Question 1:

1. (a) Justify the following:

(i) Triacylglycerol is especially suitable as a storage fuel.

- Triacylglycerols (TAGs) are highly reduced, meaning they contain many C-H bonds and relatively few oxygen atoms. This allows for a greater release of energy upon complete oxidation compared to carbohydrates or proteins.
- TAGs are hydrophobic and stored in an anhydrous form. This means they do not carry water, making them a very compact and lightweight form of energy storage compared to glycogen, which is stored hydrated and thus much heavier for the equivalent amount of energy.
- They are metabolically inert under normal conditions, meaning they are not readily broken down unless energy is required, allowing for long-term storage.

(ii) An individual with a genetic defect in enoyl CoA isomerase will have difficulty in metabolizing oil.

- Enoyl-CoA isomerase is a crucial enzyme in the β-oxidation of unsaturated fatty acids, particularly those with double bonds at odd-numbered carbons.
- These unsaturated fatty acids cannot be completely oxidized by the standard β-oxidation pathway. Enoyl-CoA isomerase converts the cis- or trans- double bond at position 3 (which is not a substrate for enoyl-CoA hydratase) to a trans-double bond at position 2, allowing the β-oxidation pathway to proceed.
- Without a functional enoyl-CoA isomerase, the breakdown of a significant portion of dietary fatty acids, especially those found in oils (which are rich in unsaturated fatty acids), would be impaired, leading to an inability to extract energy from these lipids.

(iii) Linoleic and linolenic acids are essential to mammals.

- Linoleic acid (an omega-6 fatty acid) and α-linolenic acid (an omega-3 fatty acid) are classified as essential fatty acids because mammals, including humans, cannot synthesize them de novo.
- Mammalian cells lack the desaturase enzymes required to introduce double bonds at positions beyond carbon 9 and 10 from the carboxyl end (e.g., at carbon 12 or 15).
- These essential fatty acids are precursors for the synthesis of other important polyunsaturated fatty acids (PUFAs), such as arachidonic acid (from linoleic acid) and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (from α-linolenic acid).
- These derived PUFAs are critical components of cell membranes, influencing membrane fluidity and function, and serve as precursors for eicosanoids (prostaglandins, thromboxanes, leukotrienes), which are potent signaling molecules involved in inflammation, blood clotting, and other physiological processes.

(iv) Two isoenzymes of HMG CoA synthase are known.

- Two isoenzymes of HMG-CoA synthase are known, each localized in a different cellular compartment and serving a distinct metabolic function:
    - Cytosolic HMG-CoA synthase: This isoenzyme is found in the cytoplasm and is involved in the initial steps of cholesterol synthesis. It condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA, which is then reduced by HMG-CoA reductase in the committed step of cholesterol biosynthesis.
    - Mitochondrial HMG-CoA synthase: This isoenzyme is located in the mitochondria and plays a crucial role in ketone body synthesis (ketogenesis). It catalyzes the condensation of acetoacetyl-CoA with acetyl-CoA to form HMG-CoA, which is then cleaved by HMG-CoA lyase to produce acetoacetate and acetyl-CoA. This pathway provides an alternative fuel source

(ketone bodies) for certain tissues during periods of fasting or low carbohydrate availability.

(v) Fatty acid biosynthesis in rat liver homogenate is severely inhibited when avidin is added.

- Avidin is a protein that binds very strongly and irreversibly to biotin (vitamin B7).
- Biotin is an essential prosthetic group for acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid biosynthesis.
- Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, which is the committed step and the first regulated step in fatty acid synthesis.
- When avidin is added to rat liver homogenate, it binds to and sequesters biotin, rendering acetyl-CoA carboxylase inactive. Without active acetyl-CoA carboxylase, the production of malonyl-CoA ceases, thereby severely inhibiting the entire pathway of fatty acid biosynthesis.

(vi) Brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirements.

- Brain: The blood-brain barrier is largely impermeable to long-chain fatty acids. While the brain can utilize fatty acids to a limited extent under specific conditions, its primary and preferred energy source is glucose. During prolonged starvation, the brain can adapt to utilize ketone bodies (derived from fatty acid breakdown in the liver) as an alternative fuel.
- Erythrocytes (Red Blood Cells): Mature erythrocytes lack mitochondria. Fatty acid oxidation (beta-oxidation) is an exclusively mitochondrial process. Therefore, red blood cells cannot perform fatty acid oxidation and rely solely on glycolysis for their energy needs.

- Adrenal Medulla: Cells of the adrenal medulla primarily utilize glucose as their energy source. They are highly specialized for the synthesis and secretion of catecholamines (epinephrine and norepinephrine), a process that is glucose-dependent and does not rely on fatty acid oxidation for energy.

(b) Write the reactions catalyzed by the following enzymes:

(i) HMG CoA Lyase

- HMG-CoA Lyase catalyzes the cleavage of (S)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) into acetoacetate and acetyl-CoA. This is a key step in ketogenesis, occurring in the mitochondria.
- Reaction:
    - HMG-CoA → Acetoacetate + Acetyl-CoA

(ii) Acetyl CoA carboxylase

- Acetyl-CoA carboxylase (ACC) catalyzes the irreversible carboxylation of acetyl-CoA to malonyl-CoA. This is the committed and rate-limiting step in fatty acid biosynthesis.
- Reaction:
    - Acetyl-CoA + ATP + HCO_3^- → Malonyl-CoA + ADP + P_i

Question 2: 2. Differentiate between the following:

(a) Alpha and omega oxidation.

- Alpha (α) Oxidation:
    - Location: Peroxisomes, primarily in the brain.
    - Substrate: Branched-chain fatty acids, especially those with a methyl group at the β-carbon (e.g., phytanic acid).
    - Process: Involves the removal of one carbon atom at a time from the carboxyl end (α-carbon) of the fatty acid, without the

involvement of CoA. The initial step is hydroxylation of the α-carbon, followed by decarboxylation.

- Purpose: To allow for the subsequent β-oxidation of fatty acids that cannot be processed by β-oxidation due to branching at the β-carbon.

- Omega (ω) Oxidation:
  - Location: Endoplasmic reticulum of the liver and kidneys.
  - Substrate: Primarily medium-chain fatty acids, but also long-chain fatty acids.
  - Process: Involves the oxidation of the methyl end (ω-carbon) of the fatty acid. The initial step is hydroxylation of the ω-carbon by a cytochrome P450 enzyme, followed by further oxidation to a dicarboxylic acid.
  - Purpose: Provides an alternative pathway for fatty acid degradation, particularly when β-oxidation is impaired or overwhelmed. Dicarboxylic acids can then undergo β-oxidation from both ends.

(b) Endogenous and Exogenous pathway of lipid transport.

- Endogenous Pathway of Lipid Transport:
  - Function: Transports lipids (primarily triglycerides and cholesterol) synthesized in the liver to peripheral tissues, and returns excess cholesterol to the liver.
  - Lipoproteins involved: VLDL (Very Low-Density Lipoproteins), IDL (Intermediate-Density Lipoproteins), LDL (Low-Density Lipoproteins), and HDL (High-Density Lipoproteins).
  - Process: The liver synthesizes VLDL, which transports endogenous triglycerides to peripheral tissues. After triglyceride removal by lipoprotein lipase, VLDL becomes IDL, then LDL.

LDL delivers cholesterol to peripheral cells via LDL receptors. HDL collects excess cholesterol from peripheral tissues and transports it back to the liver (reverse cholesterol transport).

- Clinical Relevance: Dysregulation can lead to elevated LDL ("bad" cholesterol), contributing to atherosclerosis.

- Exogenous Pathway of Lipid Transport:
  - Function: Transports dietary lipids (triglycerides and cholesterol) from the intestine to peripheral tissues and the liver.
  - Lipoproteins involved: Chylomicrons and chylomicron remnants.
  - Process: Dietary triglycerides and cholesterol are absorbed in the intestine and packaged into chylomicrons. Chylomicrons enter the lymphatic system and then the bloodstream, delivering triglycerides to peripheral tissues via lipoprotein lipase. After triglyceride depletion, chylomicron remnants, enriched in cholesterol, are taken up by the liver via specific receptors.
  - Clinical Relevance: Involved in postprandial lipemia.

(c) Fatty acid oxidation in mitochondria and peroxisome.

- Fatty Acid Oxidation in Mitochondria (β-oxidation):
  - Location: Mitochondria.
  - Primary Function: Major pathway for energy production from fatty acids, generating ATP via the electron transport chain.
  - Substrates: Primarily fatty acids of various lengths (short, medium, long, very long chains).
  - Initial Steps: Long-chain fatty acids require carnitine shuttle for transport into mitochondria. The first oxidative step is catalyzed

by acyl-CoA dehydrogenase, which directly produces $FADH_2$ (enters ETC).

- End Products: Acetyl-CoA (enters TCA cycle), NADH, and $FADH_2$ (both enter ETC).
- Regulation: Highly regulated by cellular energy status (ATP/AMP ratio), availability of substrates, and hormones.

- Fatty Acid Oxidation in Peroxisome:
  - Location: Peroxisomes.
  - Primary Function: Primarily involved in the oxidation of very long-chain fatty acids (VLCFAs), branched-chain fatty acids, and dicarboxylic acids. Also involved in detoxification.
  - Substrates: VLCFAs (e.g., >C20), branched-chain fatty acids (e.g., phytanic acid), dicarboxylic acids.
  - Initial Steps: The first oxidative step is catalyzed by acyl-CoA oxidase, which directly produces H_2O_2 (hydrogen peroxide), requiring catalase for its detoxification. No ATP is directly generated from this step. Carnitine shuttle is not required for transport into peroxisomes.
  - End Products: Shortened fatty acids (which are then transferred to mitochondria for further β-oxidation), acetyl-CoA (can be transported to mitochondria), and H_2O_2.
  - Regulation: Less directly regulated by cellular energy status compared to mitochondrial β-oxidation.

Question 3: 3. (a) Calculate the net number of ATP generated on complete oxidation of oleic acid.

- Oleic acid is an 18-carbon monounsaturated fatty acid with one cis double bond at Δ^9 (C9=C10).
- Steps for calculation:

- i. Activation: Oleic acid is activated to oleoyl-CoA, consuming 2 ATP equivalents (ATP → AMP + $2P_i$).

- ii. β-oxidation cycles:
  - For an 18-carbon fatty acid, $n/2 - 1 = 18/2 - 1 = 9 - 1 = 8$ cycles of β-oxidation.
  - However, due to the unsaturated bond, one $FADH_2$ is skipped.
  - An 18-carbon saturated fatty acid would yield 8 $FADH_2$ and 8 NADH.
  - Since there is one double bond at Δ^9, the first three cycles produce NADH and $FADH_2$ normally.
  - After 3 cycles: $18 - (3 \times 2) = 12$ carbons remaining. Acetyl-CoA generated: 3. NADH: 3. $FADH_2$: 3.
  - The double bond is at C9. After 3 cycles, it moves to C3 (cis-Δ^3-enoyl-CoA).
  - Enoyl-CoA isomerase converts cis-Δ^3-enoyl-CoA to trans-Δ^2-enoyl-CoA. This step bypasses the acyl-CoA dehydrogenase step, so no $FADH_2$ is generated in this specific cycle.
  - The remaining 5 cycles produce NADH and $FADH_2$.
  - Total NADH: $8 = 3 + 5$
  - Total $FADH_2$: $7 = 3 + 4$ (one $FADH_2$ skipped due to enoyl-CoA isomerase)
  - For each $FADH_2$, 1.5 ATP are generated (from oxidative phosphorylation).

- For each NADH, 2.5 ATP are generated (from oxidative phosphorylation).
- Total ATP from NADH: $8 \times 2.5 = 20$ ATP
- Total ATP from $FADH_2$: $7 \times 1.5 = 10.5$ ATP

iii. Acetyl-CoA molecules:

- An 18-carbon fatty acid yields $n/2 = 18/2 = 9$ molecules of Acetyl-CoA.
- Each Acetyl-CoA molecule entering the TCA cycle produces 10 ATP (3 NADH, 1 $FADH_2$, 1 GTP/ATP).
- Total ATP from Acetyl-CoA: $9 \times 10 = 90$ ATP

iv. Net ATP:

- Total ATP generated: ATP from NADH + ATP from $FADH_2$ + ATP from Acetyl-CoA
- Total ATP generated = 20 + 10.5 + 90 = 120.5 ATP
- Subtract ATP used for activation: 120.5 - 2 = 118.5 ATP

- Net ATP generated on complete oxidation of oleic acid = 118.5 ATP.

(b) Give an overview of the digestion and absorption of lipids.

- Digestion of Lipids:

ii. Mouth: Lingual lipase, secreted by glands in the mouth, initiates some hydrolysis of short- and medium-chain triglycerides. This activity is more significant in infants.

iii. Stomach: Gastric lipase, secreted by the stomach, continues the hydrolysis of short- and medium-chain triglycerides. The churning action of the stomach also helps in emulsification.

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iv. Small Intestine (Duodenum): This is the primary site of lipid digestion.

- Bile Salts: Secreted from the liver (and stored in the gallbladder), bile salts emulsify large lipid globules into smaller micelles. This increases the surface area for enzyme action.
- Pancreatic Lipase: Secreted by the pancreas, pancreatic lipase (along with colipase) is the most important enzyme for triglyceride digestion. It hydrolyzes triglycerides at the 1 and 3 positions, primarily producing 2-monoacylglycerol (2-MAG) and free fatty acids (FFAs).
- Cholesterol Esterase: Hydrolyzes cholesterol esters into cholesterol and fatty acids.
- Phospholipase A2: Hydrolyzes phospholipids (e.g., lecithin) into lysophospholipids and fatty acids.

- Absorption of Lipids:
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iii. Micelle Formation: The products of lipid digestion (2-MAG, FFAs, cholesterol, lysophospholipids, fat-soluble vitamins) are hydrophobic and relatively insoluble in the aqueous environment of the intestinal lumen. They spontaneously form mixed micelles with bile salts.

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iv. Uptake by Enterocytes: These micelles diffuse to the brush border of the intestinal epithelial cells (enterocytes). The contents of the micelles (but not the bile salts) diffuse across the cell membrane into the enterocytes. Bile salts are reabsorbed in the ileum and returned to the liver (enterohepatic circulation).

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v. Re-esterification in Enterocytes: Inside the enterocytes, the absorbed fatty acids (short- and medium-chain can directly enter the portal blood) and 2-MAG are re-esterified to form triglycerides in the smooth endoplasmic reticulum. Cholesterol and lysophospholipids are also re-esterified to form cholesterol esters and phospholipids.

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v. Chylomicron Formation: The newly synthesized triglycerides, cholesterol esters, phospholipids, and fat-soluble vitamins are packaged with specific apolipoproteins (e.g., apoB-48) within the enterocytes to form large lipoprotein particles called chylomicrons.

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v. Transport: Chylomicrons are too large to directly enter the bloodstream. They are secreted into the lymphatic system via the lacteals and eventually enter the systemic circulation through the thoracic duct, delivering dietary lipids to peripheral tissues.

(c) With the help of a diagram explain the process by which a fatty acid is transported to mitochondria for its complete oxidation.

- *Please note: As per your instructions, I cannot provide a diagram. I will explain the process of fatty acid transport into the mitochondria.*
- Process of Fatty Acid Transport to Mitochondria (Carnitine Shuttle):

- Long-chain fatty acids (LCFAs, 14 carbons or more) cannot directly cross the inner mitochondrial membrane for β-oxidation. They require a specialized transport system known as the carnitine shuttle (or carnitine acyltransferase system).

- iv. Activation in Cytosol: Fatty acids in the cytosol are first activated by acyl-CoA synthetase (also called fatty acyl-CoA ligase). This enzyme catalyzes the attachment of coenzyme A (CoA) to the fatty acid, forming fatty acyl-CoA. This step consumes 2 ATP equivalents (ATP → AMP + PPi, followed by PPi hydrolysis).
    - Fatty acid + CoA + ATP → Fatty acyl-CoA + AMP + PPi

- v. Outer Mitochondrial Membrane Transport: Fatty acyl-CoA is then transported across the outer mitochondrial membrane.

- vi. Carnitine Acyltransferase I (CAT I or CPT I): This enzyme, located on the outer mitochondrial membrane, transfers the fatty acyl group from CoA to carnitine, forming fatty acylcarnitine. CoA is released into the cytosol. This is a crucial regulatory step.
    - Fatty acyl-CoA + Carnitine → Fatty acylcarnitine + CoA

- vi. Translocase: Fatty acylcarnitine is then transported across the inner mitochondrial membrane into the mitochondrial matrix by a carnitine-acylcarnitine translocase (a carrier protein). Simultaneously, a

molecule of free carnitine is moved from the matrix back to the intermembrane space.

- vi. Carnitine Acyltransferase II (CAT II or CPT II): This enzyme, located on the inner face of the inner mitochondrial membrane within the matrix, transfers the fatty acyl group from fatty acylcarnitine back to a molecule of mitochondrial CoA, regenerating fatty acyl-CoA within the matrix. Free carnitine is released.
  - Fatty acylcarnitine + CoA → Fatty acyl-CoA + Carnitine
- vi. β-oxidation: The fatty acyl-CoA is now in the mitochondrial matrix and can enter the β-oxidation pathway for complete degradation to acetyl-CoA, NADH, and $FADH_2$, which then feed into the citric acid cycle and oxidative phosphorylation for ATP production.

- Short-chain and medium-chain fatty acids (up to 12 carbons) can cross the inner mitochondrial membrane without the need for the carnitine shuttle. They are activated to their CoA derivatives in the mitochondrial matrix.

Question 4: 4. Give the following conversions:

(a) Acetyl CoA to mevalonate

- This is the committed step in cholesterol biosynthesis.
- b. Two molecules of Acetyl-CoA condense to form acetoacetyl-CoA (catalyzed by Thiolase).

c. Acetoacetyl-CoA condenses with a third molecule of Acetyl-CoA to form (S)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (catalyzed by HMG-CoA synthase).

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d. HMG-CoA is then reduced by NADPH to form mevalonate (catalyzed by HMG-CoA reductase). This is the rate-limiting step of cholesterol synthesis.

- Overall Reaction (simplified):
    - 3 Acetyl-CoA + 2 NADPH + 2 H^+ → Mevalonate + 3 CoA + 2 $NADP^+$

(b) Dihydroxyacetone phosphate to phosphatidic acid

- This is a key step in glycerolipid biosynthesis (synthesis of triacylglycerols and glycerophospholipids).

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c. Dihydroxyacetone phosphate (DHAP) is reduced to sn-glycerol 3-phosphate (catalyzed by glycerol-3-phosphate dehydrogenase, using NADH).

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d. sn-Glycerol 3-phosphate then undergoes two successive acylations with fatty acyl-CoAs (catalyzed by acyltransferases) to form phosphatidic acid. The first acylation occurs at C1, and the second at C2.

- Overall Reaction (simplified):
    - Dihydroxyacetone phosphate + NADH + H^+ → sn-Glycerol 3-phosphate + NAD^+
    - sn-Glycerol 3-phosphate + 2 Fatty acyl-CoA → Phosphatidic acid + 2 CoA

(c) CDP-diacylglycerol to phosphatidylethanolamine

- This is a pathway for the synthesis of phosphatidylethanolamine, particularly in bacteria and some eukaryotic cells (alternative pathway also exists).
- CDP-diacylglycerol reacts with ethanolamine (or serine, which is then decarboxylated to ethanolamine) to form phosphatidylethanolamine.
- Overall Reaction:
    - CDP-diacylglycerol + Ethanolamine → Phosphatidylethanolamine + CMP

Question 5: 5. (a) What are the various classes of lipoproteins? How do they differ from each other in terms of lipid composition?

- Lipoproteins are complex particles composed of lipids (triglycerides, cholesterol, phospholipids) and proteins (apolipoproteins) that transport lipids through the aqueous bloodstream. They are classified based on their density, which is determined by their lipid-to-protein ratio.
- Various classes of lipoproteins and their lipid composition:
    - i. Chylomicrons:
        - Density: Lowest density (largest in size).
        - Origin: Intestine (transport dietary lipids).
        - Primary Lipid Composition: Extremely rich in triglycerides (about 85-90% by weight). Also contain cholesterol, cholesterol esters, and phospholipids.
        - Main Apolipoproteins: ApoB-48, ApoC-II, ApoE.

ii. VLDL (Very Low-Density Lipoproteins):

- Density: Very low density (smaller than chylomicrons).
- Origin: Liver (transport endogenously synthesized triglycerides).
- Primary Lipid Composition: Rich in triglycerides (about 50-60% by weight). Also contain cholesterol, cholesterol esters, and phospholipids.
- Main Apolipoproteins: ApoB-100, ApoC-I, ApoC-II, ApoC-III, ApoE.

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iii. IDL (Intermediate-Density Lipoproteins):

- Density: Intermediate density.
- Origin: Formed from VLDL after partial hydrolysis of triglycerides by lipoprotein lipase.
- Primary Lipid Composition: Reduced triglyceride content compared to VLDL, but relatively enriched in cholesterol and cholesterol esters.
- Main Apolipoproteins: ApoB-100, ApoE. (Some ApoC proteins may be lost).

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vii. LDL (Low-Density Lipoproteins):

- Density: Low density.
- Origin: Formed from IDL by further triglyceride removal and removal of most ApoC and ApoE.
- Primary Lipid Composition: Relatively rich in cholesterol and cholesterol esters (about 45% by weight). Low triglyceride content.

- Main Apolipoproteins: Primarily ApoB-100.

vii. HDL (High-Density Lipoproteins):

- Density: Highest density (smallest in size).
- Origin: Liver and intestine (nascent HDL), mature in circulation.
- Primary Lipid Composition: Relatively rich in protein (highest protein content among lipoproteins, ~50% by weight). Rich in phospholipids and unesterified cholesterol. Cholesterol esters increase as they pick up cholesterol. Low triglyceride content.
- Main Apolipoproteins: ApoA-I (major), ApoA-II, ApoC-I, ApoC-II, ApoC-III, ApoE.

(b) What are Ketone bodies and what are their uses? What is the significance of ketone bodies during diabetes and starvation?

- What are Ketone bodies?
  - Ketone bodies are water-soluble molecules produced in the liver from fatty acids when carbohydrate availability is low (e.g., during prolonged fasting, starvation, or uncontrolled diabetes). They serve as an alternative fuel source for various tissues.
  - The three main ketone bodies are:
    1. Acetoacetate (a β-keto acid)
    2. β-hydroxybutyrate (a reduced form of acetoacetate)

3. Acetone (a volatile, non-metabolized breakdown product of acetoacetate)

- What are their uses?
    - Fuel for peripheral tissues: Tissues such as skeletal muscle, heart muscle, kidneys, and especially the brain (during prolonged fasting) can utilize ketone bodies for energy. The brain, which normally relies on glucose, can adapt to derive up to 70% of its energy from ketone bodies during starvation, thus sparing glucose for other critical functions (e.g., red blood cells).
    - Transportable energy: Ketone bodies are water-soluble and can be transported easily in the bloodstream to various tissues, unlike fatty acids which require albumin binding.
    - Glucose sparing: By providing an alternative fuel source, ketone bodies reduce the reliance on glucose, thereby conserving glucose for tissues that are obligate glucose users.

- Significance of ketone bodies during diabetes and starvation:
    - Significance during Starvation:
        - During prolonged fasting or starvation, glycogen stores are depleted, and blood glucose levels start to fall.
        - To maintain energy supply for glucose-dependent tissues (like red blood cells) and to provide an alternative fuel for the brain, the liver dramatically increases fatty acid oxidation and ketogenesis.
        - Ketone bodies become a crucial energy source for many tissues, effectively "sparing" protein (reducing gluconeogenesis from amino acids) and prolonging survival.

        - Levels typically remain within a physiological range in healthy starvation (nutritional ketosis).
    - Significance during Diabetes (Type 1 Diabetes Mellitus):
        - In uncontrolled Type 1 Diabetes Mellitus (T1DM), there is an absolute deficiency of insulin. This leads to:
            - High blood glucose (hyperglycemia) because glucose cannot enter cells for utilization.
            - Increased lipolysis in adipose tissue, releasing large amounts of free fatty acids into circulation.
            - Unrestrained fatty acid oxidation in the liver, leading to massive production of acetyl-CoA.
            - Overproduction of ketone bodies by the liver, as the capacity of the TCA cycle to process acetyl-CoA is overwhelmed.
        - This excessive production of ketone bodies, coupled with impaired utilization by peripheral tissues, leads to ketoacidosis (diabetic ketoacidosis, DKA). Ketone bodies are acidic, causing a drop in blood pH, which can be life-threatening if untreated.
        - The presence of ketone bodies in urine (ketonuria) and breath (fruity smell due to acetone) are characteristic signs of DKA.

(c) What are the various ways by which cholesterol biosynthesis is regulated? Elaborate.

- Cholesterol biosynthesis is tightly regulated to maintain appropriate cellular and systemic cholesterol levels. The primary regulatory point is the enzyme HMG-CoA reductase, which catalyzes the committed and rate-limiting step of the pathway (conversion of HMG-CoA to mevalonate).

- Various ways by which cholesterol biosynthesis is regulated:
    - ii. Transcriptional Control (Regulation of HMG-CoA Reductase Gene Expression):
        - SREBP-2 (Sterol Regulatory Element-Binding Protein 2) is the master regulator.
        - When cholesterol levels are low: SREBP-2, along with its escort protein SCAP (SREBP Cleavage-Activating Protein), moves from the ER to the Golgi apparatus. In the Golgi, SREBP-2 is proteolytically cleaved to release its active N-terminal domain.
        - This active SREBP-2 fragment translocates to the nucleus and binds to Sterol Regulatory Elements (SREs) in the promoter regions of genes encoding HMG-CoA reductase and other cholesterol synthesis enzymes, as well as the LDL receptor gene.
        - This binding increases the transcription of these genes, leading to increased synthesis of HMG-CoA reductase and increased cholesterol synthesis, as well as increased uptake of LDL-cholesterol from the blood.
        - When cholesterol levels are high: Cholesterol binds to SCAP, preventing its dissociation from SREBP-2 and their translocation to the Golgi. This inhibits the cleavage of SREBP-2, reducing the transcription of cholesterol synthesis genes and the LDL receptor gene, thereby decreasing cholesterol synthesis and uptake.
    - iii. Hormonal Regulation:

- Insulin and Thyroid Hormones: Promote cholesterol synthesis by increasing the expression and activity of HMG-CoA reductase. This aligns with a state of energy abundance and anabolic processes.
- Glucagon and Glucocorticoids: Inhibit cholesterol synthesis by decreasing the expression and activity of HMG-CoA reductase. This aligns with states of energy scarcity or stress.

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iv. Proteolytic Degradation of HMG-CoA Reductase:

- HMG-CoA reductase itself is a sterol-sensing protein in the ER membrane.
- High cholesterol levels (particularly oxysterols derived from cholesterol) cause a conformational change in HMG-CoA reductase.
- This conformational change makes the enzyme a target for ubiquitination by an E3 ligase, leading to its degradation by the proteasome.
- This rapid degradation helps to quickly reduce cholesterol synthesis when levels are high.

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viii. Covalent Modification (Phosphorylation/Dephosphorylation):

- HMG-CoA reductase activity is regulated by phosphorylation.
- Phosphorylation of HMG-CoA reductase by AMP-activated protein kinase (AMPK) inactivates the enzyme. AMPK is activated when cellular AMP levels are high (low

energy state), thus turning off an energy-expensive process like cholesterol synthesis.

- Dephosphorylation by a phosphatase activates the enzyme.

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viii. Feedback Inhibition by Cholesterol (Allosteric and Product Inhibition):

- While not the primary regulatory mechanism, high levels of cholesterol can allosterically inhibit some enzymes in the pathway.
- Cholesterol (or its derivatives) can also directly inhibit the activity of HMG-CoA reductase.

Question 6: 6. Write short notes on the following:

(a) Plasmalogen Synthesis

- Plasmalogens are a unique class of glycerophospholipids characterized by the presence of an ether bond at the sn-1 position of the glycerol backbone, rather than the ester bond found in other phospholipids. Specifically, they have an α,β-unsaturated ether linkage (vinyl ether). The sn-2 position typically contains a fatty acyl ester, and the head group (e.g., ethanolamine, choline) is at the sn-3 position.
- Synthesis occurs in both peroxisomes and the endoplasmic reticulum:

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iii. Peroxisomal Steps: The initial steps of plasmalogen synthesis occur in the peroxisomes.

- Dihydroxyacetone phosphate (DHAP) is acylated at C1 by acyl-CoA:DHAP acyltransferase (an ester linkage).

- The acyl group at C1 is then replaced by a long-chain fatty alcohol via an ether linkage, catalyzed by alkyl-DHAP synthase. This is the defining step for ether lipid formation.
- This alkyl-DHAP is then reduced to 1-alkylglycerol-3-phosphate.

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iv. Endoplasmic Reticulum Steps: The 1-alkylglycerol-3-phosphate is then transferred to the ER.

- Acylation occurs at the sn-2 position to form 1-alkyl-2-acylglycerol-3-phosphate.
- The head group is then added (e.g., ethanolamine phosphate or choline phosphate) to form 1-alkyl-2-acylglycerophosphoethanolamine or 1-alkyl-2-acylglycerophosphocholine.
- Finally, the crucial vinyl ether bond (the characteristic of plasmalogens) is formed by a desaturation reaction at the C1 position, catalyzed by a specific desaturase.

- Role: Plasmalogens are particularly abundant in neural tissues (brain, myelin) and heart muscle. They are thought to play roles in membrane integrity, as antioxidants (due to the vinyl ether bond being susceptible to oxidation, protecting other membrane lipids), and in signal transduction pathways. Deficiencies in plasmalogen synthesis are associated with peroxisomal disorders like Zellweger syndrome.

(b) Fatty Acid Synthase Complex

- The Fatty Acid Synthase (FAS) Complex is a multi-enzyme system responsible for the de novo synthesis of long-chain saturated fatty acids, primarily palmitate (16:0), from acetyl-CoA and malonyl-CoA.

In mammals, it is a large, dimeric protein where each monomer contains all the enzymatic activities required for synthesis.

- Location: Cytosol of liver, adipose tissue, and lactating mammary glands.
- Substrates: Acetyl-CoA, malonyl-CoA, and NADPH.
- Process (repeated 7 times for palmitate synthesis):
    - iv. Acetyl-CoA and Malonyl-CoA are loaded onto acyl carrier protein (ACP) and ketoacyl synthase (KS) domains, respectively.
    - v. Condensation: Acetyl group (from KS) condenses with malonyl group (from ACP), releasing CO_2 and forming a β-ketoacyl-ACP. This step is catalyzed by β-ketoacyl synthase.
    - v. Reduction (First): The β-keto group is reduced to a β-hydroxyl group, using NADPH, catalyzed by β-ketoacyl reductase.
    - ix. Dehydration: Water is removed, forming a trans-α,β-unsaturated acyl-ACP, catalyzed by β-hydroxyacyl dehydratase.
    - ix. Reduction (Second): The double bond is reduced to a saturated single bond, using NADPH, catalyzed by enoyl reductase.

- The now saturated four-carbon acyl group (butyryl) is transferred from ACP to the KS domain, and a new malonyl-CoA molecule is loaded onto ACP, initiating another cycle. This process repeats six more times.

- Final Product: After 7 cycles, a 16-carbon saturated fatty acid (palmitate) is formed and then released from the enzyme by a thioesterase domain.
- Regulation: FAS activity is highly regulated, primarily by nutritional status and hormones. Insulin stimulates its activity (in fed state), while glucagon and starvation inhibit it. The availability of malonyl-CoA (regulated by acetyl-CoA carboxylase) also controls the flux through FAS.

(c) Atherosclerosis

- Atherosclerosis is a chronic inflammatory disease of the arteries characterized by the buildup of fatty, fibrous plaques (atheromas) within the arterial walls. It is a major underlying cause of cardiovascular diseases, including heart attacks, strokes, and peripheral artery disease.
- Pathogenesis:
    - v. Endothelial Injury: The process often begins with damage or dysfunction of the arterial endothelium (inner lining), caused by factors like high blood pressure, elevated LDL cholesterol, smoking, diabetes, and inflammation.
    - vi. LDL Accumulation and Oxidation: Damaged endothelium becomes more permeable, allowing LDL particles to enter the sub-endothelial space (intima). Here, LDL can become oxidized, which makes it highly pro-inflammatory and toxic.

- vi. Immune Response and Macrophage Infiltration: Oxidized LDL triggers an immune response. Monocytes are recruited to the site, differentiate into macrophages, and then avidly take up oxidized LDL through scavenger receptors, becoming "foam cells" (lipid-laden macrophages).

- x. Fatty Streak Formation: Accumulation of foam cells in the intima forms visible lesions called fatty streaks, the earliest stage of atherosclerosis.

- x. Plaque Development: Smooth muscle cells from the media (middle layer) migrate to the intima, proliferate, and synthesize collagen and other extracellular matrix components. This forms a fibrous cap over the lipid-rich core (composed of foam cells, dead cells, and cholesterol crystals). This entire structure is called an atherosclerotic plaque.

- vii. Plaque Progression and Complications: Plaques can grow, narrowing the arterial lumen (stenosis) and impeding blood flow. More dangerously, plaques can become unstable and rupture. A ruptured plaque exposes its thrombogenic contents to the blood, leading to the formation of a blood clot (thrombus).

- Consequences: A thrombus can completely block the artery (e.g., myocardial infarction if in a coronary artery, stroke if in a cerebral artery) or detach and travel to occlude a distant vessel (embolism).

Chronic atherosclerosis can also lead to aneurysm formation due to weakening of the arterial wall.

- Risk Factors: High LDL cholesterol, low HDL cholesterol, high blood pressure (hypertension), diabetes, smoking, obesity, physical inactivity, genetic predisposition, and chronic inflammation.