Biochemistry

Unit 3 · Lesson 2 · Fats, soap and phospholipids

The notes for Lesson 2 of Unit 3: chapters 3.3 to 3.4 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 3 | 2 chapters | Chapters 3.3 to 3.4

About these notes

These are the notes for **Lesson 2 · Fats, soap and phospholipids** of the University free n easy video lessons on Biochemistry Unit 3, Lipids: Structure and Classification. They are chapters 3.3 to 3.4 of the unit book, unchanged, so a page here is the same page in the full book.

3.3 Triacylglycerols, oils and waxes; saponification

Triacylglycerols (TAGs, also called triglycerides) are glycerol with three fatty acids attached by ester bonds. They are the body's main energy store and the main component of every cooking fat and oil. Waxes are the other simple lipids: one long fatty acid joined to one long alcohol, made for waterproofing rather than fuel. Boiling a fat with alkali breaks its ester bonds and gives glycerol and soap; this reaction, saponification, is also the basis of the number that labs use to judge a fat, the saponification value.

You need: fatty acid names from 3.2; ester hydrolysis from first-year chemistry.

Triacylglycerols

Glycerol (propane-1,2,3-triol) has three –OH groups. Each can form an ester with the –COOH of a fatty acid, losing one water molecule. One fatty acid gives a monoacylglycerol, two a diacylglycerol, three a triacylglycerol. The carbons of glycerol are numbered sn-1, sn-2 and sn-3 (sn = stereospecific numbering), because the middle carbon becomes chiral when the outer two fatty acids differ.

- A **simple TAG** has three identical fatty acids, such as tripalmitin or triolein.

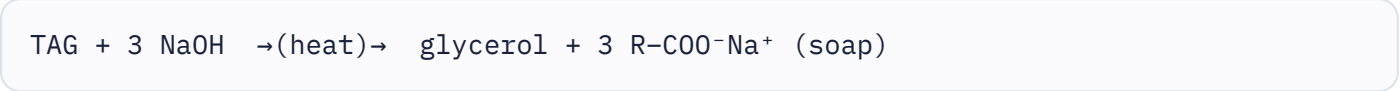
- A **mixed TAG** has two or three different fatty acids. Almost all natural fats are mixtures of mixed TAGs.

TAGs have no charge and no polar head left, so they are **neutral lipids**, completely insoluble in water. That is why they are ideal for storage: they pack into anhydrous droplets in fat cells (adipocytes) without carrying water. They are also more reduced than sugars, so they give about 9 kcal per gram against about 4 kcal per gram for carbohydrate. Fat under the skin also insulates the body, which is why seals and penguins carry thick blubber.

A **fat** is a TAG mixture that is solid at room temperature; it is rich in saturated fatty acids (ghee, butter, lard). An **oil** is liquid at room temperature; it is rich in unsaturated fatty acids (sunflower, groundnut, mustard oil). Palm and coconut oil are exceptions: they are plant oils high in saturated fat.

Saponification and other reactions of fats

Heating a TAG with sodium or potassium hydroxide hydrolyses all three ester bonds. The products are glycerol and three salts of fatty acids, which are **soaps**:



Sodium soaps are hard (bar soap); potassium soaps are soft (liquid soap, shaving cream). A soap anion is amphipathic: its tail dissolves in grease and its $–COO^-$ head faces water, so soap forms micelles that carry oil away. In hard water, Ca^{2+} and Mg^{2+} form insoluble salts with the soap, which is the grey scum in a bucket.

The reactions to know are:

1. **Hydrolysis** by lipases or acid: TAG → glycerol + free fatty acids. In digestion, pancreatic lipase cuts at sn-1 and sn-3 and leaves 2-monoacylglycerol.
2. **Saponification** by alkali, as above.
3. **Hydrogenation**: H_2 with a nickel catalyst adds across C=C, turning oil into a solid fat (vanaspati); partial hydrogenation forms trans fats.
4. **Halogenation**: iodine or iodine monochloride adds across each C=C. The amount taken up measures unsaturation.
5. **Oxidation**: oxygen attacks PUFAs and gives peroxides, then aldehydes and ketones with a bad smell. This is **oxidative rancidity**. **Hydrolytic rancidity** is lipase action releasing free fatty acids; butyric acid makes stale butter smell. Antioxidants such as vitamin E, BHA and BHT slow oxidation.

6. **Acrolein test**: heating fat with potassium bisulfate dehydrates the glycerol to acrolein, which has a sharp, irritating smell. It detects glycerol, so it is positive for fats and negative for waxes.

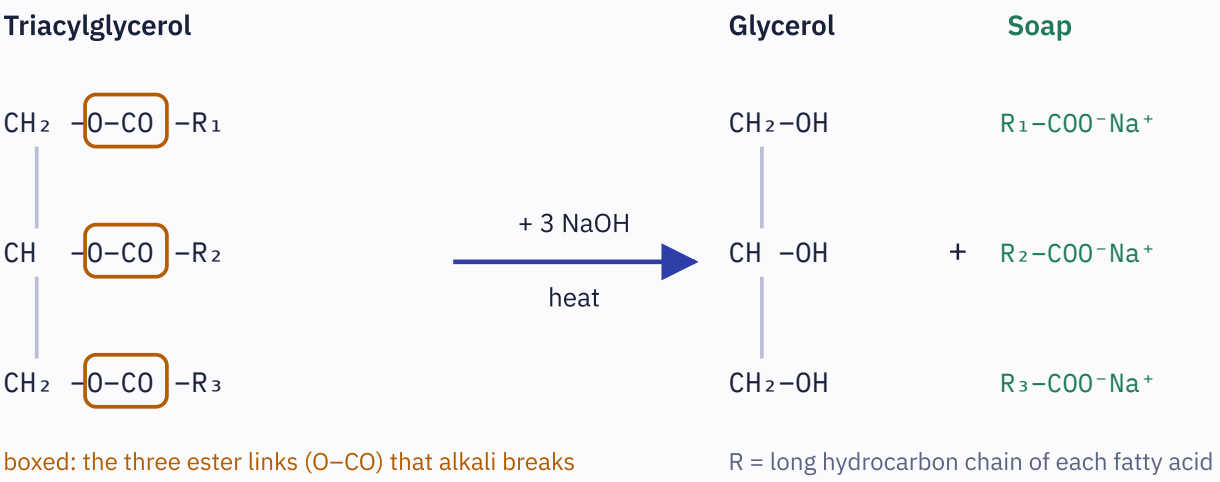


Figure 3.3.1 Saponification. Alkali cuts each of the three ester bonds (boxed), releasing glycerol and one soap anion per fatty acid. Notice the ratio: one mole of TAG uses three moles of hydroxide, which is the 3 in the saponification value formula below.

Saponification value and the other fat constants

The **saponification value (SV)** is the number of milligrams of KOH needed to saponify 1 g of fat. Because each TAG uses three KOH whatever its size, a fat of short chains has more molecules per gram and a higher SV. So SV tells you the **average chain length**: SV = 3 × 56.1 × 1000 / M, where 56.1 g/mol is the molar mass of KOH and M is the average molar mass of the TAG in g/mol. Most cooking oils have SV near 190; butterfat is higher (about 220–230) and coconut oil higher still (about 250), because both contain short- and medium-chain fatty acids.

1. Weigh W grams of fat into a flask; add a measured excess of 0.5 N alcoholic KOH.
2. Reflux on a water bath for about 30–60 minutes until saponification is complete. Set up a blank with the same KOH and no fat.
3. Titrate the unused KOH in both flasks with 0.5 N HCl, using phenolphthalein, until the pink colour just disappears.
4. SV = (B − S) × N × 56.1 / W, where B and S are the mL of HCl for blank and sample and N is the normality of the HCl.

The other constants, each measuring one property:

- **Iodine value (IV)**: grams of iodine absorbed by 100 g of fat. It measures unsaturation. Coconut oil is low (below 10), olive and groundnut oils are in the 80s and 90s, sunflower oil is higher and linseed oil is highest.
- **Acid value**: milligrams of KOH to neutralise the free fatty acids in 1 g of fat. It rises as a fat turns rancid.
- **Peroxide value**: peroxides per kilogram of fat, a direct measure of oxidative rancidity.
- **Reichert–Meissl (RM) number**: millilitres of 0.1 N alkali needed to neutralise the volatile, water-soluble fatty acids distilled from 5 g of fat. Butterfat is high because of butyric acid; most vegetable oils are very low. It is used to detect vanaspati in ghee.

Books put the boundaries at slightly different values, roughly 100 and 130 to 150.

Waxes

A wax is an ester of a long-chain fatty acid (about C14 to C36) with a long-chain monohydric alcohol (about C16 to C30). Both halves are long and saturated, so waxes are solid, water-repellent and melt above body temperature. Beeswax is mainly triacontanyl palmitate (C_{30} alcohol + palmitic acid). Other waxes: carnauba from palm leaves (car polish), lanolin from wool (creams), and the waxy cuticle that keeps leaves from drying out. Waxes waterproof feathers and insect bodies. Humans cannot digest them. They saponify only with difficulty, and give a negative acrolein test because they contain no glycerol.

WORKED EXAMPLE

Question (6 marks). (a) 2.0 g of an oil was refluxed with alcoholic KOH. The blank needed 25.0 mL and the sample 11.0 mL of 0.5 N HCl. Find the saponification value and the average molar mass of its TAG. (b) Find the theoretical iodine value of triolein (M = 885.4 g/mol; I_2 = 253.8 g/mol).

1. KOH used by the oil = B − S = 25.0 − 11.0 = 14.0 mL of 0.5 N, that is 14.0 × 0.5 = 7.0 milliequivalents.
2. Mass of KOH = 7.0 meq × 56.1 mg/meq = 392.7 mg, for 2.0 g of oil. SV = 392.7 / 2.0 = **196 mg KOH/g**.
3. Average M = 3 × 56.1 × 1000 / SV = 168 300 / 196.4 ≈ **857 g/mol**, a typical long-chain vegetable oil.
4. (b) Triolein has three C=C (one per oleic acid); each takes up one I_2. Iodine per mole = 3 × 253.8 = 761.4 g.
5. IV = 761.4 × 100 / 885.4 = **86 g I_2 per 100 g**, in the non-drying range.

EXAM TRAPS

- High saponification value means **short** chains (low molar mass), not "more fat".
- Iodine value measures unsaturation, not chain length. Mixing up SV and IV is the commonest slip.
- Forgetting the blank: the formula uses (B − S), the KOH that the fat used, not the HCl in the sample flask.
- Waxes give a negative acrolein test; fats and oils give a positive one.
- Favourite question: "Define saponification value, iodine value, acid value and RM number and state their significance."

CHECK YOURSELF

– REMEMBER **Define saponification value.** 1 mark

Milligrams of KOH needed to completely saponify 1 g of fat or oil.

– REMEMBER **What is the main component of beeswax?** 1 mark

Triacontanyl palmitate: an ester of palmitic acid with a C_{30} alcohol.

– UNDERSTAND **Why are TAGs better than glycogen for long-term energy storage?** 3 marks

They are more reduced (about 9 against 4 kcal/g) and, being non-polar, are stored without water, whereas glycogen carries about twice its weight of bound water. So the same energy weighs far less as fat.

– UNDERSTAND **Distinguish hydrolytic and oxidative rancidity.** 3 marks

Hydrolytic: lipases (with water) split TAGs into free fatty acids; short ones like butyric smell bad. Oxidative: oxygen attacks double bonds of PUFAs, giving peroxides, aldehydes and ketones. The first raises acid value, the second peroxide value.

– APPLY **Calculate the saponification value of tripalmitin (M = 807.3 g/mol).** 3 marks

SV = 3 × 56.1 × 1000 / 807.3 = 168 300 / 807.3 = **208.5 mg KOH/g**.

– APPLY **A ghee sample has a much lower RM number than pure ghee. What do you conclude?** 2 marks

It is probably adulterated with vanaspati or another vegetable fat, which has very few volatile short-chain fatty acids.

– ANALYZE **Coconut oil has a high SV but a low IV; sunflower oil has a normal SV and a high IV. What does each tell you about their fatty acids?** 4 marks

Coconut oil: high SV means many short and medium chains (lauric, myristic); low IV means few double bonds, so it is mostly saturated. Sunflower oil: normal SV means mainly C18 chains; high IV means many double bonds, mainly linoleic acid.

BEYOND THE SYLLABUS

Transesterification of TAGs with methanol gives fatty acid methyl esters, which is biodiesel, with glycerol as the by-product; it is the same ester chemistry as saponification. Lipases are used industrially to make cocoa butter substitutes by swapping fatty acids at sn-1 and sn-3. Oleaginous yeasts and microalgae are being developed to make TAGs for fuel. This is not for the exam; search "enzymatic interesterification" and "oleaginous yeast" to go further.

3.4 Phospholipids: lecithins and cephalins

Phospholipids are compound lipids that carry a phosphate group. The commonest ones, glycerophospholipids, are built like a triacylglycerol with the third fatty acid replaced by phosphate plus a small polar alcohol. That swap turns a neutral storage fat into an amphipathic molecule with a charged head and two oily tails, the molecule that forms the bilayer of every cell membrane. Lecithin (head group choline) and cephalin (head group ethanolamine) are the two that examiners name; the same plan, with other heads, gives the signalling lipids and the lipids of mitochondria.

You need: TAG structure and sn numbering from 3.3; amphipathic molecules from 3.1.

The common plan: phosphatidic acid plus a head

Start with glycerol 3-phosphate. Esterify sn-1 and sn-2 with fatty acids and you have **phosphatidic acid**, the parent of all glycerophospholipids. Then join a polar alcohol (X) to the phosphate by a second ester bond. The phosphate now links two alcohols, so it is a **phosphodiester**.

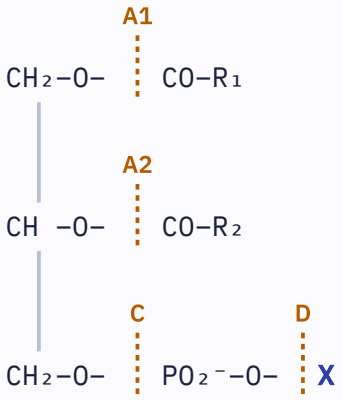
The fatty acid at sn-1 is usually saturated (palmitic, stearic) and the one at sn-2 usually unsaturated (oleic, linoleic, arachidonic). The sn-2 position is where the body stores arachidonic

acid, which becomes the eicosanoids of 3.7.

The head group X names the phospholipid:

- **X = choline**: phosphatidylcholine (PC), called **lecithin**. Net charge 0 at pH 7 (phosphate −, choline +).
- **X = ethanolamine**: phosphatidylethanolamine (PE), called **cephalin**. Net charge 0.
- **X = serine**: phosphatidylserine (PS). Net charge −1. Older books group it with the cephalins.
- **X = inositol**: phosphatidylinositol (PI). Net charge −1. Its phosphorylated form PIP_2 is split in cell signalling to IP_3 and diacylglycerol.
- **X = phosphatidylglycerol**: diphosphatidylglycerol, called **cardiolipin**, found in the inner mitochondrial membrane and in bacteria.

Glycerophospholipid



HEAD GROUP X	LIPID	COMMON NAME
choline	**PC**	lecithin
ethanolamine	**PE**	cephalin
serine	**PS**	cephalin group
inositol	**PI**	signalling
H (no head)	**PA**	phosphatidic acid

sn-1: R_1 usually saturated · sn-2: R_2 usually unsaturated
dashed lines: bonds cut by phospholipases A1, A2, C, D

Figure 3.4.1 One plan, many heads. Two fatty acids sit on sn-1 and sn-2 of glycerol, and sn-3 carries a phosphodiester to the head group X. The dashed lines mark where each phospholipase cuts: A1 and A2 release a fatty acid, C releases the phosphorylated head, D releases the free head.

Lecithins and cephalins

Lecithins (PC) are the most abundant phospholipids of animal cell membranes, concentrated in the outer leaflet. Egg yolk and soybean are rich sources; commercial soy lecithin is used as an emulsifier in chocolate, ice cream and medicines. Lecithin is also:

- **Lung surfactant.** Dipalmitoyl-phosphatidylcholine (DPPC, both chains palmitic) lines the alveoli and lowers surface tension, so the alveoli do not collapse on breathing out. Premature babies may lack it and develop respiratory distress syndrome. A lecithin-to-sphingomyelin (L/S) ratio of 2 or more in amniotic fluid indicates mature lungs.

- **A source of choline** for acetylcholine and for methyl groups.
- **A fatty acid donor** in blood: the enzyme LCAT (lecithin–cholesterol acyltransferase) moves the sn-2 fatty acid of lecithin onto cholesterol in HDL.

Cephalins (PE, and PS in the older grouping) take their name from the Greek for "head", because they were first found in brain. They sit mostly in the inner leaflet of the plasma membrane. When a platelet is activated, PS flips to the outer face and gives clotting factors a surface to assemble on. PS on the outside of a cell is also an "eat me" signal for cells dying by apoptosis.

Properties and phospholipases

Because each phospholipid has two tails, its shape is roughly cylindrical. Cylinders pack side by side into a **bilayer**, heads facing water on both sides and tails meeting in the middle. (A single-tailed fatty acid or soap is cone-shaped and forms micelles instead.) Phospholipids are also good emulsifiers, and they are part of the surface coat of lipoproteins and bile micelles.

Plasmalogens are glycerophospholipids whose sn-1 chain is joined by an ether (vinyl ether) bond instead of an ester. They are common in heart and nerve tissue. Platelet-activating factor is a related ether lipid.

The **phospholipases** each cut one bond (Figure 3.4.1):

1. **Phospholipase A1** removes the sn-1 fatty acid.
2. **Phospholipase A2** removes the sn-2 fatty acid, leaving a **lysophospholipid** (lysolecithin from lecithin). Lysolecithin is a detergent that breaks red cell membranes, which is why snake and bee venoms rich in PLA2 cause haemolysis. Cellular PLA2 releases arachidonic acid for eicosanoids.
3. **Phospholipase C** cuts between glycerol and phosphate, giving diacylglycerol plus phosphocholine (or IP_3 from PIP_2). The α-toxin of *Clostridium perfringens* in gas gangrene is a phospholipase C.
4. **Phospholipase D** cuts between phosphate and the head, giving phosphatidic acid plus free choline.

How cells make these lipids (via CDP-diacylglycerol or CDP-choline) is in Unit 6, Synthesis of triacylglycerols, phospholipids and cholesterol.

WORKED EXAMPLE

Question (6 marks). (a) 1-Palmitoyl-2-arachidonoyl-phosphatidylcholine is treated separately with phospholipases A2, C and D. Give the products of each. (b) Amniotic fluid from a pregnancy at 33 weeks contains lecithin 4.2 mg/dL and sphingomyelin 1.5 mg/dL. Should the doctors expect the baby's lungs to be mature?

1. PLA2 cuts the sn-2 ester: **arachidonic acid + 1-palmitoyl-lysophosphatidylcholine** (lysolecithin).
2. PLC cuts the glycerol–phosphate bond: **1-palmitoyl-2-arachidonoyl-glycerol (a diacylglycerol) + phosphocholine**.
3. PLD cuts the phosphate–choline bond: **phosphatidic acid (same two chains) + choline**.
4. (b) L/S = 4.2 / 1.5 = **2.8**. This is above 2, so the lungs are likely mature and enough surfactant is being made.

EXAM TRAPS

- Lecithin's head is choline, cephalin's is ethanolamine. Swapping them is the classic slip.
- PLA2 releases a fatty acid, not the head group; PLD releases the head group; PLC releases the phosphorylated head.
- Phosphatidic acid is the parent molecule, not a breakdown "acid" of lecithin by hydrolysis of fatty acids.
- Sphingomyelin is a phospholipid but not a glycerophospholipid; it is covered in 3.5.
- Favourite question: "Draw the structure of lecithin and give its functions", often with "action of phospholipases".

CHECK YOURSELF

– REMEMBER **What are the hydrolysis products of lecithin?** 2 marks

Glycerol, two fatty acids, phosphoric acid and choline.

– REMEMBER **Which phospholipid is the main component of lung surfactant?** 1 mark

Dipalmitoyl-phosphatidylcholine (DPPC), a lecithin.

– UNDERSTAND **Why do phospholipids form bilayers while soaps form micelles?** 3 marks

A phospholipid has two tails of similar width to its head, so it is cylindrical and packs flat into a sheet. A soap has one tail and a wide head, so it is cone-shaped and packs into a sphere, a micelle.

– UNDERSTAND **Why does phosphatidylserine on the outer surface of a cell matter?** 2 marks

PS is normally kept in the inner leaflet. On the outer face it provides the negatively charged surface for clotting factors on activated platelets, and it marks apoptotic cells for removal by macrophages.

– APPLY **Give the net charge at pH 7 of PC, PE and PS.** 3 marks

PC 0 (phosphate – and quaternary N^+); PE 0 (phosphate – and NH_3^+); PS –1 (phosphate –, carboxyl –, NH_3^+).

– APPLY **A snakebite victim's urine turns red. Link this to a phospholipid.** 3 marks

Venom phospholipase A2 converts membrane lecithin to lysolecithin, a detergent that lyses red cells. Released haemoglobin appears in urine (haemoglobinuria).

– ANALYZE **Compare a TAG and a lecithin with the same two fatty acids at sn-1 and sn-2. Why is one a fuel and the other a membrane builder?** 4 marks

The TAG has a third fatty acid at sn-3, so it has no polar head: it is neutral, insoluble and packs into droplets for storage. Lecithin has phosphocholine at sn-3, a charged head that faces water, making it amphipathic and cylindrical, so it assembles into bilayers. One change at sn-3 switches function.

BEYOND THE SYLLABUS

Liposomes, vesicles made of lecithin and cholesterol, carry drugs into cells, and the lipid nanoparticles of the mRNA vaccines used in COVID-19 contain a phosphatidylcholine (DSPC) as a helper lipid. Synthetic lung surfactants are given to premature babies. Lipid flippases and scramblases, which control membrane asymmetry, are an active research area. This is not for the exam; search "lipid nanoparticle composition" and "phospholipid scramblase" to go further.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Saponification value	mg KOH per g fat; SV = 3 × 56.1 × 1000 / M; lab: (B – S) × N × 56.1 / W	3.3
Iodine value	g I_2 per 100 g fat; measures unsaturation	3.3
Acid value, RM number	free fatty acids (rancidity); volatile soluble fatty acids (ghee purity)	3.3
Lecithin / cephalin	phosphatidylcholine / phosphatidylethanolamine	3.4
Phospholipases	A1, A2 remove fatty acids; C gives DAG + phospho-head; D gives PA + head	3.4

Glossary (terms used in this lesson)

Amphipathic Having a polar and a non-polar part in one molecule.

Arachidonic acid 20:4(Δ5,8,11,14), ω-6; precursor of series-2 prostanoids.

Cephalin Phosphatidylethanolamine (older use includes phosphatidylserine).

Eicosanoid Signalling molecule made from a C20 polyunsaturated fatty acid.

Iodine value Grams of iodine taken up by 100 g of fat; measures unsaturation.

Lecithin Phosphatidylcholine.

Lipoprotein Particle of lipid and apolipoprotein that carries lipids in blood.

Lysophospholipid Phospholipid that has lost one fatty acid, for example lysolecithin.

Phosphatidic acid Glycerol-3-phosphate with two fatty acids; parent of glycerophospholipids.

Rancidity Spoilage of fat by hydrolysis or oxidation.

Saponification Alkaline hydrolysis of a fat into glycerol and soap.

Saponification value mg KOH needed to saponify 1 g of fat.

Trans fat Unsaturated fat with trans double bonds, formed in partial hydrogenation.

Triacylglycerol Glycerol esterified with three fatty acids; storage fat.

Wax Ester of a long-chain fatty acid with a long-chain alcohol.

The full unit book

Every chapter of Unit 3, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 3 · Lipids: Structure and Classification (one file, works offline).

Sources and further reading

Textbooks

- D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, W. H. Freeman; the chapter on lipids and the chapter on lipid biosynthesis (from memory; check).
- J. M. Berg, J. L. Tymoczko, G. J. Gatto and L. Stryer, *Biochemistry*, W. H. Freeman; chapters on membrane lipids and lipid metabolism (from memory; check).
- D. Voet and J. G. Voet, *Biochemistry*, Wiley (from memory; check).
- U. Satyanarayana and U. Chakrapani, *Biochemistry*, a widely used Indian textbook; the chapter on lipids (from memory; check).
- V. W. Rodwell and others, *Harper's Illustrated Biochemistry*, McGraw-Hill, for lipoproteins and clinical aspects (from memory; check).

Open web resources

- LIPID MAPS, Lipid Classification System: lipidmaps.org/resources/education/classification (the eight lipid categories).
- FSSAI, news compilation on the trans fat regulation of December 2020: fssai.gov.in (PDF) (limit of 2 percent in oils and fats from January 2022).

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

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