
- **HDL protects by reverse cholesterol transport**: ABCA1 loads cell cholesterol on to apoA-I; LCAT esterifies it; CETP exchanges it for triglyceride in VLDL/LDL; the liver takes it up via SR-B1. Tangier disease is ABCA1 deficiency (orange tonsils, low HDL).
- Lp(a) is an LDL particle with apo(a), homologous to plasminogen; an independent thrombotic and atherogenic risk; PCSK9 inhibitors reduce it modestly.

Dyslipidaemias (Fredrickson)

Type	Defect	Lipids	Features
I	**Lipoprotein lipase or apoC-II**	Chylomicrons, TG very high	Pancreatitis, eruptive xanthomas, lipaemia retinalis
IIa	**LDL receptor** (also apoB-100, PCSK9 gain of function)	LDL high, TG normal	**Tendon (Achilles) xanthomas, corneal arcus, premature coronary disease**
IIb	Usually familial combined hyperlipidaemia (hepatic overproduction of apoB and VLDL; polygenic)	LDL and VLDL high	Premature CAD; the commonest familial dyslipidaemia
III (dysbetalipoproteinaemia)	ApoE2/E2 homozygosity (recessive, needs a second hit such as obesity, diabetes, hypothyroidism)	Chylomicron and VLDL remnants (IDL); broad β band	Palmar (striate) and tuberoeruptive xanthomas, premature CAD and peripheral vascular disease
IV	Overproduction of VLDL	TG high	Pancreatitis risk, diabetes, obesity
V	Raised VLDL and chylomicrons (often a genetic tendency with diabetes, alcohol, obesity)	TG very high	Pancreatitis, eruptive xanthomas, lipaemia retinalis

- Familial hypercholesterolaemia is autosomal (semi)dominant: LDL receptor (most), apoB-100 or PCSK9 gain of function. **Heterozygotes: classically 1 in 500** (current estimates about 1 in 250–300), total cholesterol about 300 mg/dL, LDL roughly 190–400 mg/dL, coronary disease in the 30s–50s; **homozygotes** (about 1 in 300,000–1,000,000): LDL usually above 500 mg/dL (total cholesterol 600–1,000), xanthomas and MI in childhood. Treat: high-intensity statin plus ezetimibe, PCSK9 inhibitors (evolocumab, alirocumab), inclisiran (siRNA), lomitapide and LDL apheresis in homozygotes.
- Statins: myopathy risk, raised transaminases; bempedoic acid is a newer ATP-citrate lyase inhibitor (cuts events, CLEAR Outcomes 2023). Ezetimibe blocks NPC1L1 (intestinal cholesterol uptake). Fibrates (PPAR-α) lower TG; niacin raises HDL but adds no outcome benefit to a statin (AIM-HIGH, HPS2-THRIVE), so it is no longer used for that. LDL goals in current practice (ESC/EAS 2019): below 55 mg/dL in very high risk, below 70 in high risk, below 100 in moderate risk.

Sphingolipidoses (lysosomal storage)

Disease	Enzyme	Features
Tay–Sachs	**Hexosaminidase A** (GM2)	Cherry-red spot, hyperacusis, neurodegeneration, **no hepatosplenomegaly**; Ashkenazi Jews
Sandhoff	Hexosaminidase A and B	Like Tay–Sachs plus organomegaly
Gaucher	**Glucocerebrosidase**	Commonest; hepatosplenomegaly, pancytopenia, bone crises, **crumpled tissue-paper macrophages**, Ashkenazi; imiglucerase, eliglustat
Niemann–Pick A/B	Sphingomyelinase	Foam cells, cherry-red spot, hepatosplenomegaly (type C is a different disease: NPC1/NPC2 cholesterol transport, vertical gaze palsy)
Fabry	α-Galactosidase A (X-linked)	Ceramide trihexoside, angiokeratomas, acroparaesthesia, renal and cardiac failure; agalsidase
Krabbe	Galactocerebrosidase	Globoid cells, demyelination
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide, demyelination
Farber	Ceramidase	Joint swelling, nodules

- Lysosomal diseases are mostly autosomal recessive; **Fabry and Hunter are X-linked**.
- Cherry-red spot at the macula: Tay–Sachs, Sandhoff, Niemann–Pick (also GM1 gangliosidosis, sialidosis); not Gaucher. Hepatosplenomegaly separates Niemann–Pick and Sandhoff from Tay–Sachs.

Membranes, lipid digestion and lipid chemistry

- **Fluid mosaic model:** a phospholipid bilayer with proteins; cholesterol, unsaturated fatty acids and shorter chains regulate fluidity (cholesterol stiffens above the transition temperature and prevents crystallisation below it). Membranes are asymmetric: phosphatidylserine faces the inside (its exposure marks apoptosis), sphingomyelin and phosphatidylcholine face out, and glycolipids and carbohydrates face the outside.
- **Transport:** simple diffusion, facilitated diffusion (channels, carriers), primary active transport (Na^+/K^+-ATPase moves 3 Na^+ out and 2 K^+ in for one ATP), secondary active transport (SGLT, Na^+-glucose). **Ionophores** carry ions through membranes (valinomycin for K^+, gramicidin as a channel). **Spur cell anaemia** is acanthocyte haemolysis from excess membrane cholesterol in severe liver disease; acanthocytes in abetalipoproteinaemia come from an altered membrane lipid make-up, without haemolysis being the main problem. Failure of specific carriers causes glucose–galactose malabsorption (SGLT1), Hartnup disease (neutral amino acids) and cystinuria (dibasic amino acids).
- **Fat digestion and absorption:** emulsification by bile salts, pancreatic lipase with colipase (cleaves triglyceride at positions 1 and 3 to 2-monoacylglycerol and free fatty acids), **mixed micelles** carry products to enterocytes, resynthesis into triglyceride, chylomicron assembly with apoB-48 (MTP needed), release into lymph. Short- and medium-chain fatty acids go directly to the portal vein. Malabsorption causes steatorrhoea and fat-soluble vitamin deficiency; abetalipoproteinaemia is MTP deficiency.
- **Liposomes** are closed phospholipid bilayer vesicles used to carry drugs (liposomal doxorubicin, amphotericin B). **Micelles**

are single-layer aggregates above a critical concentration. Saponifiable lipids (triglycerides, glycerophospholipids, waxes: ester-linked fatty acids) yield soap on alkali hydrolysis; non-saponifiable ones (steroids, terpenes) do not.

- **Essential fatty acids** linoleic (ω-6) and α-linolenic (ω-3); deficiency causes scaly dermatitis and poor wound healing. Trans fats raise LDL and lower HDL. **Obesity** biochemistry: leptin (adipose, signals satiety through the hypothalamus; congenital leptin deficiency causes severe obesity and responds to leptin), ghrelin (stomach, raises appetite), and insulin resistance.
- **Alpha oxidation** (peroxisomal) removes one carbon at a time and is needed for phytanic acid (Refsum); **omega oxidation** (ER) produces dicarboxylic acids when β-oxidation fails.
- **Creatine and creatinine** (the curriculum asks muscle metabolism): creatine is made from glycine, arginine and S-adenosylmethionine (kidney then liver), phosphorylated by creatine kinase, and the spontaneous cyclisation of creatine and creatine phosphate to creatinine (about 1–2% a day) gives a daily excretion proportional to muscle mass. Raised serum creatinine reflects reduced GFR.

Amino acid metabolism and urea cycle

Classification and special roles

- Essential: **PVT TIM HALL** (phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine, lysine); **histidine is now counted essential in adults as well as children** (WHO/FAO), and **arginine is semi-essential** (needed in the diet in growth, pregnancy and illness). **Tyrosine is nonessential** (made from phenylalanine) but becomes essential in PKU.
- Purely ketogenic: **leucine and lysine**. Both: phenylalanine, isoleucine, tyrosine, tryptophan, threonine. Alanine is the main gluconeogenic amino acid.
- Branched-chain amino acids: leucine, isoleucine, valine (muscle metabolises them).
- **Charges at body pH:** aspartate and glutamate negative; **arginine the most basic (guanidinium pKa about 12.5)**, then lysine (about 10.5); histidine (pKa about 6) is mostly uncharged at pH 7.4. Histones are rich in lysine and arginine.
- **Carnitine is made from lysine (trimethylated, methyl groups from SAM) and methionine** (liver, kidney); creatine from glycine, arginine and methionine; carnosine from β-alanine and histidine.
- Methionine: SAM is the main methyl donor. Glycine: haem, purines, creatine, glutathione, bile acid conjugation. Histidine: histamine; best buffer near pH 7.
- **Creatine is made from glycine and arginine** (guanidinoacetate, in kidney) and **methylated by SAM** (methionine) in the liver; phosphocreatine is the muscle energy store, creatinine its breakdown product.
- Glutathione: glutamate, cysteine, glycine. Purine ring atoms: glycine, aspartate, glutamine, formate, CO_2. Pyrimidine: aspartate, glutamine, CO_2.
- Arginine gives nitric oxide; tryptophan gives serotonin, melatonin and niacin; tyrosine gives catecholamines, thyroid hormones and melanin; histidine histamine; glutamate GABA.
- Collagen: Gly-X-Y repeats, proline and lysine hydroxylation (vitamin C), glycosylation, lysyl oxidase (copper) cross-links. **Type I (bone, skin, tendon, sclera): osteogenesis imperfecta (blue sclerae, fractures)**; type II cartilage; type III vessels, uterus, bowel (vascular Ehlers–Danlos); type IV basement membrane (Alport, Goodpasture).

Transamination and the alanine cycle

- Aminotransferases (AST, ALT) use **pyridoxal phosphate (B6)** and α-ketoglutarate/glutamate. Glutamate dehydrogenase releases ammonia. Glutamine is the nontoxic ammonia carrier in brain, glutamine synthetase uses ATP.
- **Glucose–alanine cycle:** muscle transaminates pyruvate to alanine, which goes to the liver; ALT returns pyruvate for gluconeogenesis and **the nitrogen enters the urea cycle**. It carries amino nitrogen plus a carbon skeleton; muscle lacks glucose-6-phosphatase and cannot release glucose itself. Alanine is not ketogenic.

Urea cycle

- Liver only; the first two steps (CPS-I, OTC) are mitochondrial, the rest cytosolic. **CPS-I is rate-limiting and is activated by N-acetylglutamate** (not N-acetylcysteine). Urea's two nitrogens come from ammonia and aspartate.
- Net cost: 4 high-energy phosphate bonds.
Defect	Pattern
CPS-I	Hyperammonaemia, low citrulline, **low orotic acid**
OTC (commonest, X-linked)	Hyperammonaemia, low citrulline, **high urinary orotic acid** (carbamoyl phosphate spills into pyrimidine synthesis), low BUN
Argininosuccinate synthetase	Citrullinaemia type I
Argininosuccinate lyase	Argininosuccinic aciduria
Arginase	Hyperargininaemia, spastic diplegia, mild ammonia
- Neonate at 2–3 days, lethargic, vomiting, **respiratory alkalosis** (ammonia stimulates the respiratory centre): think urea cycle defect. Treatment: stop protein, IV glucose, sodium benzoate and phenylbutyrate (alternative nitrogen excretion), arginine, haemodialysis for very high ammonia, lactulose in adults. Organic acidaemias (propionic, methylmalonic) present with **metabolic acidosis** and raised anion gap.
- **HHH syndrome:** defect of the mitochondrial **ornithine–citrulline carrier (ORNT1, SLC25A15)**: hyperammonaemia, hyperornithinaemia and **homocitrullinuria** (carbamoyl phosphate condenses with lysine); autosomal recessive; spastic paraparesis, protein aversion.
- Hepatic encephalopathy: ammonia depletes α-ketoglutarate and raises glutamine in astrocytes; lactulose, rifaximin and L-ornithine L-aspartate.

Inborn errors

Disease	Enzyme	Key features
Classic PKU	**Phenylalanine hydroxylase** (AR; tetrahydrobiopterin defect in a minority)	Intellectual disability, fair hair and skin, eczema, **musty (mousy) odour**, seizures; Guthrie/tandem MS screening; low-phenylalanine diet, sapropterin, pegvaliase; maternal PKU is teratogenic (microcephaly, congenital heart disease); **avoid aspartame (contains phenylalanine)**; BH4 defects (malignant PKU) need BH4 and neurotransmitter precursors
Albinism	Tyrosinase	Oculocutaneous

Disease	Enzyme	Key features
Alkaptonuria	Homogentisate oxidase	**Urine darkens on standing (oxidation and polymerisation of homogentisic acid)**, ochronosis of cartilage, early arthritis; nitisinone
Maple syrup urine disease	**Branched-chain α-ketoacid dehydrogenase** (thiamine cofactor)	**Leucine, isoleucine, valine** raised; sweet urine; thiamine trial; diet
Homocystinuria	**Cystathionine β-synthase** (B6 cofactor; about half respond to B6); rarer causes are MTHFR and methionine synthase (B12) defects	**Marfanoid habit, downward (inferior) lens dislocation, intellectual disability, osteoporosis, thrombosis**; treat B6, folate, B12, betaine, low methionine diet; cysteine becomes essential
Cystinuria	Renal transporter of cystine, ornithine, lysine, arginine (COLA)	Hexagonal stones; nitroprusside positive; alkalinise urine, tiopronin
Hartnup	Neutral amino acid transporter (tryptophan)	Pellagra-like rash
Tyrosinaemia type I	Fumarylacetoacetate hydrolase	Liver failure, cabbage odour; nitisinone

- **Marfan: lens dislocates upward, normal intelligence, no thrombosis.** Homocystinuria's lens goes down.
- Urine odours: MSUD maple syrup; PKU musty; isovaleric acidaemia sweaty feet; tyrosinaemia cabbage.
- **Carcinoid syndrome** diverts tryptophan to serotonin, leaving too little for niacin, so **pellagra** (diarrhoea, dermatitis, dementia). Hartnup, isoniazid and 60 mg tryptophan = 1 mg niacin are related facts. Diet: **maize** (niacin bound as niacytin, and little tryptophan) and **jowar (sorghum**, whose excess leucine is the classic explanation) cause pellagra; nixtamalisation of maize with lime frees the niacin.
- **Mucopolysaccharidoses:** GAG degradation in lysosomes. **Hurler (α-L-iduronidase): coarse facies, corneal clouding, AR. Hunter (iduronate-2-sulfatase): X-linked, clear corneas, aggressive behaviour**, raised urinary GAGs, mother's brother affected. Sanfilippo (heparan sulfamidase): severe neurological, mild somatic. Morquio (galactosamine-6-sulfatase or β-galactosidase): skeletal dysplasia, normal intelligence. Enzyme replacement exists for Hurler (laronidase) and Hunter (idursulfase).

Mitochondrial genetic code (links with molecular biology)

- Mitochondria read UGA as tryptophan, AUA as methionine and AGA/AGG as stop. See the molecular biology section.

Protein–energy malnutrition and metabolic interrelationships

- **Kwashiorkor** is protein deficiency with adequate calories (weaning from milk to a starchy diet): **pitting oedema** (low albumin, low oncotic pressure; also low antioxidants), **fatty liver** (too little apoprotein to export VLDL), flaky-paint dermatitis, hair depigmentation, apathy and a protuberant belly, preserved subcutaneous fat. **Marasmus** is total energy deficiency: wasting, no oedema, no fatty liver, albumin near normal, alert and hungry. Marasmic kwashiorkor combines both. The classic explanation (the exam answer): in marasmus high cortisol and low insulin mobilise muscle amino acids and keep albumin up (adaptation), while in kwashiorkor carbohydrate keeps insulin relatively higher, muscle protein is not mobilised and albumin falls (dysadaptation). Current view (WHO; Lancet reviews): kwashiorkor is not explained by protein lack alone; oxidative stress, infection and the gut microbiome contribute, and both are managed as severe acute malnutrition (WHO 2023 guideline).
- **Starvation:** liver glycogen lasts about a day; gluconeogenesis from amino acids (muscle protein) and glycerol; after 2–3 days ketone bodies rise and the brain starts to use them, sparing protein; at about 3 weeks, ketones supply most of the brain's energy. **Well-fed state:** insulin-driven glycogen and fat synthesis; early fasting: glucagon-driven glycogenolysis; refeeding syndrome follows insulin-driven phosphate, potassium and magnesium shift into cells (thiamine first).
- **Reye syndrome:** after a viral illness (influenza, varicella) and **aspirin** in a child, mitochondrial damage in the liver causes **hyperammonaemia (urea cycle enzymes fail), microvesicular fatty liver, hypoglycaemia** and encephalopathy with cerebral oedema; this is why aspirin is avoided in children with viral fever.
- **γ-Glutamyl cycle and glutathione:** glutathione (γ-glutamyl-cysteinyl-glycine) is made in two ATP-requiring steps by **γ-glutamylcysteine synthetase (rate-limiting)** and glutathione synthetase; γ-glutamyl transpeptidase moves amino acids across membranes. Deficiency of glutathione synthetase causes **5-oxoprolinuria** with haemolytic anaemia, severe metabolic acidosis and neurological signs; γ-glutamylcysteine synthetase deficiency gives haemolysis and neurological damage. Glutathione peroxidase (selenium) and glutathione reductase (NADPH from the HMP shunt) protect red cells from oxidant damage.
- **Cancer cachexia:** TNF-α, IL-6 and tumour factors cause anorexia, muscle and fat loss, raised resting energy expenditure and insulin resistance; the tumour uses glucose by aerobic glycolysis (Warburg effect) and the Cori cycle wastes host ATP.

Vitamins and minerals

Water-soluble vitamins

Vitamin	Coenzyme or role	Deficiency	Test or note
B1 thiamine	TPP: PDH, α-ketoglutarate dehydrogenase, branched-chain ketoacid dehydrogenase, **transketolase**	Wet and dry beriberi, **Wernicke–Korsakoff**	**RBC transketolase activity (with and without added TPP)**
B2 riboflavin	FAD, FMN	Cheilosis, angular stomatitis, magenta tongue, corneal vascularisation	RBC glutathione reductase

Vitamin	Coenzyme or role	Deficiency	Test or note
B3 niacin	NAD^+, $NADP^+$	**Pellagra: dermatitis, diarrhoea, dementia** (death)	Tryptophan gives niacin; **nicotinic acid flushes via prostaglandin D_2 (GPR109A), blunted by aspirin; slow-release forms are hepatotoxic**, and also raise glucose and urate
B5 pantothenate	CoA, fatty acid synthase	Burning feet	
B6 pyridoxine	**PLP: transamination, decarboxylation**, haem synthesis (ALA synthase)	Sideroblastic anaemia, neuropathy, seizures in neonates, cheilosis	**Xanthurenic acid after tryptophan load**
B7 biotin	**Carboxylases** (pyruvate, acetyl-CoA, propionyl-CoA)	Dermatitis, alopecia, enteritis	Raw egg white (avidin)
B9 folate	Tetrahydrofolate: one-carbon transfer, purine and thymidylate synthesis	Megaloblastic anaemia, neural tube defects	Urinary FIGLU after histidine load
B12 cobalamin	Methylcobalamin (homocysteine to methionine); adenosylcobalamin (methylmalonyl-CoA mutase)	Megaloblastic anaemia plus **neurological damage**	**Raised methylmalonic acid**, raised homocysteine
C ascorbate	Proline and lysine hydroxylation, dopamine β-hydroxylase, iron absorption	**Scurvy**	

- **Thiamine and Wernicke encephalopathy:** triad of confusion, ataxia and ophthalmoplegia/nystagmus, usually in alcoholics. **Give IV thiamine first (before or with glucose)**, since a glucose load consumes the remaining thiamine and can precipitate or worsen the encephalopathy; never delay glucose in hypoglycaemia to wait for thiamine. Current practice gives high doses (for example 200–500 mg IV three times daily for 3–5 days; EFNS 2010, Royal College of Physicians UK); magnesium is a cofactor and is replaced. Korsakoff psychosis (confabulation, amnesia) follows if untreated. Mammillary body haemorrhages.
- **Vitamin B6 and isoniazid:** isoniazid combines with pyridoxal and increases its loss, causing peripheral neuropathy; **co-prescribe pyridoxine** (also cycloserine, hydralazine, penicillamine, and in alcoholics, malnourished, diabetics, pregnant women, HIV). The AST > ALT ratio in alcoholic liver disease partly reflects pyridoxal phosphate deficiency (ALT more dependent).
- **B12 vs folate:** both give megaloblastic anaemia, hypersegmented neutrophils and macro-ovalocytes with raised homocysteine; **only B12 deficiency raises methylmalonic acid and causes neuropathy (subacute combined degeneration)**. Folic acid corrects the anaemia but not the neurology ("folate trap" of methyl-tetrahydrofolate). Causes of B12 deficiency: pernicious anaemia (anti-intrinsic factor), ileal disease, *Diphyllobothrium latum*, strict vegan diet, metformin, nitrous oxide; stores last 3–5 years. Phenytoin, methotrexate and trimethoprim lower folate. Folic acid 400 µg daily from before conception prevents neural tube defects; 4 mg (5 mg in the UK) after a previous affected pregnancy. Serum B12 can mislead: **methylmalonic acid** (and holotranscobalamin) confirm deficiency; anti-intrinsic factor antibodies diagnose pernicious anaemia (the Schilling test is obsolete).
- **Avidin** in raw egg white binds biotin tightly, preventing absorption and giving a biotin deficiency (dermatitis, hair loss); biotin is the cofactor for carboxylation (CO_2 transfer).
- **Scurvy: vitamin C keeps the iron of prolyl and lysyl hydroxylases reduced**, so collagen's triple helix is stabilised; deficiency gives perifollicular haemorrhages, corkscrew hairs, bleeding gums, poor wound healing, subperiosteal bleeds in infants (Barlow disease). Copper is needed for lysyl oxidase (cross-links, not hydroxylation).

Fat-soluble vitamins

Vitamin	Role	Deficiency	Excess
A (retinol)	Rhodopsin (opsin plus 11-cis retinal, the prosthetic group); in the visual cycle all-trans retinal is reduced to retinol by an **NADPH**-dependent dehydrogenase in the photoreceptor, then in the pigment epithelium LRAT esterifies it and **RPE65** isomerises the ester to 11-cis retinol (biallelic RPE65 retinal dystrophy, a form of Leber congenital amaurosis, is treated by the gene therapy voretigene neparvovec, FDA 2017); epithelia	**Night blindness (earliest symptom)**, conjunctival xerosis, Bitot spots, keratomalacia, follicular hyperkeratosis	**Teratogenic** (isotretinoin), pseudotumour cerebri, hepatotoxic
D	Calcitriol: Ca and phosphate absorption	Rickets, osteomalacia; **serum 25-hydroxyvitamin D is the measure of status** (deficiency below 20 ng/mL, 50 nmol/L, in the usual teaching)	Hypercalcaemia, nephrocalcinosis
E (α-tocopherol)	Membrane antioxidant	Haemolytic anaemia (premature infants), spinocerebellar ataxia, posterior column loss, retinopathy	Bleeding tendency (antagonises vitamin K)
K	**γ-Carboxylation of factors II, VII, IX, X and proteins C and S**	Bleeding, haemorrhagic disease of newborn	

- Vitamin K1 phylloquinone (diet), K2 menaquinone (gut bacteria), K3 menadione (synthetic; haemolysis and kernicterus in newborns, so not used). Newborns are given 1 mg vitamin K1 intramuscularly at birth against vitamin K deficiency bleeding (WHO).
- **Xerophthalmia treatment (WHO):** vitamin A 200,000 IU by mouth on day 1, day 2 and day 14 (half the dose at 6–11 months, a quarter under 6 months). Prophylaxis: 100,000 IU at 6–11 months and 200,000 IU every 4–6 months from 12–59 months.

- **Warfarin inhibits vitamin K epoxide reductase (VKOR)**, so oxidised vitamin K is not recycled; it does not block the carboxylase itself. The effect takes days because existing factors must decay; factor VII and protein C fall first, which explains the early skin-necrosis risk, and why heparin bridging is used. Reversal: vitamin K, prothrombin complex concentrate. Direct oral anticoagulants (dabigatran, rivaroxaban, apixaban) act otherwise.
- Calcitriol: 25-hydroxylation in liver, 1α-hydroxylation in kidney (PTH, low phosphate stimulate). Vitamin D deficiency: low Ca/P, raised ALP, raised PTH.

Minerals and trace elements

- **Copper:** Wilson disease is **ATP7B** (hepatocyte copper transport into bile and ceruloplasmin): low ceruloplasmin, high urinary copper, Kayser–Fleischer rings, liver and basal ganglia disease; serum ceruloplasmin below 20 mg/dL (normal 20–40), 24-hour urine copper above 100 µg (40 µg in current criteria), hepatic copper above 250 µg/g dry weight; autosomal recessive, chromosome 13; diagnosed with the Leipzig score (AASLD 2022, EASL 2025); treat with penicillamine, trientine, zinc (maintenance), liver transplant for fulminant failure. **Menkes (kinky hair) is ATP7A** (intestinal copper absorption failure; copper deficiency, X-linked).
- Zinc: acrodermatitis enteropathica (periorificial dermatitis, diarrhoea, alopecia); Selenium: glutathione peroxidase, deiodinase; Keshan disease; Iodine: thyroid hormones; Fluoride: fluorosis; Molybdenum: xanthine oxidase, sulfite oxidase; Cobalt: B12; Manganese: superoxide dismutase.
- Iron: ferritin stores; transferrin transports; hepcidin lowers absorption (ferroportin degradation); haemochromatosis (HFE C282Y) with raised ferritin and saturation. Calcium and phosphate regulation by PTH, calcitriol and calcitonin.

Nucleotide metabolism

Structure

- **Nucleoside = base + sugar; nucleotide = nucleoside + phosphate.** Purines (two rings): adenine, guanine. Pyrimidines (one ring): cytosine, thymine (DNA), uracil (RNA).
- Chargaff's rule in double-stranded DNA: A = T (two hydrogen bonds) and G = C (three), so GC-rich DNA melts at a higher temperature. B-DNA is the usual right-handed form, about 10.5 base pairs a turn; Z-DNA is left-handed.
- Nucleosomes: about 146–147 bp of DNA wound round an octamer of histones H2A, H2B, H3, H4 (two each); H1 binds the linker DNA. Histones are rich in lysine and arginine (positive charge binds the phosphate).

Purine and pyrimidine synthesis

- Purines are built on ribose-5-phosphate: PRPP synthetase, then **PRPP amidotransferase (rate-limiting, committed; inhibited by AMP, GMP, IMP)**, giving **IMP, the first purine nucleotide formed**, then AMP (from aspartate, using GTP) and GMP (from glutamine, using ATP). Ring sources: glycine (C4, C5, N7), aspartate (N1), glutamine (N3, N9), N^{10}-formyl-tetrahydrofolate (C2, C8), CO_2 (C6). Two steps need folate, which is why antifolates stop purine synthesis.
- Pyrimidines: ring built first, then attached to PRPP; human **CPS-II (cytosolic, glutamine, activated by PRPP, inhibited by UTP)**, then aspartate transcarbamylase, dihydroorotase, DHO dehydrogenase, then **UMP synthase (orotate phosphoribosyltransferase + OMP decarboxylase)** to **UMP, the first pyrimidine nucleotide formed**; CTP is made from UTP. Thymidylate synthase makes dTMP from dUMP with N^5,N^{10}-methylene-tetrahydrofolate, which becomes dihydrofolate; **dihydrofolate reductase** regenerates tetrahydrofolate. CPS-II (cytosol, pyrimidines, glutamine) is not CPS-I (mitochondria, urea cycle, ammonia, needs N-acetylglutamate).
- Ribonucleotide reductase makes deoxyribonucleotides (inhibited by hydroxyurea, dATP).

Drugs acting on nucleotide synthesis

Drug	Target
Methotrexate (human), trimethoprim (bacterial), pyrimethamine (protozoal)	Dihydrofolate reductase; **leucovorin (folinic acid) rescue** bypasses the block
5-Fluorouracil, capecitabine	5-FdUMP inhibits **thymidylate synthase**
Hydroxyurea	**Ribonucleotide reductase** (also raises HbF in sickle cell disease)
Mycophenolate, ribavirin	IMP dehydrogenase (guanine nucleotides)
Leflunomide, teriflunomide	Dihydroorotate dehydrogenase
6-Mercaptopurine, azathioprine	De novo purine synthesis; inactivated by **xanthine oxidase** and TPMT
Pentostatin	Adenosine deaminase inhibitor (hairy cell leukaemia); cladribine is an ADA-resistant analogue

- **Azathioprine or 6-MP with allopurinol or febuxostat**: xanthine oxidase is blocked, the drug accumulates, marrow suppression follows; cut the dose to a quarter or avoid the combination.
- Current practice (CPIC 2018): test **TPMT and NUDT15** before thiopurines (NUDT15 variants are common in East and South Asians), and **DPYD** before fluoropyrimidines (EMA 2020), since dihydropyrimidine dehydrogenase deficiency gives severe 5-FU toxicity.

Salvage and catabolism

- Purine catabolism ends in **uric acid**: AMP to adenosine, to inosine (**adenosine deaminase**), to hypoxanthine (purine nucleoside phosphorylase); guanine to xanthine (guanase); hypoxanthine to xanthine to uric acid by **xanthine oxidase** (molybdenum). Humans and higher apes lack uricase (other mammals make soluble allantoin), so urate is near its solubility limit: **plasma saturates at about 6.8 mg/dL**. Normal serum uric acid: about 3.5–7.0 mg/dL in men, 2.5–6.0 in women. Uric acid is a plasma antioxidant.
- Pyrimidines are broken down to soluble products (CO_2, NH_3, β-alanine, β-aminoisobutyrate, the last a marker of DNA turnover), so they cause no disease like gout.
- **HGPRT** salvages hypoxanthine and guanine; **APRT** salvages adenine.
- **Lesch–Nyhan syndrome: X-linked recessive HGPRT deficiency.** PRPP accumulates, purine synthesis rises, so uric acid is high: gout, nephrolithiasis, **self-mutilation (biting lips and fingers)**, dystonia, choreoathetosis, intellectual disability; megaloblastic changes can appear. Allopurinol treats the hyperuricaemia but not the neurology. Orange "sand" (urate crystals) in the nappy is an early sign. Kelley–Seegmiller is partial HGPRT deficiency (gout and stones without the neurological picture).

- **Adenosine deaminase deficiency**: autosomal recessive SCID (toxic dATP accumulates in lymphocytes); treated with enzyme replacement or gene therapy. PNP deficiency gives T-cell deficiency.
- Hyperuricaemia in von Gierke disease has two causes: lactate (and ketones) compete with urate for renal excretion, and glucose-6-phosphate trapping depletes phosphate and ATP, so AMP is broken down to uric acid (more urate made). The same ATP breakdown explains hyperuricaemia after fructose in hereditary fructose intolerance.
- **Other salvage and catabolic disorders:** APRT deficiency gives **2,8-dihydroxyadenine stones** (radiolucent, treated with allopurinol); xanthine oxidase deficiency (xanthinuria) gives xanthine stones and a very low uric acid; myoadenylate (AMP) deaminase deficiency of muscle (purine nucleotide cycle) gives exercise cramps.

Gout drugs

Drug	Mechanism
Allopurinol (and oxypurinol)	**Inhibits xanthine oxidase**, lowering uric acid (hypoxanthine and xanthine are more soluble)
Febuxostat	Non-purine xanthine oxidase inhibitor (cardiovascular warning)
Probenecid	Blocks URAT1, increasing urate excretion (uricosuric)
Colchicine	Inhibits microtubule assembly, neutrophil migration (acute attacks and prophylaxis)
Pegloticase, rasburicase	Recombinant uricase (rasburicase for tumour lysis; both contraindicated in G6PD deficiency, since the hydrogen peroxide made causes haemolysis and methaemoglobinaemia)

- **Gout's hyperuricaemia is from underexcretion in about 90%** and overproduction in about 10% (HGPRT partial deficiency, PRPP synthetase superactivity, X-linked; myeloproliferative disease, tumour lysis, psoriasis).
- Acute gout: NSAIDs, colchicine or steroids. **Exam answer: do not start allopurinol during an attack, and do not stop it if already taken. Current practice (ACR 2020):** urate-lowering therapy may be started during a flare under anti-inflammatory cover; prophylaxis (colchicine, NSAID or low-dose steroid) for 3–6 months when starting; allopurinol begins at no more than 100 mg daily (less in chronic kidney disease) and is titrated to target. Needle-shaped, negatively birefringent crystals of monosodium urate; pseudogout has rhomboid, positively birefringent calcium pyrophosphate crystals. Target serum urate is below 6 mg/dL. ACR 2020 guidelines recommend allopurinol first-line with a low starting dose; HLA-B*58:01 screening (allopurinol hypersensitivity, SJS/TEN) in people at high risk: South-East and East Asian descent (Han Chinese, Korean, Thai) and African Americans.
- Tumour lysis syndrome: hyperuricaemia, hyperkalaemia, hyperphosphataemia, hypocalcaemia (Cairo–Bishop); prevented with hydration and allopurinol, or rasburicase in high risk. Uric acid stones are radiolucent and form in acid urine; alkalinise the urine (potassium citrate).

Orotic aciduria

- **Hereditary orotic aciduria**: UMP synthase deficiency. **Megaloblastic anaemia not responding to B12 or folate**, failure to thrive, raised urinary orotic acid, **no hyperammonaemia**. **Treat with uridine** (bypasses the block and feeds back to inhibit CPS-II); uridine triacetate is the approved form (FDA 2015). Autosomal recessive.
- Distinguish from OTC deficiency: high orotic acid too, but with **hyperammonaemia** and no megaloblastic anaemia.
- Other causes of hyperuricaemia: tumour lysis, psoriasis, alcohol, lead (saturnine gout), CKD, thiazide and loop diuretics, low-dose aspirin, **pyrazinamide** (and ethambutol), ciclosporin, ketoacidosis and lactic acidosis. Losartan, fenofibrate and SGLT2 inhibitors lower urate a little.

Molecular biology and techniques

DNA, RNA and the genetic code

- **Central dogma:** DNA to RNA (transcription) to protein (translation); reverse transcriptase (HIV, hepatitis B) makes DNA from RNA; telomerase is a reverse transcriptase that carries its own RNA template (adds TTAGGG repeats to chromosome ends; active in germ cells, stem cells and most cancers).
- **Coding strand** has the sequence of the mRNA (with T in place of U); the **template strand** is read by RNA polymerase 3′ to 5′, and RNA is made 5′ to 3′. Worked example: coding 5′-ATGCCGTA-3′ gives mRNA **5′-AUGCCGUA-3′**. Always check the 5′/3′ labels: an answer written 3′→5′ can be the same molecule reversed.
- Genetic code: triplet, nonoverlapping, degenerate (more than one codon per amino acid), almost universal, unambiguous. **AUG is the start codon (methionine; N-formylmethionine in prokaryotes)**; stops are UAA, UAG, UGA. Prokaryotes can also start with GUG or UUG.
- **Mitochondrial code differs: UGA codes for tryptophan**, AUA for methionine, AGA and AGG are stops. In nuclear mRNA UGA is a stop, and encodes selenocysteine only with a SECIS element; UAG with a downstream element gives pyrrolysine in archaea.
- Mutations: silent, missense (sickle cell, Glu to Val), nonsense (premature stop), frameshift (insertion or deletion not a multiple of 3), splice-site. Wobble at the third codon position.
- Mitochondrial DNA: circular, 16.5 kb, maternal inheritance, 37 genes, high mutation rate; heteroplasmy.

Replication

- Semi-conservative, bidirectional, from origins. Helicase unwinds, SSB proteins stabilise, topoisomerase relieves supercoils (quinolones inhibit bacterial gyrase; irinotecan topoisomerase I; etoposide topoisomerase II), primase lays an RNA primer.
- Prokaryotes: **DNA polymerase III** replicates, polymerase I removes primers (5′→3′ exonuclease) and fills; ligase joins Okazaki fragments (lagging strand). Eukaryotes: polymerase α (primase), δ (lagging), ε (leading), γ (mitochondrial); β repair.
- **Cohesin (SMC1, SMC3, RAD21) holds sister chromatids together from S phase; separase cleaves RAD21 at anaphase once the APC/C has destroyed securin;** condensin compacts chromosomes; cohesin-loader defects cause Cornelia de Lange syndrome.
- Polymerases proofread by 3′→5′ exonuclease; **Taq lacks proofreading**.

Transcription and RNA processing

- Prokaryotic RNA polymerase with σ factor; rifampicin inhibits the β subunit. Eukaryotic: Pol I (rRNA), **Pol II (mRNA, snRNA, miRNA)**, Pol III (tRNA, 5S rRNA); **α-amanitin inhibits Pol II**.

- Promoters: TATA box (−25), CAAT box. Enhancers act at a distance.
- mRNA processing in the nucleus: 5′ capping (7-methylguanosine), 3′ polyadenylation (AAUAAA signal), **splicing** by the spliceosome (snRNPs; GU…AG rule; lariat); anti-Smith and anti-snRNP antibodies in SLE and mixed connective tissue disease. Alternative splicing creates different proteins from one gene. Thalassaemia can arise from splice-site mutations.
- Translation: initiation (eIF; Shine–Dalgarno in prokaryotes, Kozak in eukaryotes), elongation (A, P, E sites; peptidyl transferase is a ribozyme), termination (release factors). Ribosomes: prokaryotic 70S (30S + 50S), eukaryotic 80S (40S + 60S). Antibiotics: aminoglycosides and tetracyclines (30S), macrolides, clindamycin, chloramphenicol, linezolid (50S).
- Post-translational: N-glycosylation on asparagine in the ER; O-glycosylation on serine/threonine in the Golgi; mannose-6-phosphate targets enzymes to lysosomes (**I-cell disease**: GlcNAc phosphotransferase defect); ubiquitin tags proteins for the proteasome; chaperones.

DNA repair

Pathway	Lesion	Disease when defective
Nucleotide excision repair	Bulky lesions, **UV pyrimidine (thymine) dimers**	**Xeroderma pigmentosum**, Cockayne syndrome
Mismatch repair (MLH1, MSH2, MSH6, PMS2)	Replication errors	**Lynch syndrome (HNPCC)**: **microsatellite instability**; colon, endometrial, ovarian cancers
Base excision repair (glycosylase removes the base, **AP endonuclease nicks the abasic site**, polymerase β fills, ligase seals)	Single deaminated or oxidised bases (uracil)	MUTYH-associated polyposis
Homologous recombination (BRCA1, BRCA2)	Double-strand breaks	Breast and ovarian cancer; PARP inhibitors (olaparib) exploit this (synthetic lethality)
Non-homologous end joining	Double-strand breaks, rejoined without a template (error-prone)	SCID with radiosensitivity (Artemis, DNA-PKcs, ligase IV)
Damage sensing for double-strand breaks	ATM kinase	**Ataxia telangiectasia** (ataxia, telangiectasia, IgA deficiency, lymphoma, raised AFP, radiosensitivity)
Interstrand crosslink repair (with homologous recombination)	FANC genes	**Fanconi anaemia** (marrow failure, absent radius and thumb, sensitivity to mitomycin and diepoxybutane)

- Lynch: a young patient with colon cancer and relatives with endometrial or ovarian cancer; **Amsterdam II criteria (3-2-1: three relatives, two generations, one before 50)** are the exam answer; **current practice** tests every colorectal and endometrial cancer for MMR deficiency (NICE DG27 2017, DG42 2020; NCCN); test tumour for MSI or immunohistochemical loss of MMR proteins; MSI-high tumours respond to immune checkpoint inhibitors (pembrolizumab).
- Xeroderma pigmentosum: sun sensitivity, freckling, skin cancers (basal and squamous cell carcinoma, melanoma), neurological problems.

Techniques

Technique	Detects
Southern blot	**DNA** (named for Southern)
Northern blot	**RNA**
Western blot	Protein (antibody)
Southwestern	DNA-binding proteins
ELISA	Antigen or antibody; HIV screening (classic: ELISA then Western blot; current: fourth-generation antigen–antibody assay, then a differentiation immunoassay, CDC 2014; WHO uses three rapid tests in a row)
FISH	Chromosomal location, translocations (BCR-ABL), microdeletions
Microarray	Gene expression or copy number
Karyotype	Gross chromosomal abnormalities
Sanger sequencing / next-generation sequencing	Sequence; NGS for panels and exomes

- **PCR:** denaturation near 95 °C, annealing of primers (50–65 °C), extension near 72 °C by **Taq polymerase from *Thermus aquaticus*, which survives repeated heating to 95 °C**; needs template, two primers, dNTPs, Mg^{2+}. Each cycle doubles the product: n cycles gives 2^n copies. Real-time (quantitative) PCR measures amplification with fluorescence; reverse transcriptase PCR starts from RNA (viral load, e.g. SARS-CoV-2). **CRISPR-Cas9** (guide RNA directs Cas9 cuts) is used for gene editing; exa-cel (an approved CRISPR therapy for sickle cell disease and β-thalassaemia in 2023).
- Restriction enzymes cut at palindromic sequences; DNA fingerprinting by VNTR/STR; RFLP; cloning vectors (plasmids, bacteriophage, cosmids, YACs).
- Epigenetics: DNA methylation (CpG islands) silences genes; histone acetylation opens chromatin; genomic imprinting in Prader–Willi (loss of paternal 15q11–13: deletion, or maternal uniparental disomy) and Angelman (loss of maternal *UBE3A*: deletion, or paternal uniparental disomy) syndromes. Trinucleotide repeat disorders: fragile X (CGG), Huntington (CAG), myotonic dystrophy (CTG), Friedreich ataxia (GAA).

Regulation of gene expression

- **Lac operon** (E. coli): the repressor (lacI) binds the operator and blocks RNA polymerase; **allolactose (the inducer; IPTG in the laboratory)** removes the repressor. **High glucose lowers cAMP, so CAP–cAMP does not activate transcription (catabolite repression)**: the operon is fully on only with lactose present and glucose absent. The trp operon is a repressible system (tryptophan activates the repressor, with attenuation as well).
- **Eukaryotes:** enhancers, silencers, transcription factors (zinc finger, leucine zipper, helix–turn–helix), chromatin remodelling, histone acetylation (opens) and DNA methylation (silences). **Steroid and thyroid hormones** enter the cell, bind intracellular receptors and the complex binds hormone response elements; peptide hormones act through cell-surface receptors and second messengers (cAMP, cGMP, IP_3 and diacylglycerol, calcium).
- **Post-transcriptional regulation:** alternative splicing, mRNA stability (the transferrin-receptor mRNA is stabilised and ferritin translation is blocked when iron is low through iron-responsive elements), microRNA and siRNA silencing. **Gene**

amplification raises copy number (dihydrofolate reductase gene amplification gives methotrexate resistance; N-myc in neuroblastoma; HER2 in breast cancer). Reverse transcriptase makes DNA from RNA (retroviruses, retrotransposons, telomerase).

- **Antibiotics acting on transcription and translation:** rifampicin (RNA polymerase β), actinomycin D (intercalates DNA), α-amanitin (Pol II); **30S**: aminoglycosides (misreading), tetracyclines (block the A site); **50S**: macrolides, clindamycin (block translocation), chloramphenicol (peptidyl transferase), linezolid (initiation); puromycin (premature chain termination); diphtheria toxin inactivates eEF-2, and ricin inactivates the 60S subunit.

Inborn errors, mutations and cancer

- **Patterns of inheritance:** autosomal recessive (most enzyme defects), autosomal dominant (structural and receptor proteins: Marfan, familial hypercholesterolaemia), X-linked (G6PD, Duchenne, Lesch–Nyhan), mitochondrial (maternal), and non-classical (genomic imprinting, trinucleotide repeat expansion with anticipation, uniparental disomy, mosaicism). **Multifactorial** (polygenic with environment): type 2 diabetes, hypertension, coronary disease, neural tube defects, cleft lip, pyloric stenosis.
- **Defects by protein class:** structural (collagen: osteogenesis imperfecta, Ehlers–Danlos; dystrophin: Duchenne, from a frameshift deletion, with raised CK; fibrillin: Marfan), receptors (LDL receptor, androgen receptor), enzymes (most inborn errors), transport proteins (CFTR, haemoglobin), growth-regulating proteins (RB and p53 tumour suppressors; RAS and MYC proto-oncogenes).
- **Molecular diagnosis:** PCR, allele-specific PCR, Southern and RFLP analysis, DNA sequencing, MLPA, array comparative genomic hybridisation, FISH, and tandem mass spectrometry of dried blood spots (newborn screening). Sickle cell disease is a point mutation (GAG to GTG, Glu6Val); **α-thalassaemias are mostly gene deletions; β-thalassaemias mostly point mutations** (splicing, promoter, nonsense, frameshift) that reduce β-globin chains.
- **Chemical carcinogens:** direct-acting (alkylating agents, nitrogen mustards) and procarcinogens needing metabolic activation (polycyclic hydrocarbons and benzo[a]pyrene, aromatic amines and azo dyes, aflatoxin B1 from *Aspergillus flavus* that causes G-to-T mutation in p53 and hepatocellular carcinoma, nitrosamines). They form **DNA adducts**; initiation is an irreversible mutation, promotion is clonal expansion. Other agents: ultraviolet light (thymine dimers; xeroderma pigmentosum), ionising radiation, oncogenic viruses (HPV, EBV, HBV, HCV, HTLV-1).
- **Plasmids and antibiotic resistance:** plasmids are circular extrachromosomal DNA that carry resistance genes (β-lactamase, aminoglycoside-modifying enzymes) and spread by conjugation, transformation or transduction; resistance (R) plasmids spread multidrug resistance between species.

Clinical biochemistry and acid–base

Acid–base analysis

- Steps: pH (acidaemia below 7.35, alkalaemia above 7.45); primary disorder from HCO_3^- and PCO_2; check compensation; calculate the anion gap; delta ratio.
- **Anion gap = Na^+ − (Cl^- + HCO_3^-); normal about 8–12 mmol/L** (without K^+; the exam value; analysers with ion-selective electrodes give lower ranges, about 3–11, so use the laboratory's own). Fall in albumin lowers the normal gap (about 2.5 mmol/L per 1 g/dL).
- **High-gap acidosis (MUDPILES):** methanol, uraemia, diabetic (and alcoholic or starvation) ketoacidosis, propylene glycol, iron/isoniazid, **lactic acidosis**, ethylene glycol, salicylates. **Current mnemonic GOLDMARK** (glycols, oxoproline from paracetamol, L-lactate, D-lactate, methanol, aspirin, renal failure, ketoacidosis) drops paraldehyde, iron and isoniazid as rare. An **osmolar gap above about 10 mOsm/kg** (measured minus 2Na + glucose/18 + BUN/2.8, in mg/dL) points to methanol or ethylene glycol.
- **Normal-gap (hyperchloraemic) acidosis:** diarrhoea (HCO_3^- lost in stool), renal tubular acidosis, acetazolamide, ureteric diversions, saline infusion. Urine anion gap is negative in diarrhoea (appropriate NH_4^+ excretion) and positive in RTA.
- **Worked example:** pH 7.28, HCO_3^- 12, PCO_2 26, Na 138, Cl 114: AG = 138 − 126 = **12 (normal)**, so a normal-gap metabolic acidosis from stool bicarbonate loss. **Winter's formula** expected PCO_2 = 1.5 × HCO_3^- + 8 (± 2) = 26, so respiratory compensation is appropriate; if the measured value is higher a superimposed respiratory acidosis exists, if lower a respiratory alkalosis.
- Metabolic alkalosis: vomiting, diuretics (loss of Cl^-, volume contraction), hyperaldosteronism; compensation raises PCO_2 by about 0.7 per mmol/L rise in HCO_3^-. Urine chloride low in vomiting (saline-responsive), high in Bartter, Gitelman and hyperaldosteronism.
- Respiratory acidosis: hypoventilation (COPD, opioids, neuromuscular disease); acute rise in HCO_3^- 1 per 10 mmHg PCO_2 rise, chronic 3.5–4. Respiratory alkalosis: hyperventilation (anxiety, pulmonary embolism, salicylate early, altitude, pregnancy, sepsis, liver failure); acute fall in HCO_3^- 2 per 10 mmHg drop, chronic 4–5. **Salicylate poisoning** gives respiratory alkalosis first, then a high-gap metabolic acidosis (mixed in adults).
- Delta ratio ($\Delta AG/\Delta HCO_3^-$): below 1 suggests a mixed normal-gap component, above 2 a coexistent metabolic alkalosis.
- Henderson–Hasselbalch: pH = 6.1 + log [HCO_3^-]/(0.03 × PCO_2). Main buffers: bicarbonate (extracellular), haemoglobin and proteins, phosphate and ammonia (urine). Kidney excretes acid as titratable acid and ammonium.

Liver enzymes

- **AST:ALT above 2 suggests alcoholic liver disease** (alcoholic hepatitis): AST rarely exceeds 300 U/L; mitochondrial AST release and pyridoxal phosphate deficiency (ALT depends more on it) explain the ratio; GGT is usually raised. In viral and autoimmune hepatitis ALT is usually the higher; AST > ALT also appears in cirrhosis and ischaemic hepatitis.
- Very high transaminases (thousands): viral hepatitis, ischaemic hepatitis, paracetamol toxicity.
- Cholestatic pattern: raised ALP and GGT (and 5′-nucleotidase) out of proportion to transaminases. Raised ALP alone with normal GGT points to bone.
- Bilirubin: unconjugated (haemolysis, Gilbert, Crigler–Najjar: UDP-glucuronosyltransferase) vs conjugated (Dubin–Johnson, Rotor, obstruction). Urobilinogen is absent from urine in complete obstruction.

Other laboratory biochemistry

- Cardiac injury: high-sensitivity troponin first-line; CK-MB for re-infarction; LDH-1 pattern in infarction is historical.

- Glucose sampling: sodium fluoride tube (enolase inhibition) so glycolysis in cells does not lower the result (unseparated blood loses about 5–7% of its glucose an hour); HbA1c reflects 2–3 months and misleads in haemolysis, recent transfusion, haemoglobin variants and iron deficiency. **Diabetes (ADA Standards of Care 2025):** HbA1c $\geq$ 6.5%, fasting plasma glucose $\geq$ 126 mg/dL (7.0 mmol/L), 2-hour glucose $\geq$ 200 mg/dL (11.1 mmol/L) on a 75 g OGTT, or random $\geq$ 200 with symptoms; prediabetes HbA1c 5.7–6.4%, fasting 100–125 mg/dL.
- Metabolic fuels in fasting: glycogen for about 24 hours, then gluconeogenesis, ketones after 2–3 days; the brain reaches ketone use by about 3 weeks of starvation.
- Heller's test and other urine protein tests are pathology/laboratory topics; modern practice uses the urine albumin–creatinine ratio (albuminuria: 30 mg/g or more, KDIGO 2024).

Normal values (adults; laboratories differ slightly)

Analyte	Conventional	SI
Fasting plasma glucose	70–99 mg/dL	3.9–5.5 mmol/L
Blood urea nitrogen (BUN)	7–20 mg/dL (blood urea is about 2.14 × BUN, 15–40 mg/dL)	2.5–7.1 mmol/L
Creatinine	0.6–1.2 mg/dL	53–106 µmol/L
Sodium / potassium / chloride	135–145 / 3.5–5.0 / 98–106 mmol/L	same
Bicarbonate	22–28 mmol/L	same
Total calcium (ionised)	8.5–10.5 mg/dL (4.5–5.3)	2.2–2.6 mmol/L (1.1–1.3)
Phosphate	2.5–4.5 mg/dL	0.8–1.45 mmol/L
Magnesium	1.7–2.4 mg/dL	0.7–1.0 mmol/L
Total protein / albumin	6–8 / 3.5–5.0 g/dL	60–80 / 35–50 g/L
Bilirubin, total (direct)	0.3–1.2 (below 0.3) mg/dL	5–21 µmol/L
Serum osmolality	275–295 mOsm/kg	same
Arterial pH, $PaCO_2$, PaO_2	7.35–7.45; 35–45 mmHg; 80–100 mmHg	$PaCO_2$ 4.7–6.0 kPa
Lipids (desirable)	Total cholesterol below 200, LDL below 100, HDL at least 40 (men) or 50 (women), TG below 150 mg/dL	
Uric acid	Men 3.5–7.0, women 2.5–6.0 mg/dL	

- Conversions: glucose mg/dL ÷ 18 = mmol/L; cholesterol ÷ 38.7; triglyceride ÷ 88.5; creatinine × 88.4 = µmol/L.

Haem synthesis, porphyrias and iron

- **Haem is made in bone marrow (mainly for haemoglobin) and liver (for cytochromes).** Succinyl-CoA and glycine form δ-aminolaevulinic acid (ALA) by **ALA synthase (mitochondrial, pyridoxal phosphate, rate-limiting, repressed by haem and induced by drugs, fasting and steroids)**; ALA dehydratase (cytosolic; inhibited by lead, zinc-dependent) makes porphobilinogen; **porphobilinogen deaminase** makes hydroxymethylbilane; uroporphyrinogen III synthase, uroporphyrinogen decarboxylase and coproporphyrinogen oxidase follow; protoporphyrinogen oxidase and **ferrochelatase (lead-sensitive; inserts Fe^{2+})** finish the pathway.
- **Porphyrias:**

Disease	Enzyme	Features
Acute intermittent porphyria (autosomal dominant)	**Porphobilinogen deaminase**	**Neurovisceral attacks:** severe abdominal pain, vomiting, constipation, neuropathy, psychosis, hyponatraemia; **no skin lesions**; urine ALA and PBG raised, **darkening to port-wine on standing**; the **Watson–Schwartz test** separates porphobilinogen (stays in the water layer after chloroform) from urobilinogen (extracted); precipitated by fasting, alcohol and enzyme-inducing drugs (barbiturates, phenytoin, sulfonamides); treat with **haem (hemin) and glucose**; givosiran (siRNA against ALA synthase) is the current preventive therapy
Porphyria cutanea tarda (commonest)	Uroporphyrinogen decarboxylase	Blistering photosensitive skin, no neurovisceral attacks; alcohol, hepatitis C, iron overload; red-pink urine fluorescence
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Severe photosensitivity, red teeth, haemolysis
Erythropoietic protoporphyria	Ferrochelatase	Painful photosensitivity, no blisters
Lead poisoning	ALA dehydratase and ferrochelatase	Basophilic stippling, microcytic anaemia, raised ALA and protoporphyrin, abdominal colic, Burton line, wrist drop

- **Haem catabolism and bilirubin:** microsomal haem oxygenase (biliverdin, CO, iron) then cytosolic biliverdin reductase give **unconjugated bilirubin**, carried on albumin; ligandin takes it into the hepatocyte, **UDP-glucuronosyltransferase 1A1 (UGT1A1)** conjugates it, **MRP2 pumps it out into bile**; gut bacteria make urobilinogen, which is excreted as stercobilin in stool (brown) and urobilin in urine.
- **Inherited hyperbilirubinaemias:**

Condition	Defect	Bilirubin	Features
Gilbert (common, 5–10%)	Reduced UGT1A1 expression (TA-repeat promoter variant)	Mild unconjugated	Rises with fasting, stress, illness; harmless; normal LFTs; no treatment
Crigler–Najjar I	**UGT1A1 absent**	Severe unconjugated (above 20 mg/dL)	Kernicterus, **no response to phenobarbital**; phototherapy then liver transplant
Crigler–Najjar II (Arias)	Reduced UGT1A1	Moderate unconjugated	**Responds to phenobarbital**
Dubin–Johnson	**MRP2 (ABCC2) canalicular export**	Conjugated	Black liver (pigment in lysosomes), normal urinary coproporphyrin total with mostly isomer I, benign
Rotor	OATP1B1/1B3 reuptake	Conjugated	No liver pigment, benign

- **Physiological neonatal jaundice** arises from low UGT1A1 activity, fast red cell turnover and enterohepatic circulation; phototherapy converts bilirubin into water-soluble lumirubin. **Prehepatic** jaundice: unconjugated, no bilirubin in urine,

raised urobilinogen; **obstructive**: conjugated, bilirubinuria, pale stools, absent urobilinogen, raised ALP.

- **Iron:** absorbed as Fe^{2+} in the duodenum (DMT1, ferroportin; vitamin C and acid help; phytate and tannin block), carried by transferrin, stored as ferritin and haemosiderin. **Hepcidin** from the liver blocks ferroportin and is high in inflammation.

Test	Iron deficiency	Anaemia of chronic disease	Iron overload
Serum iron	Low	Low	High
Total iron-binding capacity	**High**	Low or normal	Low
Transferrin saturation	Low	Low or normal	High
Ferritin	**Low (most specific)**	Normal or high	Very high
Soluble transferrin receptor	High	Normal	Normal or low

Ferritin is also an acute-phase protein, so a value below 30 µg/L diagnoses iron deficiency, while a higher cut-off (below 100) is used in inflammation or heart failure.

Lung biochemistry, oxygen transport and gases

- **Surfactant** is about 90% lipid, of which **dipalmitoylphosphatidylcholine (lecithin)** is the main component, plus surfactant proteins SP-A to SP-D; it is made by **type II pneumocytes**, lowers alveolar surface tension (Laplace law) and prevents collapse. **Respiratory distress syndrome (hyaline membrane disease)** in premature infants is surfactant deficiency, and cortisol (antenatal betamethasone) induces its synthesis; treatment is exogenous surfactant. **Lecithin : sphingomyelin ratio** in amniotic fluid of **2 or more** means mature lungs (lecithin rises sharply from about 34 weeks; sphingomyelin stays constant); phosphatidylglycerol appearing in amniotic fluid also indicates maturity. Pulmonary alveolar proteinosis is surfactant accumulation, mostly autoimmune (**autoantibodies against GM-CSF**, which alveolar macrophages need to clear surfactant), treated by whole lung lavage and inhaled GM-CSF. The lung also uses glucose, and type II cells make surfactant lipids from glucose and fatty acids.
- **Oxygen dissociation curve:** sigmoid because of cooperativity; **shifts right** (lower affinity, more O_2 delivered) with raised CO_2, H^+ (Bohr effect), temperature and **2,3-BPG**; shifts left with fetal haemoglobin (HbF binds 2,3-BPG poorly), CO, methaemoglobin and alkalosis. Carbon dioxide is carried as bicarbonate (about 70%), carbamino compounds (about 20%) and dissolved (about 10%); the **chloride shift** and **Haldane effect** (deoxygenated Hb carries more CO_2 and H^+) link the gases. Cyanosis appears when deoxyhaemoglobin exceeds 5 g/dL.
- Definitions: **standard bicarbonate** is HCO_3^- at PCO_2 40 and full saturation at 37 °C; **base excess** is the amount of strong acid or base needed to restore pH 7.4 at PCO_2 40 (normal ±2); oxygen content = 1.34 × Hb × SaO_2 + 0.003 × PaO_2. Uncompensated: pH abnormal; compensated: pH nearly normal.

Blood: coagulation and red cell metabolism

- **Coagulation:** extrinsic pathway (tissue factor, factor VII; measured by PT/INR), intrinsic (XII, XI, IX, VIII; aPTT), common pathway (X, V, II, I). Factors II, VII, IX, X (and proteins C and S) need **vitamin K (γ-glutamyl carboxylase, adding γ-carboxyglutamate that binds calcium)**; warfarin inhibits vitamin K epoxide reductase (VKORC1). Haemophilia A is factor VIII, B is factor IX (X-linked; long aPTT, normal PT); von Willebrand disease has a long aPTT and bleeding time.
- **In-vitro anticoagulants: oxalate, citrate and EDTA remove calcium**; fluoride (with oxalate) inhibits enolase and weakly binds calcium; heparin acts through antithrombin. Citrate tubes for coagulation tests, EDTA for haematology, fluoride–oxalate for glucose.
- **Fibrinolysis:** plasminogen (activated by tPA, urokinase) forms plasmin, which cleaves fibrin to D-dimer; α_2-antiplasmin and PAI-1 inhibit it; tranexamic acid blocks plasminogen's lysine-binding sites. Defects: platelet (Glanzmann, Bernard–Soulier), plasma factors, vessel wall (scurvy, Henoch–Schönlein). **Haemorrhagic disease of the newborn** (now called vitamin K deficiency bleeding) is vitamin K deficiency (breast milk low in K, sterile gut), prevented by intramuscular vitamin K at birth.
- **Metabolism of blood cells:** red cells have no mitochondria (anaerobic glycolysis, HMP shunt for NADPH, 2,3-BPG shunt, methaemoglobin reductase); reticulocytes have mitochondria and make haem and protein; leucocytes use glucose, the respiratory burst (NADPH oxidase) and myeloperoxidase; platelets use glycolysis, oxidative phosphorylation and fatty acids.

Hormones and endocrine biochemistry

- **Receptors:** steroid and thyroid hormones act through intracellular (nuclear) receptors; peptide hormones, catecholamines act through cell-surface receptors (G-protein-coupled via cAMP or IP_3; insulin and growth factors through receptor tyrosine kinases; GH and prolactin through JAK–STAT). **Insulin** lowers glucose through GLUT4 insertion and by activating glycogen synthase, glycolysis and lipogenesis; **glucagon** (α cells) raises cAMP and glucose; **somatostatin** (δ cells) inhibits both.
- **Adrenal steroidogenesis:** cholesterol to pregnenolone by **CYP11A1 (desmolase), the rate-limiting step, stimulated by ACTH through StAR**. Zones: glomerulosa (aldosterone, angiotensin II and K^+), fasciculata (cortisol, ACTH), reticularis (androgens). Cortisol effects: gluconeogenesis, protein catabolism, lipolysis, anti-inflammatory; mineralocorticoids retain Na^+ and lose K^+ and H^+.
- **Adrenal tests: Addison disease** (primary adrenal failure, now commonly autoimmune; tuberculosis in endemic areas): low cortisol, high ACTH, hyponatraemia, hyperkalaemia, hypoglycaemia, hyperpigmentation; diagnose by the **short synacthen test** (250 µg ACTH, a peak cortisol at 30 or 60 minutes below about 18 µg/dL, 500 nmol/L, is a failure; with newer, more specific immunoassays and mass spectrometry the cut-off is lower, about 14–15 µg/dL, 400–450 nmol/L), long test for secondary failure (now rarely used). **Cushing syndrome**: failure to suppress cortisol (below 1.8 µg/dL, 50 nmol/L) after **1 mg overnight dexamethasone**, raised late-night salivary cortisol and 24-hour urinary free cortisol; ACTH separates pituitary or ectopic from adrenal causes, and high-dose dexamethasone suppresses a pituitary adenoma but not ectopic ACTH. **Conn syndrome** (primary aldosteronism): hypertension with hypokalaemia, low renin and high aldosterone (aldosterone : renin ratio is the screen, confirmed by saline suppression).
- **Thyroid:** iodide trapping (NIS), oxidation and organification by thyroid peroxidase (blocked by thionamides), coupling to T3

and T4; T4 deiodinated to T3 (type 1 and 2 deiodinases, selenium); TSH is the best screen (high in primary hypothyroidism, low in hyperthyroidism); Hashimoto thyroiditis (anti-TPO), Graves (TSH-receptor antibodies), de Quervain (painful, low uptake). Iodine deficiency causes goitre and, in utero, cretinism; Nepal fortifies salt with iodine.

- **Posterior pituitary and water balance:** vasopressin (V2 receptors, aquaporin-2) and oxytocin. **Diabetes insipidus:** central (low ADH) or nephrogenic; **water deprivation test**: urine osmolality stays low in both, then rises after desmopressin in central but not in nephrogenic DI (the exam answer). Current practice adds **copeptin** (the stable fragment of the ADH precursor): a stimulated copeptin after hypertonic saline or arginine separates central DI from primary polydipsia better than the deprivation test (NEJM 2018, 2023); central DI is now also called arginine vasopressin deficiency (2022 renaming). SIADH: euvolaemic hyponatraemia with concentrated urine (urine osmolality above 100 mOsm/kg, urinary sodium above 30 mmol/L); small-cell lung cancer ectopic ADH.
- **Eicosanoids:** from arachidonic acid by cyclooxygenase (prostaglandins, **prostacyclin** from endothelium opposes **thromboxane A2** from platelets) and 5-lipoxygenase (leukotrienes, bronchoconstriction); aspirin irreversibly inhibits COX, corticosteroids inhibit phospholipase A2. Circadian rhythm: cortisol peaks early morning, melatonin at night, controlled by the suprachiasmatic nucleus.

Calcium, phosphate, muscle and nerve

- **Calcium:** 99% in bone; plasma about 45% ionised (active), 40% protein-bound (mainly albumin), 15% complexed. Corrected calcium = measured + 0.8 × (4 – albumin in g/dL). **PTH** (chief cells; raised by low ionised calcium sensed by CaSR) raises bone resorption, renal calcium reabsorption, **1α-hydroxylation of vitamin D** and phosphate excretion; **calcitriol** raises intestinal absorption of calcium and phosphate; **calcitonin** (parafollicular C cells) lowers calcium and is a tumour marker in medullary thyroid carcinoma.
- **Vitamin D:** 7-dehydrocholesterol to cholecalciferol in the skin by UVB, 25-hydroxylation in liver, **1α-hydroxylation in the kidney** (stimulated by PTH, low phosphate). Hypocalcaemia: tetany, Chvostek and Trousseau signs, prolonged QT. Hypercalcaemia: primary hyperparathyroidism (high PTH) and malignancy (low PTH, PTHrP) are the commonest; stones, bones, groans, moans. Hypophosphataemia: rickets (X-linked, FGF23); hyperphosphataemia: renal failure.
- **Muscle:** thick filaments myosin; thin filaments actin, tropomyosin, **troponin (T, I, C)**, with titin and nebulin. Contraction: calcium released from the sarcoplasmic reticulum binds troponin C, exposing actin; myosin heads cycle using ATP; relaxation needs ATP to pump calcium back (SERCA). Fuels: **resting muscle uses fatty acids, working muscle uses glucose and glycogen, creatine phosphate for the first seconds**, anaerobic glycolysis, then oxidative phosphorylation; the **purine nucleotide cycle** (AMP to IMP by AMP deaminase, then fumarate) regenerates ATP and TCA intermediates in exercise. Training raises mitochondrial number, oxidative enzymes and myoglobin. **Duchenne muscular dystrophy:** dystrophin deficiency, CK greatly raised; **hypokalaemia** causes weakness and paralysis; carnitine palmitoyltransferase II deficiency causes exercise-induced rhabdomyolysis.
- **Nervous tissue:** the brain uses glucose almost exclusively (about 120 g a day) and ketones in prolonged fasting; lipids include cerebrosides, sulphatides, gangliosides, sphingomyelin; myelin proteins (myelin basic protein, proteolipid protein); in **multiple sclerosis** autoimmune demyelination releases myelin basic protein and oligoclonal IgG bands are in the CSF. The **Na^+/K^+-ATPase** uses most of the brain's ATP and maintains the gradients for resting potential (about −70 mV). **Action potential:** depolarisation to threshold (about −55 mV) opens voltage-gated Na^+ channels, then K^+ channels cause repolarisation and hyperpolarisation; **tetrodotoxin and saxitoxin block the Na^+ channel** from outside, local anaesthetics from inside. Acetylcholine is made by choline acetyltransferase and broken down by **acetylcholinesterase** (inhibited by organophosphates, physostigmine, neostigmine); **α-bungarotoxin of snake venom blocks the nicotinic receptor** and botulinum toxin blocks vesicular release. Neurotransmitters: glutamate (excitatory), GABA (inhibitory, from glutamate with PLP), glycine, dopamine, noradrenaline, serotonin.

Kidney, electrolytes, liver function and tumour markers

- **Clearance and GFR:** clearance = (urine concentration × urine volume) / plasma concentration. **Inulin clearance is the gold standard** for GFR (about 125 mL/min), creatinine clearance slightly overestimates it (tubular secretion), and **eGFR** (CKD-EPI 2021, race-free, from creatinine and/or cystatin C) is the clinical estimate. Urea is reabsorbed passively and so is a poorer marker; BUN : creatinine ratio above 20 suggests pre-renal failure. Glucose is fully reabsorbed until the **renal threshold (about 180 mg/dL, Tm 375 mg/min)**; SGLT2 inhibitors lower the threshold.
- **Urine composition:** about 1–1.5 L a day, osmolality 50–1,200 mOsm/kg, urea (largest solute), creatinine, sodium, potassium; protein below 150 mg a day. Kidney acid–base work: HCO_3^- reclaimed in the proximal tubule by carbonic anhydrase, ammonium and titratable acid excreted distally.
- **Water, sodium and potassium:** total body water 60% of body weight in men; two-thirds intracellular. Plasma osmolality = 2Na + glucose/18 + BUN/2.8 (glucose and BUN in mg/dL; in mmol/L simply 2Na + glucose + urea). **Hyponatraemia** (below 135 mmol/L): hypovolaemic (diuretics, diarrhoea, adrenal failure), euvolaemic (SIADH, hypothyroidism, polydipsia), hypervolaemic (heart failure, cirrhosis, nephrotic syndrome); pseudohyponatraemia with high lipids or protein, and translocational with high glucose. **Correct sodium slowly** (no more than 8–10 mmol/L in 24 hours, to avoid osmotic demyelination). **Hypernatraemia**: water loss (diabetes insipidus, insensible loss), correct slowly. **Hyperkalaemia**: renal failure, ACE inhibitors, acidosis, rhabdomyolysis; ECG peaked T, loss of P, widened QRS; treat with calcium gluconate first, then insulin–glucose, salbutamol, and removal. **Hypokalaemia**: diuretics, vomiting, diarrhoea, hyperaldosteronism, insulin; flat T, U waves, weakness.
- **Liver functions:** synthesis (albumin, clotting factors, transferrin), detoxification (**phase I oxidation, reduction and hydrolysis by cytochrome P450; phase II conjugation with glucuronic acid, sulphate, glycine, glutathione and methylation**), bile acid and cholesterol synthesis, urea cycle, storage. Liver tests: bilirubin, albumin (half-life 20 days), prothrombin time (the earliest synthetic marker, as factor VII half-life is 6 hours), enzymes.
- **Tumour markers: AFP** (hepatocellular carcinoma, yolk sac tumour), **β-hCG** (choriocarcinoma, mole, germ cell), **CEA**

(colorectal cancer follow-up), **PSA** (prostate), **CA-125** (ovarian epithelial), **CA 19-9** (pancreas, biliary), **CA 15-3** (breast, for monitoring), calcitonin (medullary thyroid), thyroglobulin (follow-up of differentiated thyroid cancer), **neuron-specific enolase** (small-cell lung cancer, neuroblastoma), **ALP** (bone and liver metastases), LDH (germ cell, lymphoma), catecholamine metabolites (phaeochromocytoma, neuroblastoma). Markers monitor treatment and recurrence; most are unsuitable for screening.

- **Paraneoplastic and ectopic hormones:** small-cell lung cancer (ACTH, ADH), squamous cell lung cancer (**PTHrP**, hypercalcaemia), renal cell carcinoma (erythropoietin, PTHrP, renin), hepatocellular carcinoma (erythropoietin), hypercalcaemia of malignancy (breast, lung, myeloma, renal).
- **Laboratory methods (practicals): colorimetry and spectrophotometry** follow the Beer–Lambert law (absorbance proportional to concentration); **flame photometry** measures sodium and potassium (emission at 589 nm and 767 nm); **paper chromatography** separates amino acids and sugars (Rf = distance moved by solute / distance moved by solvent; ninhydrin makes amino acids purple); **centrifugation** separates serum or subcellular fractions; **electrophoresis** separates serum proteins by charge (albumin fastest toward the anode; a monoclonal M-band in myeloma); **ion-selective electrodes and pH meters** use a potential difference; blood gas analysers measure pH, PCO_2 and PO_2 directly and calculate bicarbonate.
- **Qualitative tests:** protein: biuret (peptide bonds, violet), ninhydrin (free α-amino groups), Millon (tyrosine), xanthoproteic (aromatic), Hopkins–Cole (tryptophan), sulfur lead acetate (cysteine); carbohydrate: Molisch (any carbohydrate, purple ring), Benedict and Fehling (reducing sugars), Barfoed (monosaccharides), Seliwanoff (ketoses, fructose red), iodine (starch blue); lipid: Sudan stain, saponification, acrolein test for glycerol. **Urine:** glucose by glucose oxidase strips (specific) versus Benedict (all reducing substances: galactose, fructose, pentose); albumin by heat and acetic acid or sulfosalicylic acid; bile salts by Hay's test; bilirubin by Fouchet or diazo (Ehrlich); urobilinogen by Ehrlich aldehyde.

Sources

- Harper's Illustrated Biochemistry; Lippincott's Illustrated Reviews: Biochemistry; Lehninger Principles of Biochemistry; Vasudevan and Sreekumari, Textbook of Biochemistry for Medical Students; Satyanarayana, Biochemistry; Harrison's Principles of Internal Medicine (inherited metabolic disease, acid–base, vitamins); Robbins and Cotran Pathologic Basis of Disease (storage diseases, Lynch syndrome); Katzung and KD Tripathi (statins, warfarin, gout drugs, isoniazid).
- First Aid for the USMLE Step 1 2022 (to check facts, not wording).
- ACR 2020 gout guideline; ESC/EAS 2019 dyslipidaemia guidelines; ADA Standards of Care 2025 and the ADA hyperglycaemic crises consensus 2024; Fourth Universal Definition of Myocardial Infarction 2018; Amsterdam II criteria and NICE DG27 (2017) for Lynch syndrome; CPIC 2018 thiopurine guideline; EFNS 2010 Wernicke guideline; WHO vitamin A guidance; AASLD 2022 Wilson disease guidance; KDIGO 2024 CKD guideline; IUBMB enzyme nomenclature (class 7, 2018).
- WHO 2023 guideline on the prevention and management of wasting and nutritional oedema; Endocrine Society guidelines on Cushing syndrome (2008), primary aldosteronism (2016) and adrenal insufficiency (2016); Fenske et al., NEJM 2018, and Refardt et al., NEJM 2023 (copeptin in polyuria–polydipsia); the 2022 joint statement renaming diabetes insipidus; CKD-EPI 2021 equation; givosiran for acute hepatic porphyria (FDA approval 2019).