MOLECULAR AND CLINICAL BIOCHEMISTRY:

FROM MECHANISMS TO CLINICAL IMPLICATIONS, ADVANCED ANALYTICAL TECHNOLOGIES, AND ARTIFICIAL INTELLIGENCE

Editor: SERAP ÖZER YAMAN

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Molecular and Clinical Biochemistry: From Mechanisms To Clinical Implications, Advanced Analytical Technologies, And Artificial Intelligence

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PREFACE

This book consists of original chapters prepared with the contributions of expert academicians, aiming to address the multidimensional relationships between metabolic regulation, molecular biochemical mechanisms, and clinical applications. The work comprehensively covers the fundamental components of biochemistry from cellular energy metabolism to the molecular basis of protein structure, from enzyme mechanisms to the metabolic regulation of ion channels together with their clinical implications.

The chapters elaborate on current and clinically significant topics such as novel approaches in the treatment of atherosclerosis, the physiological and clinical importance of anti-Müllerian hormone, and the role of omentin-1 in the postmenopausal period. In addition, the increasing importance of flow cytometry analysis in clinical laboratory practice and the role of LC-MS/MS technology in modern toxicological evaluation covering screening, confirmation, and clinical applications are discussed, emphasizing the contribution of advanced analytical methods to diagnostic processes. Furthermore, artificial intelligence and machine learning–based predictive models are evaluated in the context of understanding and managing metabolic diseases. In this respect, the book presents a broad perspective extending from basic sciences to clinical applications.

The authors have carefully presented their topics with scientific accuracy and in light of current literature, using a clear, fluent, and educational language. It is our greatest hope that this book will contribute to researchers working in clinical biochemistry, molecular biology, internal medicine, and related health sciences, and inspire future studies.

I would like to express my sincere gratitude to all contributing authors for their valuable efforts in the preparation of this work, and to Bidge Publishing for their contributions throughout the publication process.

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CHAPTER 1

MOLECULAR BASES AND CLINICAL IMPLICATIONS OF PROTEIN STRUCTURE, ENZYME MECHANISMS AND CELLULAR ENERGY METABOLISM

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Introduction

The living cell may be considered a highly organized chemical factory that is supported by an extremely intricate infrastructure. The glycolytic pathway, which is initiated by the phosphorylation of glucose catalyzed by hexokinase, simultaneously produces free energy in the form of adenosine triphosphate (ATP), carbon skeletons for biosynthetic reactions, reducing equivalents (NADH), and signaling molecules. The structural framework for the chemical factory is the proteins. Enzymes catalyze reactions to increase their rates. The carrier proteins transport the substances to the correct cellular compartments. The structural proteins make up

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the membranes and the cytoskeleton. The receptors convey chemical signals from the outside of the cell to the inside.

This biochemical cascade emphasizes the point that cellular metabolism is not a simple sum of independent processes, but rather a highly integrated system, in which the product of one reaction becomes the substrate for the next, a signal from one point is transmitted to the next, and a local disturbance can lead to a general disequilibrium involving many processes. The biochemical cascade can be schematically represented as follows: Glucose, after being trapped within the cell by the action of the enzyme hexokinase, is converted into glucose-6-phosphate, which is subsequently converted into fructose-1,6-bisphosphate by the action of the enzyme phosphofructokinase-1 (PFK-1), which is the rate-controlling, most stringently controlled step of the glycolytic pathway. PFK-1 is a biochemical sensor of the cell\'s energy status, as the ratio of ATP to AMP dictates the allosteric control of the enzyme, with high energy status (high ratio of ATP to AMP) causing inhibition of the enzyme by ATP, whereas the converse effect is caused by the presence of the molecule AMP, which acts as a co-activator. The same signal is transduced through the insulin/glucagon hormonal system, as the intracellular concentration of the molecule fructose-2,6-bisphosphate, the most potent allosteric activator of PFK-1, is under the control of the hormonal status of the pancreas and liver (Zong et al., 2024).

At the end point of this pathway, the end product of glycolysis, pyruvate, arrives at a critical branch point in the pathway where it is converted into acetyl-CoA by the mitochondrial pyruvate dehydrogenase complex (PDC) in the presence of oxygen and enters the tricarboxylic acid (TCA) cycle. In the absence of oxygen, it is reduced by lactate dehydrogenase (LDH) into lactate, which has the net effect of regenerating cytosolic NAD^+ from NADH. The TCA cycle is the mitochondrial component of the pathway (Stacpoole &

McCall, 2023). In this pathway, the carbon backbone of acetyl-CoA is converted into CO_2 over eight consecutive steps. At the same time, oxaloacetate is used for gluconeogenesis; succinyl-CoA is used for heme synthesis; citrate is used for fatty acid synthesis; and α-ketoglutarate is used for amino acid synthesis. The electrons from NADH and $FADH_2$ are passed on to the mitochondrial electron transport chain (ETC). In this pathway, complexes I-V are used for the transfer of these high-energy electrons from higher electrochemical potential to lower electrochemical potential from coenzymes NADH and $FADH_2$ to molecular oxygen. This creates a proton gradient or proton motive force (Δp) that drives the synthesis of high-energy phosphate bonds by the F_1F_0-ATP synthase or complex V. This was first proposed by Mitchell in 1961.

The reactive oxygen species (ROS) that are inevitably produced during the ETC process include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). ROS exert concentration-dependent bimodal effects. At normal concentrations, ROS transiently inhibit tyrosine phosphatases to enhance growth factor signaling; activate hypoxia-inducible factor 1 alpha (HIF-1α) and AMP-activated protein kinase (AMPK); and stimulate the nuclear translocation of the Nrf2 transcription factor to initiate antioxidant response element (ARE) gene expression. However, when ROS generation outpaces antioxidant defenses, oxidative stress occurs. The resulting lipid peroxidation, protein oxidation, and mtDNA damage are a common pathophysiological substrate for Parkinson's disease, Alzheimer's disease, diabetes mellitus, and cardiovascular disease (Sies et al., 2022).

Disruption in any of these steps leads to clinical disease. The Glu6Val substitution in the hemoglobin β-chain subunit results in polymerization in a deoxygenated environment and leads to the full spectrum of sickle cell disease. A mutation in the active site of IDH1/2 results in the diversion of TCA cycle metabolism and leads

to the oncometabolite D-2-hydroxyglutarate (D-2HG), which reprograms the epigenome and results in glioma or acute myeloid leukemia. The m.3243A>G transition in mtDNA results in decreased synthesis of $tRNA^{Leu}$, leading to decreased synthesis of OXPHOS subunits and resulting in MELAS syndrome. The loss of succinate dehydrogenase results in the accumulation of succinate, leading to HIF-1α stabilization and paradoxical angiogenesis in tumors. Loss-of-function mutations in SCN1A encoding the voltage-gated sodium channel Nav1.1 result in decreased firing of inhibitory GABAergic interneurons, leading to Dravet epileptic encephalopathy (Esteban-Amo et al., 2024).

This chapter will explore this cascade in detail through six major sections, each of which will highlight the causal pathway from molecular mechanism through clinical phenotype in an integrated fashion. Section 2 will focus on the four levels of protein structure, chaperone-mediated protein folding, allosteric control through the hemoglobin and PFK-1 examples. Section 3 will highlight glycolysis control points, glycolysis cancer metabolism, and pyruvate dehydrogenase complex. Section 4 will discuss TCA cycle control, IDH mutations and the concept of oncometabolites, and the pathological consequences of succinate and fumarate accumulation. Section 5 will offer a detailed discussion of the mechanism of OXPHOS, hereditary mitochondrial disorders, and the two faces of reactive oxygen species: antioxidant defense systems/physiological oxidative stress cascade. Section 6 will conclude this chapter with membrane protein biochemistry: ion transport, voltage-gated channels, calcium signaling, and disease.

Molecular Foundations of Protein Structure and Allosteric Regulation

The Four Levels of Organization and Their Relationship to Disease

The structure of proteins is generally investigated on four levels of hierarchy: primary, secondary, tertiary, and quaternary structure. The primary sequence of amino acids dictates all other levels of structure, and therefore, a single amino acid substitution can result in major clinical consequences. The prototype of this is hemoglobin S (HbS), where a substitution of Glu6Val at position 6 of the β-globin chain changes a charged residue exposed to solvent into a hydrophobic residue. In its deoxygenated form, HbS molecules make hydrophobic interactions with other molecules of HbS, leading to polymerization into elongated structures that cause deformation of red blood cells into sickled shapes, resulting in vaso-occlusive crises (Bellelli & Tame, 2022).

Secondary structural elements such as α-helices and β-sheets are stabilized primarily through hydrogen bonding patterns, hydrophobic interactions , and van der Waals forces. Aberrant folding or misfolding of proteins plays a key role in the formation of pathological protein aggregates. The conversion of α-synuclein to β-sheet rich amyloid fibrils in Parkinson's disease and the aggregation of amyloid-β (Aβ) peptide in Alzheimer's disease are some of the more well-recognized manifestations of changes in secondary structural propensities. In prion diseases, the conformational switch from PrP^{C} to PrP^{Sc}, characterized by a shift from alpha helix to beta sheet content, triggers an autocatalytic aggregation cascade (Baiardi et al., 2023).

Tertiary structure refers to the three-dimensional globular form of a polypeptide chain, which is maintained by hydrophobic interactions, disulfide bridges formed between two cysteine residues with the help of protein disulfide isomerase in the endoplasmic reticulum, hydrogen bonds, and coordination bonds found in metalloproteins. Experiments conducted by Anfinsen were instrumental in showing that the tertiary structure of a protein is indeed contained within the primary sequence itself. The

experiments were recognized with the Nobel Prize in 1972. The quaternary structure refers to the formation of an oligomer from two or more polypeptide chains. Quaternary structure is the basis for allosteric control mechanisms.

Thermodynamics of Protein Folding and Chaperone Systems

The folding process is thermodynamically favored by a reduction in Gibbs free energy: $\Delta G = \Delta H - T\Delta S < 0$. The energy difference between the folded and unfolded conformations is 5-60 kJ/mol. The folding process is driven by the burial of hydrophobic residues and the formation of hydrogen bonds (Albakova et al., 2022). The folding process is facilitated by molecular chaperones known as the heat shock proteins (HSPs), which require ATP for the folding process. The HSPs inhibit misfolding and aggregation. The process is facilitated by the action of Hsp70 (DnaK in bacteria), which binds substrates at high affinity in the ADP-bound state. The ATP-binding causes a reduction in the affinity for the substrate. The folding process is facilitated by the action of Hsp90 in the activation of client proteins such as hormone receptors and signaling kinases. Geldanamycin and radicicol, inhibitors of Hsp90, have potential anticancer properties. The GroEL/GroES complex (Hsp60/Hsp10) encloses the substrates in a cylindrical cage with a hydrophobic interior surface known as the Anfinsen cage. The 26S proteasome recognizes misfolded proteins with high specificity and degrades them in an ATP dependent reaction. The proteasome targets misfolded proteins for proteolytic degradation. The unfolded protein response (UPR) is activated in conditions where misfolded proteins accumulate in excess in the endoplasmic reticulum. UPR activation in pancreatic β-cells ultimately triggers apoptosis, thereby reducing insulin secreting cell mass and contributing to the pathogenesis of the type 2 diabetes mellitus (Liu et al., 2024a).

Hemoglobin: The Classical Model of Allostery

The $\alpha_2\beta_2$ hemoglobin tetramer has a low affinity for O_2 in the T (tense) or deoxygenated state and a high affinity for O_2 in the R (relaxed) or oxygenated state (Bellelli & Tame, 2022). The binding of the first O_2 molecule results in movement of the Fe^{2+} ion into the porphyrin plane, which causes the proximal histidine from F8 to be displaced and the salt bridges at the $\alpha_1\beta_1/\alpha_2\beta_2$ intarfaces to be disrupted; this results in a series of conformational changes that increase the affinity for the remaining O_2 molecules. This transition from the T/R state was described by Monod et al. in 1965 using the MWC model. The binding of O_2 is described by the Hill equation:

$$\theta = pO_2^n / (P_{50}^n + pO_2^n)$$

For hemoglobin, $n \approx 2.8$; for myoglobin, $n = 1$ (hyperbolic curve; no cooperativity). The P_{50} for hemoglobin is about 26 mmHg, indicating efficient release of oxygen in tissues. Myoglobin has a P_{50} value of about 1 mmHg and is nearly saturated at low partial pressures (Bellelli & Tame, 2022).

Heterotropic allosteric effectors regulate the physiological integration of hemoglobin. The Bohr effect: is mediated by the increased concentrations of CO_2 and H^+ in peripheral tissues, indicating increased metabolic rate, stabilizes the T conformation and facilitates the release of oxygen. The mechanism: Protons bind to the C-terminal His146 in the beta subunit and His122 in the alpha subunit and form additional salt bridges. The increased concentration of CO_2 reacts with the amino groups at the N-terminus and forms carbamino compounds. The T conformation is favored in both cases. 2,3-Bisphosphoglycerate (2,3-BPG) binds in the central cavity of deoxyhemoglobin and favors the T conformation. The electrostatic interactions between 2,3-BPG and deoxyhemoglobin reduce the affinity for oxygen. At high altitudes, the concentration of 2,3-BPG increases to maintain tissue oxygen supply. Fetal hemoglobin (HbF: $\alpha^2\gamma^2$) has a P_{50} value that is substantially lower (~19 mmHg vs. 26 mmHg for adult hemoglobin) due to the reduced

affinity for 2,3-BPG by the γ chains. The difference in the partial pressures maintains the concentration gradient for the transfer of oxygen from mother to fetus (Benner et al., 2026).

Hemoglobinopathies: Systemic Consequences of a Single Amino Acid Substitution

HbS: The Glu6Val mutation is responsible for sickle cell disease (SCD). In deoxy form, Val6 interacts with a hydrophobic pocket on a neighboring HbS molecule, consisting of Val1, Phe85, and Leu88, resulting in polymerization and causing sickle-shaped, rigid cells. The clinical features include chronic anemia, painful crises, splenic sequestration, and multi-organ infarcts. In heterozygotes, resistance to Plasmodium falciparum infection provides a survival advantage, leading to a high frequency in malaria-endemic areas (Brandow & Liem, 2022).

HbC / HbE: The Glu6Lys mutation in HbC results in a different tendency for crystal formation with a milder course of the disease, and compound heterozygotes for S and C have a moderate sickling disorder. The Glu26Lys mutation in HbE is common in Southeast Asia, where it results in a severe form of anemia when combined with beta-thalassemia (Sabath, 2023).

Methemoglobin: Methemoglobinemia occurs when heme iron is oxidized from Fe^{2+} to Fe^{3+}, making it incapable of binding oxygen. It may result from a deficiency of NADH-cytochrome b5 reductase or oxidizing agents like primaquine, dapsone, or benzocaine. Methemoglobin levels above 70% of total hemoglobin are life-threatening, and treatment with methylene blue activates NADPH-methemoglobin reductase (Sabath, 2023).

Phosphofructokinase-1: The Allosteric Regulator of Glycolysis

PFK-1 catalyzes the ATP-consuming conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, the committed and rate-

limiting step of glycolysis. This homotetrameric enzyme contains a catalytic site and several allosteric binding sites on each of its subunits. ATP functions as a substrate and an inhibitor. When ATP levels are high, it binds to the allosteric inhibitory site on the enzyme. This binding stabilizes the T state. ATP binding to the allosteric site shifts the sigmoidal substrate saturation curve to the right. AMP binding to the allosteric site replaces ATP. This binding stabilizes the R state.

The increased AMP to ATP ratio directly signals a cell-wide energy deficit. This increased AMP to ATP ratio activates the rate of glycolysis. Citrate binding to the allosteric site inhibits the activity of the PFK-1 enzyme. This inhibition suppresses the rate of glycolysis. This is the molecular basis of the Pasteur effect. Fructose-2,6-bisphosphate is the most potent allosteric activator of the PFK-1 enzyme. The concentration of this allosteric activator is regulated by the activity of the PFK-2 enzyme. This activity is regulated by the insulin to glucagon ratio (Lynch et al., 2024).

Tarui disease (Glycogen storage disease type VII):

Deficiency of the muscle isozyme of the PFK-1 enzyme (PFKM) leads to a complete absence of lactate production during exercise. This leads to muscle weakness and hemolytic anemia. McArdle disease is caused by the absence of the muscle glycogen phosphorylase enzyme (Li & Ye, 2024).

Glycolysis and Pyruvate Metabolism

Regulatory Control Points of Glycolysis

The ten-step glycolytic pathway yields a net of 2 ATP and 2 NADH per glucose molecule. Flux and directionality are governed by three irreversible enzymes: hexokinase/glucokinase (step 1), phosphofructokinase-1 (step 3), and pyruvate kinase (step 10). These enzymes are functionally replaced by their gluconeogenic

counterparts (glucose-6-phosphatase, fructose-1,6-bisphosphatase, and pyruvate carboxylase/PEPCK) preventing the simultaneous operation of both pathways and the energy dissipation of a futile cycle. Glucokinase, expressed in hepatocytes and pancreatic β-cells, displays sigmoidal kinetics with a Km of approximately 8--10 mM and functions as a glucose sensor; gain-of-function mutations cause persistent hyperinsulinemic hypoglycemia, whereas loss-of-function mutations underlie maturity-onset diabetes of the young type 2 (MODY-2). Erythrocyte pyruvate kinase (PK) deficiency is the most common hereditary glycolytic enzymopathy: reduced ATP production compromises Na^+/K^+-ATPase activity, impairing erythrocyte morphology and survival (Li & Ye, 2024).

Glycolysis and Cancer Metabolism

In 1924, Otto Warburg observed that tumor cells produce lactate even in the presence sufficient oxygen. This is also known as aerobic glycolysis or the Warburg effect. This was originally believed to be caused by defective mitochondria; however, it is now believed that the majority of cancer cells have normal mitochondria. The three key advantages for cancer cells to engage in aerobic glycolysis are: 1. increased ATP production rate, because the glycolytic rate of ATP production per unit time exceeds that of oxidative phosphorylation, despite the lower per molecule yield; 2. provision of biosynthetic precursors, as intermediates of glycolysis are diverted to the pentose phosphate pathway to produce NADPH and ribose-5-phosphate, glycine synthesis, and lipid biosynthesis; 3. creation of an acidic tumor environment by the secretion of lactate, which inhibits immune cell function and tumor invasion (Li & Ye, 2024).

The isoform of pyruvate kinase that is highly expressed in cancer cells is PKM2. It is allosterically activated by fructose-1,6-bisphosphate. Inhibition of PKM2 by tyrosine phosphorylation

(downstream of EGFR) and by phenylalanine reduced pyruvate production, thereby diverting upstream glycolytic intermediates towards biosyntheticpathways. This is an important adaptive mechanism that allows cancer cells to cope with hostile growth conditions such as hypoxia and nutrient starvation. Under hypoxic conditions, the expression of lactate dehydrogenase A and pyruvate dehydrogenase kinase 1 is increased by HIF-1α. The increased rate of lactate production and reduced rate of pyruvate entry into the TCA cycle minimize the rate of oxygen consumption by mitochondria in a hypoxic environment (Pavlova et al., 2022).

The Pyruvate Dehydrogenase Complex and Its Regulation

The pyruvate dehydrogenase complex, consisting of E1 (pyruvate decarboxylase), E2 (dihydrolipoyl transacetylase), and E3 (dihydrolipoyl dehydrogenase), is responsible for the irreversible conversion of pyruvate into acetyl-CoA, CO_2, and NADH. Five cofactors are required: thiamine pyrophosphate, lipoate, CoA, FAD, and NAD^+. The product inhibition of PDC is very potent, with increased levels of acetyl-CoA, NADH, and ATP activating PDC kinase, which phosphorylates E1, resulting in its inactivation, whereas low energy charge activates PDP, which dephosphorylates E1, restoring its activity. Ca^{2+} activates PDP, inhibiting PDK, thus increasing glucose oxidation in myocardium and skeletal muscles during exercise. Thiamine deficiency leads to PDC dysfunction, resulting in carbohydrate loading-induced Wernicke encephalopathy (confusion, ophthalmoplegia, and ataxia) and beriberi (neuropathy) (Stacpoole & McCall, 2023).

PDC deficiency: The disorder is inherited as an autosomal recessive trait, E1α subunits following an X-linked inheritance pattern, with clinical manifestations of lactic acidosis, hypotonia, encephalopathy, and developmental delay. A high-fat, low-carbohydrate diet is beneficial for increasing ketone bodies, which

are an alternative mitochondrial substrate that bypasses PDC, thus having therapeutic value.

The Tricarboxylic Acid (TCA) Cycle: The Hub of Central Metabolism

Cycle Chemistry and Anaplerotic Reactions

The TCA cycle consists of eight sequential steps that produce 3 NADH, 1 $FADH_2$, and 1 GTP for each acetyl-CoA fed into the cycle, which is sufficient for producing 10 ATP molecules. The TCA cycle is not only important for the breakdown of acetyl-CoA but also for biosynthetic purposes. Citrate is exported to the cytosol for fatty acid biosynthesis. Succinyl-CoA is committed to heme biosynthesis. Oxaloacetate is used for gluconeogenesis via the PEPCK pathway. α-Ketoglutarate is used for glutamate/glutamine biosynthesis and transamination of amino acids. The anaplerotic pathways are also important for replenishing the TCA cycle intermediates. The most important anaplerotic reaction is catalayzed by pyruvate carboxylase, a biotin dependent, ATP consuming enzyme expressed predominantly in the liver and kidney (Chenna et al., 2022). This enzyme converts pyruvate to oxaloacetate. Deficiency of biotin results in hypoglycemia and lactic acidosis.

IDH Mutations and Oncometabolites

The enzyme isocitrate dehydrogenase (IDH) is responsible for the oxidative decarboxylation reaction of isocitrate to alpha-ketoglutarate, CO2, and NADPH in the third step of the TCA cycle. Mutations in IDH1 were first identified in glioblastoma by genomic sequencing in 2008 and have since been recognized as key drivers in the development of glioma, acute myeloid leukemia (AML), cholangiocarcinoma, and chondrosarcoma (Solomou et al., 2023). Mutations in IDH1 and IDH2 that affect arginine residues critical for catalytic activity at arginine 132 in IDH1 and arginine 140 and 172

in IDH2 have a neomorphic enzymatic effect: instead of oxidizing isocitrate, the mutant enzyme reduces α-KG to D-2-hydroxyglutarate (D-2HG) (Solomou et al., 2023).

$$\alpha\text{-KG} + \text{NADPH} \rightarrow(\text{mutant IDH})\rightarrow \text{D-2HG} + \text{NADP}^{+}$$

While D-2HG is detectable only at trace levels in normal cells, IDH-mutant tumor cells contain D-2HG at millimolar concentrations, 50-fold above baseline levels. Due to structural similarity to α-KG, D-2HG acts as a competitive inhibitor of α-KG-dependent enzymes. The TET family of DNA demethylases is inhibited, impairing 5-methylcytosine hydroxylation and thereby promoting CpG hypermethylation. Similarly, KDM/JHDM histone demethylases and PHD-mediated prolyl hydroxylation of HIF-1α are inhibited, locking tumor cells into a hypermethylation phenotype that blocks differentiation. This epigenomic phenotype is designated the CpG Island Methylator Phenotype (G-CIMP). G-CIMP is associated with poor prognosis (Solomou et al., 2023).

Ivosidenib (IDH1 inhibitor) and enasidenib (IDH2 inhibitor) are FDA-approved drugs for D-2HG suppression and resolution of the leukemic differentiation block via a differentiation therapy approach (Wouters, 2021; Thomas et al., 2023).

Pathological Consequences of Succinate and Fumarate Accumulation

Succinate dehydrogenase (SDH/Complex II), being the sole complex common to both the TCA cycle and ETC, catalyzes succinate-to-fumarate conversion with the transfer of electrons directly to ubiquinone, without the transfer of protons, resulting in a lower yield of ATP for each FADH2 transferred. Inactivating mutations in SDHB, SDHC, and SDHD cause hereditary paraganglioma and pheochromocytoma, and in some cases GISTs. The pathway leading to these tumors involves: loss of SDH → succinate accumulation → competitive inhibition of prolyl

hydroxylase (PHD) → HIF1α stabilization → increased levels of VEGF and PDGF → pathological angiogenesis (Li & Ye, 2024). Inactivating mutations in fumarate hydratase cause fumarate accumulation, leading to hereditary leiomyomatosis and renal cell carcinoma (HLRCC). In addition, fumarate releases Nrf2 from Keap1, leading to activation of the antioxidant response pathway.

Oxidative Phosphorylation and Mitochondrial Disease

The Electron Transport Chain and ATP Synthesis

Oxidative Phosphorylation (OXPHOS) is the terminal reaction in the metabolic pathway wherein the reducing equivalents NADH and FADH2 generated in glycolysis and the TCA cycle are transferred to molecular oxygen. The liberated free energy is utilized to produce ATP. This is achieved by four integral protein complexes in the inner mitochondrial membrane (Complexes I to IV) and two mobile electron carriers: ubiquinol (CoQ_{10}) and cytochrome c. Complex I: NADH:ubiquinone oxidoreductase has 45 subunits. This complex transfers two electrons from NADH to ubiquinol. The reaction is catalyzed with the help of FMN and 7-8 iron-sulfur centers. The complex translocates four H^+ from the matrix to the intermembrane space. Complex II: Succinate:ubiquinol oxidoreductase transfers two electrons from FADH2 to ubiquinol. Complex III: ubiquinol:cytochrome c reductase translocates four H^+ from the matrix to the intermembrane space with the help of the Q-cycle mechanism. Complex IV: Cytochrome c oxidase catalyzes the reaction: $4^{e-} + O^2 \rightarrow 2H_2O$. This complex translocates four H^+ from the matrix to the intermembrane space (Blanc & Hummer, 2024). The proton motive force is:

$$\Delta p = \Delta\Psi - (2.303 \times RT/F) \times \Delta pH \ (\sim 180\text{-}220 \text{ mV})$$

85 to 90% of Δp is accounted for by the membrane potential (ΔΨ). The ATP synthase (V complex or F_1F_0 ATPase) is a rotary molecular motor that utilizes the rotation caused by the movement

of $3H^+$ to rotate the c-ring by 120° to synthesize one ATP from ADP and Pi (Boyer-Walker mechanism). The coupling is so tight that when ADP is depleted, Δp increases to slow Complex IV and thereby O_2 consumption. Uncoupling Protein 1 (UCP1)/thermogenin is found in brown adipose tissue and is responsible for dissipating the proton gradient to warm the body. This is particularly important for neonatal thermoregulation (Blanc & Hummer, 2024).

ETC Inhibitors and Therapeutic Connections

Each ETC complex has specific inhibitors, some of which have both toxicological and therapeutic relevance. Complex I: rotenone (insecticide; associated with Parkinson's disease); metformin (low-level Complex I inhibition → AMPK activation → suppression of hepatic gluconeogenesis; first-line therapy for type 2 diabetes mellitus). Complex III: antimycin A (research tool). Complex IV: cyanide (CN^- binds cytochrome a_3 → lactic acidosis and coma; antidote: hydroxocobalamin); carbon monoxide (CO, binds hemoglobin and cytochrome oxidase); azide (N_3^-). ATP synthase: oligomycin (research inhibitor); bedaquiline (targets mycobacterial ATP synthase; approved for tuberculosis treatment (Zhang et al., 2024). Uncouplers: dinitrophenol (DNP; historically used as a weight-loss agent, causing fatal hyperthermia); FCCP (laboratory tool). Importantly, the Complex I inhibition exerted by metformin is modest and tissue-specific; the risk of lactic acidosis is therefore confined to conditions of impaired renal function (Di Mauro et al., 2022).

Mitochondrial DNA and Hereditary Mitochondrial Diseases

Human mtDNA (16,569 bp, circular, maternally inherited) encodes 13 OXPHOS subunits, 22 tRNAs, and 2 rRNAs; the remaining approximately 1,500 mitochondrial proteins are nuclear-encoded, synthesized in the cytosol, and imported into the mitochondrial matrix via N-terminal targeting sequences.

Heteroplasmy (the co-existence of wild-type and mutant mtDNA within the same cell) accounts for clinical heterogeneity through a threshold effect: symptoms typically become apparent when the mutant mtDNA burden exceeds 60-80% (Zong et al., 2024).

MELAS (m.3243A>G): A point mutation in MT-TL1 damages $tRNA^{Leu(UUR)}$, impairing translation of Complex I subunits. Clinical features include stroke-like episodes, lactic acidosis, myopathy, sensorineural hearing loss, and diabetes mellitus. Diagnosis is supported by an elevated CSF lactate/pyruvate ratio (>20) and mtDNA mutational analysis.

MERRF (m.8344A>G): A mutation in $tRNA^{Lys}$ causes myoclonic epilepsy, cerebellar ataxia, and ragged-red fibers on Gomori trichrome-stained muscle biopsy.

LHON (m.11778G>A): A point mutation in the Complex I ND4 subunit gene produces bilateral central visual loss; predominantly affects young males, reflecting the high mitochondrial density of retinal ganglion cell axons.

Leigh syndrome: Mutations in SURF1, SDH, SDHA, or PDC subunits cause bilateral necrotic lesions in the brainstem and basal ganglia, with progressive neurodegeneration in early childhood (Khan et al., 2023).

Reactive Oxygen Species, Antioxidant Defense, and Oxidative Stress

Reactive oxygen species (ROS) are inevitable by products of aerobic respiration that, depending on their concentrations, manifest their roles either as essential second messengers or as potent tissue damaging agents. This dualistic character of ROS biology is arguably one of the most complex and clinically relevant issues in the study of medical biochemistry (Jomova et al., 2023).

In the context of oxidative phosphorylation, a percentage of the total electrons that are transported during the ETC process is transferred to molecular oxygen in a single step reaction to form the superoxide anion ($O_2^{\bullet -}$), with the percentage of total oxygen consumed under physiological conditions ranging from 0.2 to 2%. The sites of electron leakage to form the superoxide anion are two: the FMN prosthetic group and iron-sulfur clusters on the matrix face of Complex I under conditions of high NADH/NAD^+ ratios and high membrane potential. The elevated NADH/NAD^+ ratio is particularly important, as it promotes substantial reverse electron transport from the electron transport chain to Complex I as a result of the high membrane potential. Under conditions where the membrane potential exceeds 180 mV, this drives Complex I to the thermodynamic limit. This results in the direct and proportional relationship between mitochondrial overload and ROS formation.

The hierarchy of the chemical reactivity of ROS can be briefly characterized as follows. Superoxide anion ($O_2^{\bullet -}$) shows both oxidizing and reducing properties. Its half-life is on the order of microseconds. Superoxide anion is not capable of crossing cell membranes. H_2O_2 is more stable (its half-life ranges from milliseconds to seconds) and can cross membranes freely. It may act as a second messenger. The hydroxyl radical ($OH^{\bullet}$) is the most reactive ROS. Its half-life is on the order of nanoseconds. It can react with its target molecules at a rate approaching the diffusion rate. Peroxynitrite ($ONOO^-$) is formed via a reaction between superoxide anion and nitric oxide ($NO^{\bullet}$) occurring at a rate approaching the diffusion rate (Jomova et al., 2023). This reaction leads to the quenching of NO signaling as well as the nitration of protein tyrosine residues:

$$2\ O_2^{\bullet -} + 2H^+ \rightarrow (SOD) \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + Fe^{2+} \rightarrow (Fenton) \rightarrow OH^{\bullet} + OH^- + Fe^{3+}$$

$O_2^{\bullet -} + NO^{\bullet} \rightarrow ONOO^-$ (peroxynitrite, $k \approx 1.9 \times 10^{10}\ M^{-1}s^{-1}$)

The antioxidant defense system is organized into multiple enzymic and non-enzymic levels. The first line of antioxidant defense is provided by the superoxide dismutases (SODs). SOD2 is located in the mitochondrial matrix. SOD1 is located in the cytosol and the intermembrane space of mitochondria. Mutations of SOD1 are responsible for 20% of all familial ALS cases. SOD3 is located in the extracellular compartment and vascular wall. The second line of antioxidant defense is the elimination of H_2O_2. Catalase is located predominantly in hepatic peroxisomes;in erythrocytes, it is present in the cytosol (Liu et al., 2023). Catalase converts high concentrations of H_2O_2 to water and O_2. The Km for the reaction is so high that the rate of the reaction is proportional to substrate concentration. The glutathione peroxidases (GPx) are selenocysteine-containing enzymes (or cysteine containing in some isoforms) that reduce lipid hydroperoxides (LOOH) and H_2O_2 to their corresponding alcohols using reduced glutathione (GSH). GPx1 is located in the cytosol and mitochondria and reduces both LOOHs and H_2O_2. GPx4 reduces phospholipid hydroperoxides.

$$2\ GSH + H_2O_2 \rightarrow (GPx) \rightarrow GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \rightarrow (GR) \rightarrow 2\ GSH + NADP^+$$

A GSH/GSSG ratio below approximately 30:1 is generally considered indicative of oxidative stress. The third level of antioxidant defense is represented by the thioredoxin system, thioredoxin reductase, and peroxiredoxin, where thioredoxins (Trx1 in cytoplasm, Trx2 in mitochondria) are required for maintaining redox potential through NADPH, and peroxiredoxins (Prx1-6) are required for removing H_2O_2 and $ONOO^-$ at exceptionally high rates of catalysis ($k > 10^7\ M^{-1}s^{-1}$) (Jomova et al., 2023).

Non-enzymatic antioxidants: Vitamin E, also referred to as α-tocopherol, is a lipophilic membrane antioxidant that neutralizes

lipid peroxyl radicals (LOO•). Vitamin C, also referred to as ascorbate, regenerates Vitamin E. Ubiquinol, also referred to as $CoQ_{10}H_2$, is an antioxidant that acts in the mitochondrial membrane and cytosol and is under clinical development for mitochondrial disorders and heart failure. Lipoic acid is an antioxidant that functions in its oxidized and reduced forms, used clinically in diabetic neuropathy (Esteras & Abramov, 2022).

The transcription factor Nrf2 is the master antioxidant switch of the cell. Under normal circumstances, Nrf2 is bound to its adaptor protein Keap1, which is targeted for proteolytic degradation by the Cullin3-E3 ubiquitin ligase complex. Oxidative stress or electrophilic substances react with critical cysteine residues of Keap1 (Cys151, Cys273, Cys288), causing disruption of its interaction with Nrf2, which is released into the nucleus where it coordinates expression of antioxidant genes through its binding site antioxidant response element (ARE), including SOD2, catalase, GPx, heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), γ-glutamate-cysteine ligase (γ-GCL, rate-controlling enzyme of GSH synthesis), and ferritin. The Nrf2/ARE pathway is an important pharmacological target: sulforaphane, derived from glucosinolates in broccoli, is in phase II clinical trials, bardoxolone methyl, and dimethyl fumarate (Tecfidera, approved for multiple sclerosis) activate this pathway (Esteras & Abramov, 2022).

There are three major mechanisms of damage caused by oxidative stress. Lipid Peroxidation: The hydrogen atom from a polyunsaturated fatty acid (PUFA) is removed by either OH• or $ONOO^-$, leading to the formation of a lipid free radical (L•), which subsequently reacts with O_2 to form a peroxyl free radical (LOO•) that continues the free radical chain reaction by reacting with nearby PUFAs. Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are measurable indicators of lipid peroxidation, with 4-HNE also affecting the activity of enzymes by covalently modifying the lysine,

histidine, and cysteine residues. Protein oxidation: This involves the formation of a carbonyl group (C=O), the oxidation of methionine and cysteine residues, as well as the cross-linking and aggregation of proteins. The content of carbonyl groups in proteins, as measured by ELISA, is a common measure of oxidative stress. DNA oxidation: This involves the removal of a hydrogen atom from the DNA backbone by the hydroxyl free radical, leading to base modifications, as well as the oxidation of the sugar-phosphate backbone. The major oxidative product of DNA, 8-oxo-2'-deoxyguanosine (8-oxodG), is the most common form of oxidative DNA damage, which results in G:C → T: A transversions, a mutagenic event. 8-oxodG is a measure of oxidative stress, as its content can be detected in the urine (Chenna et al., 2022; Jomova et al., 2023).

The pathological impact of oxidative stress is well established in a variety of disease states. In the context of Parkinson\'s disease, Complex I activity in substantia nigra dopaminergic neurons is reduced by 30-35%, with the carbonylation and nitration of α-synuclein promoting the formation of Lewy bodies (Drouin-Ouellet, 2023). In type 2 diabetes mellitus, the hyperglycemia that is a hallmark of the disease leads to the formation of advanced glycation end products (AGEs), which binds to their receptor (RAGE) to activate NF-Kb and cytokine release. This is the molecular mechanism underlying diabetic vascular complications. In atherosclerosis, oxLDL is the initiating event for the formation of foam cells from macrophages. The protective effect of SOD3 in the vasculature has been confirmed. In Alzheimer\'s disease, amyloid-β inhibits Complex IV of the electron transport chain, leading to an imbalance in the Ca^{2+} gradient in the neuron. This activates the cascade leading to the energy failure and apoptosis of the neuron. Ischemia-reperfusion injury is a paradox in the context of ROS. The moment of tissue reoxygenation is the moment of maximal ROS formation. Hypoxanthine is accumulated during the ischemic event.

This is converted to $O_2\bullet^-$ by xanthine oxidase when reperfusion occurs. The abrupt restoration of the mitochondrial proton gradient is a second source of ROS.

The idea of redox signaling demonstrates that ROS have regulatory activities at physiological concentrations: "H_2O_2 transiently oxidizes the catalytic cysteine residue (Cys-SOH) of tyrosine phosphatases to enhance kinase-mediated signal transduction." The H_2O_2 burst caused by the activation of the PDGF receptor, EGF receptor, and insulin receptor is an enhancement of downstream signaling via this mechanism (Sies et al., 2022). The stabilization of HIF-1α, the activation of AMPK, and the translocation of Nrf2 are all ROS-induced adaptive responses. The balance between physiological redox signaling and pathological oxidative damage, within this narrow range of concentrations, is one of the most important homeostatic controls in the life of a cell.

Membrane Protein Biochemistry: Ion Channels, Pumps, and Disease

Na^+/K^+-ATPase and Cellular Ion Homeostasis

Na^+/K^+-ATPase is a P-type ion pump located in the plasma membrane of all animal cells. The hydrolysis of one ATP molecule results in the electrogenic extrusion of $3Na^+$ and the electrogenic uptake of $2K^+$. The electrochemical gradient is essential for nerve impulse conduction, muscle contraction, secondary active transport mechanisms such as SGLT (Na^+-coupled glucose absorption) and NKCC ($Na^+/K^+/2Cl^-$ cotransport for renal salt balance), and cell volume control. Between 20 and 40% of the basal energy expenditure in the brain is spent in maintaining this pump. During action potentials in neurons, this percentage is transiently increased to meet the high ATP demands. The cardiac glycosides digoxin and digitoxin inhibit the E2 conformation of the enzyme at the K^+ binding site. The increased intracellular concentration of Na^+ slows

the Na^+/Ca^{2+} exchanger (NCX) and increases Ca^{2+}. The increased Ca^{2+} has a positive inotropic effect. The therapeutic dose is very narrow. The toxic effects include ventricular arrhythmias, nausea, and visual disturbances. In primary hyperaldosteronism or Conn syndrome, increased aldosterone causes increased expression of Na^+/K^+-ATPase in the collecting duct. The increased Na^+/K^+-ATPase causes increased Na^+ retention and K^+ loss. The increased aldosterone causes hypernatremia and hypokalemia and hypertension. The most common cause is a suprarenal adenoma or Conn adenoma (Zhang et al., 2024).

Voltage-Gated Ion Channels and Channelopathies

The voltage-gated Na^+ channel has four homologous domains (I-IV) that each have six segments (S1-S6) that traverse the membrane. The arginine residues on S4 segments act as voltage sensors. S5-S6 segments contain a Na^+ filter (DEKA motif), whereas the III-IV linker is responsible for fast inactivation by occluding the pore (ball-and-chain model). Loss of function mutations in SCN1A (Nav1.1) predominantly affect GABAergic inhibitory interneuron firing patterns, resulting in a deranged balance between excitatory and inhibitory input, leading to Dravet syndrome (a severe febrile-onset epileptic encephalopathy). Gain-of-function mutations in SCN4A (Nav1.4) result in abnormal muscle Na^+ influx, leading to paramyotonia congenita or hyperkalemic periodic paralysis (Montserrat-Canals et al., 2025). SCN5A (Nav1.5) channel dysfunction leads to two distinct syndromes: long QT syndrome type 3 a gain-of-function defect causing prolonged action potentials and torsades de pointes; and Brugada syndrome, a loss-of-function defect manifesting as ST elevation in right precordial leads and a high risk of sudden cardiac death.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a Cl^- channel belonging to the ABC transporter

superfamily. The channel gating is regulated by the phosphorylation of the R domain by PKA and ATP binding to NBD1/NBD2. The ΔF508 mutation causes misfolding of the CFTR protein in the endoplasmic reticulum, resulting in degradation by the proteasome and a consequent reduction of the amount of mature CFTR on the surface membrane. The resulting thick mucus plug formation in the pancreas, lungs, liver, and reproductive organs is referred to as cystic fibrosis. Correctors such as lumacaftor, tezakaftor, and elexacaftor correct protein misfolding; ivacaftor is a potentiator that increases channel open probability; and the triple combination therapy Trikafta (elexacaftor/tezakaftor/ivacaftor) has shown dramatic results for patients with the ΔF508 mutation (Liu et al., 2024a).

Ca^{2+} Signaling, SERCA, and Clinical Implications

The intracellular Ca^{2+} concentration (\[Ca^{2+}\]) increases from 100 nM at rest to the micromolar range upon stimulation. The 10,000-fold dynamic range in Ca^{2+} concentration makes Ca^{2+} a potent second messenger. The main Ca^{2+} entry pathways are: 1. voltage-gated Ca^{2+} channels (VGCCs), which trigger contraction in cardiac and skeletal muscles. The dihydropyridine receptor is a VGCC that operates as a mechanosensor in skeletal and as an L-type Ca^{2+} channel in cardiac muscle. 2. Inositol 1,4,5-trisphosphate receptors (IP_3Rs) located in the ER membrane. IP_3R is activated via the phospholipase C-γIP_3 pathway. 3. Ryanodine receptors (RYRs), which trigger contraction in skeletal muscle via a mechanical link to the dihydropyridine receptor and in cardiac muscle via Ca^{2+}-induced Ca^{2}^+^ release (CICR). The sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} back into the ER/SR lumen, consuming ATP in the process, returning [Ca^{2+}] to baseline levels; phospholamban tonically inhibits SERCA in cardiomyocytes, whereas β-adrenergic agonists increase Ca^{2+} release into the cytoplasm through PKA-dependent phosphorylation of phospholamban relieving this inhibition. Calcineurin is a

Ca^{2+}/calmodulin-dependent Ser/Thr phosphatase that dephosphorylates NFAT, allowing its translocation into the nucleus where it activates cytokine (IL-2) and cardiac hypertrophy genes; immunosuppressive agents cyclosporin and tacrolimus block this pathway by inhibiting calcineurin phosphatase activity. In malignant hyperthermia, gain-of-function mutations in RYR1 result in uncontrolled Ca^{2+} release from the sarcoplasmic reticulum, triggering sustained muscle contraction and life threatening hyperthermia (Zhang et al., 2024).

Conclusion

In this chapter, five fundamental aspects of medical biochemistry, namely, protein structure and allosteric regulation, cellular energy metabolism, the tricarboxylic acid cycle and oncometabolism, oxidative stress, and membrane protein physiology, are discussed in an integrative framework in which each level of mechanism is directly linked to clinical disease. In the biochemical cascade, each step is linked to its antecedent and consequent steps, and pathology is felt as a whole, not in isolation.

The aforementioned examples, including the single amino acid substitution in the hemoglobin β-chain that changes the shape of the erythrocyte and affects circulatory function, the process by which molecular chaperones prevent misfolding and disease, and the consequences when these processes fail, the pathway by which an IDH mutation causes a buildup of a single TCA metabolite that leads to epigenomic reprogramming of the cancer cell, the dual role of ETC-derived ROS as both signal molecules and insults, the integrated regulation of antioxidant genes through the Nrf2/ARE pathway, and the process by which a single amino acid substitution in an ion channel causes systemic channelopathy.

Advances in next-generation sequencing, proteomics, and metabolomics are enabling the molecular characterization of an

ever-expanding repertoire of diseases. Therapeutic approaches that are directly derived from biochemical mechanisms, such as inhibitors of IDH, correctors of CFTR, activators of Nrf2, cardiac drugs targeting Na^+/K^+-ATPase, and RYR1 modulators, are examples of this trend. Understanding, interpreting, and translating these advances into clinical practice, as ever, demands a strong and deep biochemical foundation, which remains the bedrock of medical education and science.

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CHAPTER 2

METABOLIC REGULATION OF ION CHANNELS

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Introduction

The development of metabolic disorders is a result of aberrant intracellular signaling pathways. Approximately 75% of more than 500 million people living with diabetes worldwide have cardiovascular and/or renal complications; this figure is continually rising and underlines the need for developing novel therapeutic strategies that go beyond glucose control and target pathological pathways at the level of ion channels. In this context, ion channels initiate signal transduction pathways by transporting ions across cell membranes and control energy homeostasis. The decades long use of ion channel modulators in diabetes treatment represents the most clinically tangible manifestation of this regulatory role (Wu et al., 2023). There are two main mechanisms that control ion channel functions in relation to energy homeostasis and the development of metabolic disorders: protein-channel interactions and post-translational modifications (Wu et al., 2023).

The wide range of ion channels, including the classical voltage-gated channels (Kv, Nav, Cav), two-pore-domain potassium channels (K2P), ATP-sensitive KATP channels, the transient receptor potential channels (TRPC), the hyperpolarization-activated cyclic nucleotide-gated channels, and the epithelial sodium channels (ENaC), forms the basis of the multilayered interface between cellular metabolism and electrical activity, as mediated through the ATP/ADP ratio, the NAD^+/NADH redox pair, ROS, phosphoinositide microdomain physics, lipid rafts, and protein kinase cascades. The advent of the use of cryo-electron microscopy (cryo-EM) in recent years has shed new light on the mechanisms of the interface between channel subunits and their ligands, as elucidated through this technique (Driggers & Shyng, 2023; Patton et al., 2024).

Biophysical Foundations: Channel Structure, Gating Kinetics, and Metabolic Sensitivity

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Voltage-gated channels (VGC) have a four-subunit structure that spans the membrane and intracellular C and N termini that include phosphorylation sites for protein kinases, surfaces for binding phosphoinositides, and redox-sensitive cysteine residues that form the structural basis for channel metabolic sensitivity. The foundational description of channel gating kinetics in VGCs was established by Hodgkin and Huxley through voltage clamp experiments conducted in squid giant axons. The Hodgkin-Huxley formulation provided a mathematical basis for the kinetics of Na^+ activation (m), Na^+ inactivation (h), and K^+ activation (n) channels in VGC and is still used to describe the collective behavior of these channels. More recently, multistate Markov kinetic models have been employed to characterize the gating kinetics of individual channels.

The Goldman Hodgkin Katz (GHK) current equation is the fundamental biophysical mechanism underlying the regulation of membrane potential and ionic permeability. Metabolic variables modulate this system through the channel permeability coefficient or changes in intracellular or extracellular ion concentration (Wu et al., 2023). PIP2 is a critical lipid second messenger in the regulation of the function of KATP channels, Kir channels, TRP channels, and TREK channels. The concentration of PIP2 (phosphatidylinositol 4,5-bisphosphate) in the cell membrane is dynamic, fluctuating in response to the cellular metabolic state via the PI3K-PTEN-PLC signaling cascade. This is the fundamental biophysical mechanism through which the cell membrane integrates the effects of metabolism and electrical activity (Jensen et. al., 2022). The role of the K134 residue of the SUR1 subunit of the PIP2-sensitive KATP channel has been defined at the atomic level by recent cryo-EM structures (Driggers & Shyng, 2023; Patton et al., 2024).

Metabolic Sensor Channel Families: KATP, TRP, K2P, and HCN

This section describes the fundamental structural and functional characteristics of these four metabolic sensor channels, followed by a discussion of the role of each of these families of channels in diabetic pathology, as well as their assessment as potential targets for drug therapy.

KATP Channels: From Structure to Tissue Specificity

ATP-sensitive potassium (KATP) channels are the most studied group of channels that have been shown to be involved in a direct relationship between cellular energy status and membrane excitability. The KATP channel is a hetero-octamer that contains four Kir6.x subunits and four sulfonylurea receptor subunits in a 4:4 stoichiometric ratio. The high-resolution structure of KATP channels has been resolved by cryo-electron microscopy (Patton et al., 2024). In pancreatic beta cells, KATP channels containing Kir6.2 and SUR1 subunits detect increased ATP levels due to glucose metabolism and close in response to increased ATP levels, causing depolarization and insulin release. In pathological states characterized by impaired glucose metabolism, reduced ATP levels fail to promote KATP channel closure, thereby impairing insulin release (Ashcroft F.M., 2023). In cardiac myocytes, KATP channels containing Kir6.2 and SUR2A subunits open in response to a rapid decline in ATP/ADP ratio during ischemia and reduce action potential duration, thereby exerting a cardioprotective role (Brennan et al., 2024). In neurons, KATP channels are abundantly expressed in the

hippocampus, amygdala, and substantia nigra and reduce neuronal firing rates in response to hypoxic stress and exert a neuroprotective role (Lv et al., 2022).

TRP Channel Family: Sensors of Redox Stress, Hypoxia, and Lipotoxicity

The transient receptor potential (TRP) channel family is a group of multimodal sensor proteins activated under metabolic stress conditions, (including oxidative load, hypoxia, and lipotoxicity) and transduce these stimuli into Ca^{2+} signals (Wu et al., 2024). TRPM2 is a Ca^{2+} permeable cation channel activated by the production of ADP-ribose (ADPR) under the influence of accumulated intracellular ROS, resulting in apoptosis of pancreatic beta cells and initiating mitochondrial fragmentation. TRPA1 is a highly cysteine oxidation-sensitive redox sensor whose activity is suppressed under normoxic conditions and markedly enhanced under hypoxic conditions. It is involved in the mediation of diabetic neuropathy-induced pain by methylglyoxal. TRPV4 exacerbates mitochondrial dysfunction by increasing the intracellular Ca^{2+} content under lipotoxicity caused by palmitate and diacylglycerol, resulting in glycolytic reprogramming (Wu et al., 2024).

K2P Channels: Background Currents and Metabolic Sensing

The family of two-pore domain potassium channels, comprised of KCNK1-KCNK18, is responsible for transmitting voltage-insensitive leak potassium currents, which help to maintain the membrane potential and provide a counterbalance against depolarizing influences. TASK-1 (K2P3.1) and TASK-3 form the main constituents of the carotid body chemoreceptor mechanism, which is sensitive to extracellular pH and oxygen tension. Significantly, TASK-1 knockout mice have shown a reduced hypoxic ventilatory response by approximately 68%, emphasizing the importance of this channel in metabolic hypoxia sensing (Buehler et al., 2017). TREK-1, a multifunctional K2P channel, is modulated by arachidonic acid, mechanical stretch, and lysophospholipids. Studies focused on modulating TREK-2 using nanobodies have provided a framework for selective pharmacological targeting of these channels (Rödström et. al., 2024).

HCN Channels: Pacemaker Activity and Metabolic State

Hyperpolarization-activated cyclic nucleotide-gated channels (HCN1-4) differ from classical voltage-gated channels in that they open in hyperpolarized states and are sensitive to intracellular signals from cellular metabolism via the cyclic nucleotide-binding domain (CNBD). Binding of cyclic adenosine monophosphate (cAMP) to hyperpolarization-activated cyclic nucleotide-gated channels 2 and 4 shifts the activation curve to more depolarized membrane voltages and increases heart rate, thereby providing a molecular basis for adrenergic and parasympathetic control of heart rhythm. Two independent studies have demonstrated cooperative cAMP binding to closed HCN channels (Porro et al., 2024; Kuschke et al., 2024). In addition, the reduction in amyloid-β peptide formation by Aβ40 and Aβ42 upon silencing HCN2 suggests that modulation of hyperpolarization-activated cyclic nucleotide-gated channels may be a strategy in treating neurodegenerative disorders.

Voltage-Gated Na^+ and K^+ Channels: Isoform Specificity and Metabolic Regulation

Nav Channels: Ischemic Cascade, Diabetic Nociception, and Remodeling

Nine isoforms of voltage-gated sodium channels (Nav), Nav1.1 to Nav1.9, have been found in vertebrates. These isoforms have different tissue expression, inactivation properties, and post-translational regulation capacity. The pathophysiology of Nav channels in ischemic heart disease has been extensively reviewed (Zang et al., 2025). Nav1.5, encoded by the SCN5A gene, is the sodium channel involved in the rapid depolarization phase of the cardiac action potential. This sodium channel is rendered dysfunctional through at least three parallel metabolic pathways in ischemic heart disease: (i) the reduction of cellular energy through the depletion of adenosine tri-phosphate activates AMP-activated protein kinase, leading to the phosphorylation of Nav1.5 at the N-terminal threonine 101 residue and subsequent targeting of the sodium channel for degradation by the lysosome; (ii) the accumulation of lactic acid in ischemic heart disease reduces the extracellular pH, and the protonation of the sodium channel at the critical arginine and histidine residues at positions 373 and 880, respectively, increases the arrhythmogenic late sodium current; (iii) the increase in the intracellular concentration of reactive oxygen species activates the enzyme CaMKII, leading to the phosphorylation of Nav1.5 at Ser571 and increasing the arrhythmogenic late sodium current (Zang et al., 2025). In the context of diabetic hyperglycemia, methylglyoxal modifies Nav1.8 through the formation of MG-arginine adducts, which reduce the channel's inactivation rate; LTGO-33, a selective Nav1.8 inhibitor with a distinctive pharmacological profile, acts by counteracting this mechanism (Gilchrist et al., 2024).

Kv Channels: Repolarization Dynamics and Multilayered Metabolic Coupling

The regulation of Kv channel functions by metabolism follows four main axes: pyridine nucleotide redox sensitivity of Kvβ auxiliary subunits; phosphorylation by protein kinases such as PKA, PKC, CaMKII, and AMPK; dependence upon PIP2 and lipid bilayer environment; and finally, redox regulation by cysteine oxidation and S-nitrosylation. The role of Kv1.5 in generating ultrarapid delayed rectifier current in human atrial myocytes and that of NAD(P)H binding by Kvβ2 in accelerating inactivation kinetics of Kv1 channels are two examples that connect cardiac energy metabolism with electrical functions in the heart (Kim et. al., 2025). The slow delayed rectifier current in ventricular myocytes is generated by a complex between Kv7.1 and KCNE1; in this context, a high genome-wide association study significance of KCNQ1 gene variants such as rs2237892 and rs2237895 with type 2 diabetes underlines its role in this context in a mechanistic framework (Lubberding et al., 2022). The hERG channel is downregulated by 20-30% in models of diabetic cardiomyopathy; however, post-transcriptional downregulation by miR-1 and miR-133a represents the epigenetic component in this context (Lubberding et al., 2022). The transient outward current in ventricular myocytes in the heart is generated by a complex between members of the Kv4 family such as Kv4.2 and Kv4.3 and channel-interacting proteins such as KChIP2 and KChIP3; significant downregulation of KChIP2 in models of diabetic cardiomyopathy leads to a reduction in transient outward current density and prolongation in action potential duration, thereby reducing the arrhythmia threshold in this context; this underlines the importance of the Kv4 family in this context (Wu et. al., 2023).

Redox-Sensitive Calcium Channels: Cav1.2 and NMDA

The L-type voltage-dependent calcium channel Cav1.2 is still vulnerable to the functional inactivation by S-glutathionylation and S-nitrosylation in the context of chronic hyperglycemia and oxidative stress. The above post-translational modifications trigger a vicious cycle characterized by increased levels of diastolic Ca^{2+} loading, cell death, and caspase activation. The regulatory potential of thiol-based redox switch in Nav, Kv, and TRP channels has been reviewed (Orfali et. al., 2025). In the context of hypoxic and oxidative stress conditions, the NMDA receptor/channel complex is subjected to hyperactivation due to the inhibition of the zinc-mediated regulatory mechanism and the cysteine-based nitrosylation.

Metabolic Regulation of Channel Trafficking and Membrane Surface Expression

The functional characteristics of ion channels are also regulated by the density of the channel proteins in the cell membrane. The remodeling of ion channels is a dynamic process in which there is a constant interplay between endoplasmic reticulum quality control, Golgi complex trafficking, anterograde transport to the membrane, and reversible endocytosis. The role of the ubiquitin-proteasome pathway is critical in regulating ion channel density in the membrane. The ubiquitin–proteasome pathway operates through E1-E2-E3 enzyme complex-mediated ubiquitination of lysine residues in channel proteins, thereby activating either endocytosis or lysosomal degradation (Foot et. al., 2017). The Nedd4/Nedd4-2 ubiquitin ligase family is implicated in the endocytosis of KATP, Nav, Kv7 (KCNQ), and ENaC channels through the binding of the PPXY motif in the C-terminal region of the channel proteins. Nedd4-2 is regulated by SGK1 and AMPK in the context of diabetic hyperglycemia and metabolic disorders. Liddle syndrome arises from loss-of-function mutations in Nedd4-2, resulting in ENaC retention at the plasma membrane and consequent hypertension (Kim et. al., 2025). Deubiquitination approaches using nanobody deubiquitinase fusion proteins have been effective in correcting the delayed rectifier K^+ currents and action potential duration in a long QT syndrome type 1 cardiomyocyte model (Darko-Boateng et.al., 2025, Foot et. al., 2017). The quality control mechanism in the endoplasmic reticulum indicates that the glycosylation patterns in the context of diabetes mellitus affect the membrane density of hERG, CFTR, and Nav1.5. This is reversed by pharmacological chaperones such as ivacaftor in the context of specific mutations in the CFTR channel. This reverses the delayed rectifier K^+ currents and action potential duration in a LQT type 1 cardiomyocyte model (Darko-Boateng et.al., 2025, Foot et. al., 2017).

Mıtochondrial Ion Channels: Structure, Metabolic Control, And Cardioprotection

mitoKATP: Molecular Identity Debate and Functional Significance

ATP-sensitive potassium (mitoKATP) channels were first recorded in rat liver mitoplasts in 1991. Kir6 and SUR proteins have no mitochondrial targeting signal, and their presence is not recorded in any mitochondrial proteome databases. However, there is evidence that short splice variants of SUR2 may exist in mitochondria. While pharmacology data provide increasing support for the idea that respiratory chain Complex II (succinate dehydrogenase) is a component of mitoKATP, the molecular identity of these channels remains a mystery. The function of these channels includes opening during ischemic preconditioning (IPC), mild

depolarization of mitochondrial matrices, refinement of specificity of reactive oxygen species (ROS) signals, and cardioprotective signal cascades (Hausenloy et al., 2020).

MCU Complex: Subunit Architecture and Ca^{2+} Gating Control

The molecular composition of the mitochondrial calcium uniporter (MCU) complex was identified in 2011 by two separate studies. The complex contains the calcium-permeable channel subunit MCU and its dominant-negative isoform MCUb, the obligatory regulator subunit EMRE, the MICU1 and MICU2 Ca^{2+} gating control subunit heterodimer, and the positive regulator subunit MCUR1, which form a heterogeneous complex in the inner mitochondrial membrane with a mass of 450-800 kDa. In low Ca^{2+} conditions, MICU2 has a negative effect that keeps MCU in a closed state; in high Ca^{2+} conditions in the cytosol, MICU1 activates the channel to open it. In pancreatic beta cells, MCU and MICU1-mediated Ca^{2+} accumulation regulates glucose metabolism and insulin release; in cardiac ischemia/reperfusion injury, MCU-mediated Ca^{2+} uptake leads to the opening of mPTP and release of cytochrome c into cytosol (Hausenloy et al., 2020).

mPTP: Molecular Identity and Causal Significance in Cardiac Injury

The molecular structure of the mitochondrial permeability transition pore (mPTP) continues to be an area of active research. The current data provide the foundation for the proposal that the c-subunits of the ring structure of the enzyme ATP synthase may form the mPTP. Cyclophilin D (CypD) is a well-characterized regulatory subunit that modulates the mPTP by binding to the adenine nucleotide translocator (ANT); inhibition or elimination of this regulatory subunit has significant cardioprotective effects in the setting of cardiac ischemia/reperfusion injury. Pharmacological inhibition of the mPTP by the use of the CypD inhibitors cyclosporin A, NIM811, and Debio-025 positions this pathway as a potential therapeutic target for cardioprotection (Hausenloy et. al., 2020).

Ion Channel Pathology in Diabetes And Metabolic Syndrome

Metabolic syndrome is a complex clinical concept that includes insulin resistance, hyperglycemia, hyperlipidemia, and hypertension. Its presence in the domain of ion channel disorders is multifaceted. Hyperglycemia-induced arrhythmic substrates, together with impaired insulin release, are determined by the two main electrophysiological parameters of metabolic syndrome: KATP channel malfunction in pancreatic beta cells, and increased late Na^{+} current through Nav1.5 channels in cardiac myocytes. The mechanism of diabetic cardiomyopathy, together with its management, is reviewed elsewhere (Julian et. al., 2024).

Hypersensitization of TRPA1 and TRPV1 channels to metabolite activation is well established in models of diabetic neuropathy, with methylglyoxal being a key metabolite that activates TRPA1 channels, thus establishing its role as a potential therapeutic target (Wu et al., 2024). ENaC and SGLT2 dysfunctions contribute to hyperfiltration, proteinuria, and tubular damage. The cardio-renal benefits of SGLT2 inhibitors, such as dapagliflozin, empagliflozin, and canagliflozin, have been demonstrated in RCTs, while their wide range of mechanisms, including effects on KATP and ENaC, is currently under investigation (Bhatt et al., 2021). AMPK modulates Kir6.2, Nav1.5, ENaC, CFTR, TREK-2, and Kv7.1 channels through direct phosphorylation or activation of the Nedd4-2 ubiquitin ligase, providing a wide range of

adaptive responses to metabolic stress, thus providing a comprehensive response to metabolic disorders (Kim et. al., 2025).

Therapeutic Strategies: From Channel Modulation to Gene Therapy

Among these approved ion channel modulators, those with the most extensive experience are the sulfonylureas, such as glibenclamide and glipizide, which have reduced KATP channel activity in beta cells, increasing insulin secretion for about six decades (Ashcroft, F.M., 2023). Nicorandil, which activates KATP channels while releasing nitric oxide, has cardioprotective effects, while ranolazine reduces arrhythmic risk in diabetic cardiomyopathy by inhibiting Nav1.5, a late sodium current (Zang et al., 2025). The cardiorenal benefits of SGLT2 inhibitors have already been established clinically by the EMPA-REG OUTCOME and DAPA-HF trials, while the wide range of their mechanisms of action, including KATP and ENaC channels, is still an active area of investigation (Bhatt et al., 2021).

Ivacaftor reduces heart rate by selectively inhibiting the HCN4 pacemaker channel. Among these agents, those undergoing investigation, such as the Nav1.8 selective inhibitor LTGO-33, have shown promising efficacy in models of diabetic pain (Gilchrist et al., 2024). Finally, antagonists of TRPA1, such as those targeting methylglyoxal-induced pain, have a prominent place on the research agenda, especially as these agents were the first to be discovered, targeting diabetic neuropathic pain (Wu et al., 2024).

The PIP2-dependent modulation of ion channels forms an overarching theory for the complex relationship between lipid metabolism and channel function (Jensen et. al., 2022). Thiol redox switches are specific targets for pharmacological modulation of redox status; NRF2 activators increase intrinsic antioxidant pathways and thus indirectly alleviate channelopathies (Orfali et. al., 2025). Combination therapy approaches include the co-administration of an SGLT2 inhibitor and ranolazine for the synergistic treatment of diabetic cardiomyopathy, as this strategy simultaneously targets metabolic and arrhythmia-related determinants. Clinical trial outcomes also define the current borders of this field. The lack of efficacy for Nav1.7 inhibitors in human studies for diabetic neuropathy, even in rodent models for pain, emphasizes the need for species-specific considerations and the fact that channelopathies cannot be assessed in isolation but must be placed in the context of their networks (Zang et al., 2025). Regarding gene therapy approaches for channelopathies, KCNJ11/Kir6.2 and ABCC8/SUR1 are the specific genes targeted by base editing and prime editing approaches. The proven efficacy of canonical base editors CBE and ABE in preclinical studies has fueled interest in these approaches for channelopathies (Lv et al., 2022).

Research Methods: Patch-Clamp, cryo-EM, and iPSC Models

The patch-clamp technique remains the main methodological paradigm in the study of ion channels, with the whole-cell configuration commonly used for the measurement of the macroscopic current density, as well as the kinetics of activation and inactivation, while the cell-attached and inside-out configurations are the preferred choices for the measurement of the kinetics of single channels as well as the single channel conductance. The significant technical requirements for the manual patch-clamp technique have led to the development of automated patch-clamp technology, which is now widely used in drug development as well as in the

evaluation of cardiac safety, including hERG testing, while the integration of the latter with cryo-EM structural data has revitalized the structure-based drug design approach, significantly speeding up the drug discovery process targeting ion channels (Rogers et al., 2024).

The use of cryo-electron microscopy allows for the visualization of the structure of channel proteins at the atomic level under conditions closely resembling the physiological lipid environment. This helps in the determination of the structure and the sites of action of drugs for KATP, HCN, TRP, and Nav channels. One of the important technical advances in the study of metabolic channels has been the use of the lipid nanodisc technique for the reconstitution of the channel protein and its subsequent visualization. This technique allows the native lipid environment of the channel, composed of PIP2, cholesterol, and sphingomyelin, to be preserved and the impact of metabolic ligands such as ATP, ADP, and long-chain acyl-CoA on the channel structure to be studied. With the increasing size of cryo-EM data sets, artificial intelligence-assisted classification approaches that are able to distinguish different conformational states such as closed, open, and inactive states in heterogeneous data sets (demonstrated by cryoDRGN and 3D variability analysis) begin to emerge as tools for the resolution of ion channel structure. In the field of electrophysiology, machine learning-based current analysis approaches are used for the detection of gating state transitions in single-channel patch clamp data sets more efficiently and with higher accuracy than human-based approaches (Rogers et al., 2024). Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and neurons (iPSC-Ns) retain patient-specific genetic information, thus narrowing the gap between conventional animal models and human clinical observations. The use of metabolic maturation media, nanopatterning, and electrical stimulation allows the Na^+, K^+, and Ca^{2+} current density of iPSC-CMs to more closely resemble those of mature cardiomyocytes, making such models more clinically relevant to drug-induced cardiotoxicity studies (Lesche et al., 2025). Techniques such as cryoDRGN and 3D variability analysis are being increasingly used in ion channel studies; such techniques allow multiple functional states to be resolved from a single dataset.

Conclusion

The translational failure rate for drugs targeting ion channels is known to be substantial. Pharmacological divergence of KATP channels in rodent models is pronounced when compared with human KATP channels, and the inability of Nav1.7 inhibitors to meet primary endpoints in clinical trials for diabetic neuropathy, where analgesic efficacy is demonstrated both in human and rodent models, underscores the fact that the modulatory network in vivo is not fully understood (Zang et al., 2025). The common denominator for the failure of drugs targeting ion channels includes problems of species specificity as well as the inadequate modeling of the coordination of different channel types. Thus, the use of human genetics, cryo-EM structure, animal models, and induced pluripotent stem cell technology is becoming more and more crucial for the success of clinical trials (Rogers et al., 2024).

The coordination and interplay between the various mechanisms of metabolic sensitivity and their cross-talk between various ion channel types have remained a central question that has yet to be fully resolved; specifically, how various types of ion channels coordinate in a single cell type remains a question that has yet to be sufficiently addressed by computational models (Wu et al., 2023). The epigenetic aspect of this coordination and interplay between

various ion channels has also become more apparent in that hyperglycemia can lead to alterations in the promoter methylation status of genes such as KCNJ11, KCNK3, and SCN5A and thereby create a long-term memory for channel expression. Thus, it appears that transient normalization of glycemia may be insufficient to reverse this epigenetic memory in ion channels (Lubberding et al., 2022; Zang et al., 2025).

The stratification of patients based on biomarkers has been shown to play an important role in the closure of the translational gaps. The current research efforts are centered around the level of MG-arginine adducts in Nav1.8, the mutation burden of the KATP channel subunit Kir6.2, and the expression profile of the mitochondrial calcium unipporter complex subunits MICU1 and MICU2 as potential pharmacodynamic biomarkers. The integration of precise genetic diagnosis with molecularly targeted therapy approaches, such as base editing of the KATP channel and the development of Nav1.8 inhibitors, has the potential to revolutionize the field of ion channel therapeutics in the context of metabolic diseases. From the findings reviewed in this chapter, it is evident that the metabolic regulation of ion channels represents a multidimensional system with interconnected biophysical, biochemical, and epigenetic levels. Channel cross-talk needs to be included in the current models, the predictive capacity of human iPSC-based studies needs to be enhanced, and biomarker-based patient stratification needs to be an integral part of the clinical development programs for success.

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CHAPTER 3

The Multidimensional Relationship Between Low LDL Cholesterol and Type 2 Diabetes: Mechanisms, Evidence, and Clinical Implications

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Introduction

Type 2 diabetes mellitus (T2DM) is a multifaceted metabolic condition defined by persistent hyperglycemia arising from insulin resistance and/or inadequate insulin secretion. Cardiovascular diseases rank among the primary contributors to illness and death in individuals with T2DM, and low-density lipoprotein cholesterol (LDL-C) is widely regarded as a key biomarker in the evaluation of cardiovascular risk. While the beneficial effects of lowering LDL-C on cardiovascular event prevention are firmly supported by evidence, emerging research indicates that extremely low LDL-C

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levels might be linked to additional health risks, such as cancer. In a prospective cohort study, Yang et al. (2014) demonstrated that, among patients with T2DM, LDL-C levels <2.8 mmol/L in combination with low triglyceride levels or the presence of albuminuria synergistically increased cancer risk. However, this elevated risk was significantly attenuated in individuals receiving renin–angiotensin system inhibitors (Yang et al., 2014). This evidence suggests that extremely low LDL-C levels could pose increased risks for specific patient populations.

Over the past several years, the association between type 2 diabetes and low LDL-C concentrations has gained increasing attention as a key area of investigation, particularly regarding cardiovascular risk and metabolic outcomes (Vrablík & Tůmová, 2016). Diabetic dyslipidemia is defined not only by alterations in LDL-C but also by disturbances in triglyceride and high-density lipoprotein cholesterol (HDL-C) levels, and it plays a central role in the development of atherosclerotic cardiovascular disease (Vrablík & Tůmová, 2016). Lowering LDL-C is regarded as a fundamental approach for preventing cardiovascular events in individuals with T2DM and is strongly endorsed by current clinical practice guidelines (Swartz Ethan et al., 2025). Lipid- lowering therapies, including statins and the more recently introduced proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, are widely utilized to effectively reduce LDL-C concentrations and significantly lower the incidence of atherosclerotic events (Lotta & Griffin, 2017).

In summary, while low LDL-C levels have long been linked to cardiovascular benefits, recent findings point to a previously unrecognized relationship between reduced LDL-C concentrations and a higher likelihood of developing type 2 diabetes. This observation underscores the need to reassess the interplay between

lipid metabolism and glucose homeostasis and has positioned the topic as an increasingly prominent focus of multidisciplinary research (Föger, 2011). Considering the extensive utilization of LDL-C- reducing agents like statins, the clinical significance of this link has grown substantially. This review examines the multifaceted interplay between low LDL-C concentrations and type 2 diabetes in the context of existing evidence, with a specific focus on the underlying biological pathways, genetic and epigenetic factors, lipid particle characteristics, clinical findings, and variations across different ethnic groups.

Epidemiological and Clinical Findings Regarding the Link Between Low LDL-C and Type 2 Diabetes

Epidemiological data support an association between LDL cholesterol levels and the development of diabetes. Recent Mendelian randomization studies and controlled trials involving statins have yielded consistent findings indicating that low LDL-C levels may be associated with a higher incidence of diabetes (Cariou et al., 2023). This observation is supported by multiple investigations showing that diminished LDL-C concentrations could contribute to the onset of type 2 diabetes (Brinton, 2021; Föger, 2011; Kechagia et al., 2024; Nadar et al., 2025; Ruscica et al., 2014). Of particular interest, an elevated risk of T2D has also been documented in individuals with hypobetalipoproteinemia disorder marked by inherently low LDL-C levels (Cariou et al., 2023). Collectively, these data suggest that LDL cholesterol exerts a more complex biological role not only in cardiovascular risk but also in the development and metabolic course of type 2 diabetes.

Specifically, long-term studies on statin use have shown that marked reductions in LDL-C levels may be linked to a higher frequency of new-onset type 2 diabetes (Brinton, 2021). Clinical

data reinforce this relationship, revealing that among individuals beginning statin therapy, low baseline LDL-C concentrations and prediabetic conditions serve as important predictors for the development of diabetes (Nadar et al., 2025). Furthermore, evidence suggests that as more aggressive lipid-lowering approaches achieve increasingly lower LDL-C targets, the likelihood of developing diabetes rises in a dose-dependent fashion. These observations highlight the importance of further investigating the biological pathways through which reduced LDL-C levels may contribute to the onset of diabetes (Brinton, 2021).

A six-year prospective investigation by Lembo and colleagues revealed a pronounced inverse relationship between LDL-C levels and the development of type 2 diabetes. Their findings indicated that low LDL-C concentrations were linked to a higher risk of T2D, regardless of statin treatment (Lembo et al., 2025). Similarly, results from the ATTICA cohort study conducted by Kechagia et al. demonstrated that LDL cholesterol functions as an independent predictor of new-onset type 2 diabetes and offers meaningful added benefit for risk classification (Kechagia et al., 2024). These results suggest that low LDL-C levels may influence diabetes risk not only through pharmacological interventions but also via genetic or metabolic determinants.

Additionally, Ruscica and colleagues highlighted the connection between statin treatment and the emergence of new-onset type 2 diabetes. According to their findings, the elevated risk was particularly evident among women, older adults, individuals with a familial predisposition to diabetes, and those of Asian descent. The researchers further observed that the diabetogenic impact of statins seemed to reflect a class-wide phenomenon, rather than being linked to any particular statin type or dose. Nevertheless, based on their findings, they concluded that the cardiovascular benefits of statin

therapy outweigh the associated diabetes risk, while recommending regular monitoring of glucose metabolism during treatment (Ruscica et al., 2014).

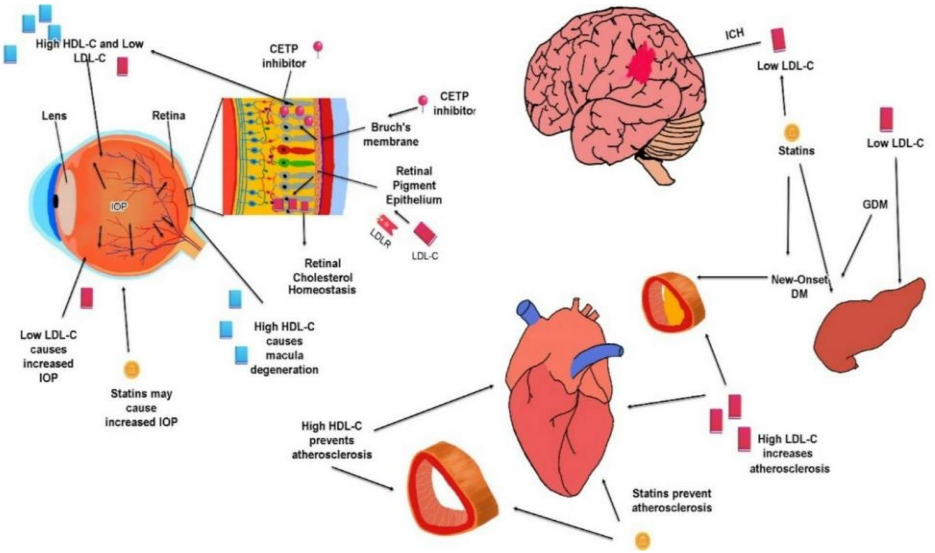

Figure 1. Impact of LDL-C and Statins on Metabolic Functions

Abbreviations: CETP, cholesteryl ester transfer protein; GDM, gestational diabetes mellitus; ICH, intracerebral hemorrhage; IOP, intraocular pressure.

Biological Pathways Linking Reduced LDL-C to Type 2 Diabetes Insulin Resistance and Beta-Cell Dysfunction

The mechanisms by which statins exert their diabetogenic effects are believed to involve mainly the promotion of insulin resistance and the deterioration of pancreatic β-cell function düşünülmektedir (Brinton, 2021; Brunham et al., 2010). Indeed, intensive statin regimens, such as high-dose atorvastatin therapy, have been shown to significantly increase insulin resistance

(Brinton, 2021). In addition, experimental studies have demonstrated that statins may adversely affect pancreatic β-cell function, leading to alterations in insulin secretion (Brinton, 2021).

These metabolic effects may be related to changes in cellular cholesterol homeostasis. Brunham and his team demonstrated that the buildup of cholesterol inside pancreatic β-cells leads to impaired cellular function and programmed cell death, with cholesterol transport proteins like ATP-binding cassette transporter A1 (ABCA1) playing an essential part in this mechanism (Brunham et al., 2010).

Moreover, the roles of mitochondria and peroxisomes in lipid metabolism may influence diabetes risk, particularly in the context of hormonal alterations. Antelo-Cea et al. emphasized that estrogen deficiency may disrupt peroxisome proliferator-activated receptor (PPAR) regulation, thereby promoting lipid accumulation and insulin resistance (Antelo-Cea et al., 2024).

Collectively, these findings suggest that cholesterol metabolism requires a finely tuned balance to maintain β-cell integrity and that excessive suppression of LDL cholesterol levels may pose potential risks to β-cell health.

Ethnic Differences and Genetic Background

The relationship between reduced LDL-C concentrations and type 2 diabetes seems to differ across ethnic groups ((Rahmoun et al., 2019; Soremekun et al., 2022). Evidence derived from genetic studies further strengthens the link between reduced LDL-C concentrations and increased T2D risk. Genome-wide association studies (GWAS) have uncovered 31 genetic loci that are linked to both reduced LDL-C concentrations and a higher susceptibility to type 2 diabetes (Klimentidis et al., 2019).

Furthermore, blocking HMGCR (3-hydroxy-3-methylglutaryl–coenzyme A reductase) the primary enzyme targeted by statins has also been found to elevate the risk of type 2 diabetes in individuals of African descent (Soremekun et al., 2022). A Mendelian randomization analysis focusing on African-ancestry populations revealed that genetically influenced low LDL-C levels were linked to a higher likelihood of developing T2D; interestingly, this pattern diverged from observations made in European-ancestry groups (Soremekun et al., 2022).

Additionally, some studies have indicated that the association between type 2 diabetes and lipid profiles may not always follow conventional patterns in specific populations. Rahmoun et al. observed that a substantial proportion of diabetic patients in Algeria exhibited lower-than- expected LDL cholesterol levels (Rahmoun et al., 2019). Although Mendelian randomization analyses suggest that low LDL-C may represent a causal risk factor for T2D, interpretation remains complex due to potential gene pleiotropy (Klimentidis et al., 2019).

Collectively, these observations underscore the complex interaction between hereditary susceptibility and environmental influences in determining how LDL-C concentrations relate to the risk of type 2 diabetes.

Epigenetic Regulation

While the precise mechanisms responsible for the diabetogenic effects of statins remain incompletely understood, emerging evidence suggests that their influence on glucose metabolism may involve epigenetic modifications. In a study conducted by Ochoa-Rosales and colleagues, alterations in specific DNA methylation sites were identified among individuals receiving statin therapy. Ochoa-Rosales and colleagues (2020) showed that

statin treatment is associated with elevated DNA methylation at the ABCG1 gene locus, a modification correlated with diminished gene expression and potentially involved in the pathogenesis of insulin resistance (Ochoa-Rosales et al., 2020). These findings indicate that the effects of statins extend beyond the inhibition of cholesterol synthesis and may involve modulation of epigenetic mechanisms regulating gene expression.

Association with Inflammatory and Immune Mechanisms

Inflammatory and immune mechanisms also appear to contribute to this association. In recent years, particularly in the context of the COVID-19 pandemic, the interplay between cholesterol metabolism and diabetes has gained renewed attention. Stepanova and Ghosal reported that cholesterol-rich lipid rafts play a critical role in the cellular entry of SARS-CoV-2 and suggested that impaired cholesterol metabolism in individuals with diabetes may exacerbate disease severity (Stepanova & Ghosal, 2025).

Furthermore, Calverley et al. observed that diabetic individuals with low LDL cholesterol levels exhibited increased platelet Fc receptor expression, which may influence the risk of vascular complications (Calverley et al., 2006). These findings suggest that the interaction between immune responses and lipid metabolism may be a key determinant of clinical outcomes in this context.

LDL Particle Characteristics and Metabolic Alterations in Type 2 Diabetes

A large volume of clinical and experimental data has established that lipid metabolism is fundamentally involved in the pathogenesis of type 2 diabetes. In individuals with T2D, evaluation of LDL cholesterol extends beyond absolute LDL-C levels, with

particle structure and its relationship to apolipoprotein B (ApoB) emerging as important parameters (Viktorinova et al., 2024). Current evidence suggests that, in assessing T2D risk, the qualitative properties of LDL particles and associated lipid parameters may be more informative than the absolute LDL- C concentration alone.

Small, dense LDL (sd-LDL) particles—frequently elevated in individuals with T2D are closely associated with insulin resistance and exhibit greater atherogenic potential (Larcher et al., 2024).The ApoB/LDL-C ratio reflects LDL particle number and the predominance of smaller particles. This ratio has been observed to be markedly higher in individuals with type 2 diabetes and has demonstrated independent predictive value for cardiovascular outcomes (Larcher et al., 2023; Larcher et al., 2024). Accordingly, in diabetic individuals, LDL particles exhibit not only quantitative alterations but also marked structural and functional changes.

In the study by Bonilha et al., insulin resistance and hyperglycemia were reported to promote the formation of smaller and denser LDL particles, prolonging their residence time in circulation and facilitating their uptake by macrophages. Additionally, enrichment of LDL particles with triglycerides and ceramides was shown to impair lipoprotein function and increase atherogenic potential (Bonilha et al., 2021). Supporting these observations, Viktorinova and colleagues found that an LDL-to-ApoB ratio below 1.2 in women with type 2 diabetes reflects a predominance of small, dense LDL particles and correlates with heightened cardiovascular risk (Viktorinova et al., 2024).

Concentrations of oxidized LDL (OxLDL), which serve as an indicator of oxidative stress, are increased in patients with type 2 diabetes and show a positive association with insulin resistance (Banerjee et al., 2019). The triglyceride to LDL-C ratio has been

recognized as a highly useful marker for detecting the presence of small, dense LDL particles in individuals with T2D (Ouchi et al., 2021). Additionally, various lipid ratios including total cholesterol/HDL-C, triglyceride/HDL-C, and LDL-C/HDL-C have been shown to correlate meaningfully with insulin resistance measured via HOMA-IR (Novida et al., 2014; Tangvarasittichai et al., 2010).

These findings demonstrate that not only the quantity of LDL cholesterol, but also the qualitative characteristics of LDL particles, are critically important from a clinical perspective.

Effects of Low LDL Cholesterol and Lipid-Lowering Therapies on Diabetes Risk

The connection between reduced LDL cholesterol concentrations and type 2 diabetes has garnered growing interest, especially given the extensive utilization of lipid-lowering treatments. Statins rank among the most potent pharmaceutical options for decreasing LDL cholesterol and are regarded as a foundational approach for preventing cardiovascular complications in patients with diabetes (Swartz Ethan et al., 2025).

Nevertheless, recent meta-analyses and clinical investigations have suggested that high-dose statin regimens, in particular, may negatively impact glycemic regulation and elevate the likelihood of developing new-onset diabetes. A meta-analysis by Cai and associates revealed that more pronounced reductions in LDL-C, lower on-treatment LDL-C concentrations, and the use of high-intensity statin therapy were each linked to elevations in HbA1c levels. Importantly, intensive strategies designed to achieve aggressive LDL-C targets were associated with greater worsening of glycemic control (Cai et al., 2016). These findings suggest that in T2DM, statin therapy may worsen glycemic control in a manner

dependent on the achieved LDL-C level and the magnitude of LDL-C reduction.

Brandts and Müller-Wieland reported that the diabetogenic effects of statins may be mediated through disruption of hepatic and pancreatic cholesterol homeostasis, alterations in the function of small G proteins, and changes in calcium signaling pathways (Brandts & Müller-Wieland, 2025). These mechanisms suggest that excessive lowering of LDL cholesterol levels may exert unforeseen effects on glucose metabolism.

Clinical studies have further substantiated the influence of statin treatment on glucose metabolism. Kameda and colleagues showed that transitioning Japanese patients with type 2 diabetes to low-dose rosuvastatin led to a marked decrease in LDL cholesterol levels, alongside a rise in HbA1c concentrations. This effect was reported to be more pronounced in patients with elevated fasting glucose levels (Kameda et al., 2017). Similarly, Breuker and colleagues observed that achievement of LDL targets in diabetic patients at very high cardiovascular risk may vary according to sex, with women exhibiting a higher rate of failure to reach LDL goals (Breuker et al., 2018).

The relationship between reduced LDL-C levels and type 2 diabetes seems to be intensified by the presence of metabolic syndrome components. Non-alcoholic fatty liver disease (NAFLD), in particular, appears to serve as a key mediating factor in this association. Research has shown that among individuals beginning statin treatment, a high Fatty Liver Index an indicator of hepatic fat accumulation was associated with a threefold increase in the risk of developing type 2 diabetes (Anastasiou et al., 2023). These findings suggest that the diabetogenic effects of low LDL-C levels or statin

therapy may be more pronounced in individuals with underlying metabolic disturbances.

The need for alternative lipid-lowering agents due to statin intolerance or statin-related adverse effects is progressively increasing (Gunta et al., 2023). Data from the study have demonstrated that many patients fail to achieve LDL-C targets despite statin use, highlighting the necessity for additional lipid-lowering approaches. Novel small molecules such as bempedoic acid offer advantages regarding muscular adverse effects due to their selective activation in the liver. Furthermore, PCSK9 inhibitors and RNA-based therapies hold promise for more effective reduction of LDL-C levels (Ruscica et al., 2014).

A multicenter observational study carried out in the Middle East revealed that less than half of the patients, only 48% achieved their targeted LDL-C levels. In a parallel finding, a quality improvement initiative in the United States indicated that 57% of individuals failed to meet the LDL-C goal of below 70 mg/dL. These findings indicate that even among high cardiovascular risk groups, target attainment rates remain low and that additional lipid-lowering treatment options such as ezetimibe or PCSK9 inhibitors are underutilized (Shehab et al., 2018; Swartz Ethan et al., 2025). In a large-scale analysis by Rossi and colleagues, although the proportion of diabetic patients achieving LDL targets has increased over the years, a significant subset of very high-risk patients still remains above the target values (Rossi et al., 2025). Collectively, these data demonstrate that despite lipid-lowering therapy, LDL-C target attainment rates in patients with T2DM remain inadequate.

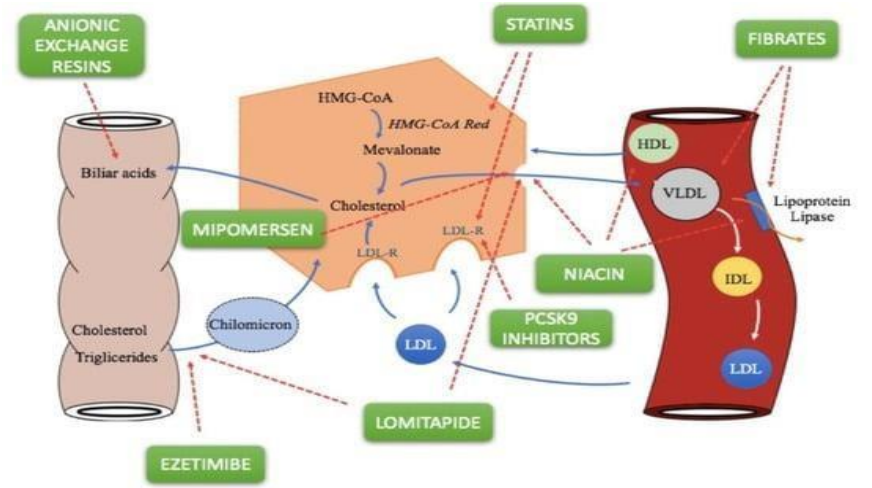

Figure 2. Overview of cholesterol metabolism and the mechanisms of action of lipid-lowering agents (Hegele, 2009; Tiwari & Khokhar, 2014; Zodda et al., 2018).

A sub-analysis of the CEPHEUS study revealed that a considerable number of patients with type 2 diabetes and dyslipidemia failed to reach their LDL-C goals, with adherence to therapy emerging as a major contributing factor (Shehab et al., 2018). Similarly, studies conducted in newly diagnosed T2DM patients have revealed challenges in achieving lipid control and underscore the critical role of treatment adherence in target attainment (García-Ulloa et al., 2021). Comparative studies with PCSK9 inhibitors have demonstrated that these agents not only lower LDL-C more effectively than statins but also reduce inflammation (Wang et al., 2022). Lavie and colleagues observed that ezetimibe acts by inhibiting intestinal cholesterol uptake, PCSK9 inhibitors function by enhancing the availability of hepatic LDL receptors, and bempedoic acid works by suppressing cholesterol production in the liver. The authors highlighted that these

agents are effective in reducing LDL-C levels and are typically associated with favorable tolerability profiles (Gunta et al., 2023).

A wide range of pharmacological strategies is currently accessible for lowering LDL cholesterol in individuals with type 2 diabetes. Although statins continue to serve as the foundation of lipid- lowering treatment, their use warrants caution given the potential for unfavorable effects on glycemic regulation (Cai et al., 2016; Ochoa-Rosales et al., 2020; Ruscica et al., 2014).

While newer agents such as PCSK9 inhibitors have demonstrated superior efficacy in reducing LDL cholesterol, it has been noted that their long-term implications for glucose homeostasis remain insufficiently understood (Rossi et al., 2025).

Conclusion

Contemporary evidence clearly indicates that the connection between low LDL cholesterol concentrations and type 2 diabetes reaches beyond the traditional framework of lipid-related cardiovascular risk. Although LDL cholesterol has long been evaluated primarily as an indicator of atherosclerotic cardiovascular disease risk, contemporary data reveal a multifaceted and bidirectional interaction of this lipoprotein with glucose metabolism, insulin resistance, and pancreatic beta-cell function. In this context, low LDL cholesterol levels should not invariably be interpreted as a metabolically "protective" state; the individual clinical context and accompanying metabolic characteristics must be taken into account. Aggressive reduction of LDL cholesterol levels may disrupt cholesterol homeostasis in hepatic and pancreatic cells, potentially leading to adverse consequences on insulin secretion and insulin sensitivity. This situation underscores the necessity of personalizing lipid targets, particularly in individuals at high risk for developing diabetes.

The relationship between low LDL cholesterol levels and type 2 diabetes reflects a multidimensional and bidirectional interaction rather than unidirectional causality, possessing a complexity that cannot be adequately assessed through a single biochemical parameter or treatment target alone. In clinical practice, when establishing LDL cholesterol goals, an individual's diabetes risk, current glycemic status, the type and dose of lipid-lowering therapy used, sex, age, and concomitant metabolic disorders should be considered collectively. This holistic approach will contribute to the development of a more balanced and personalized strategy in type 2 diabetes management, enabling both the maintenance of cardiovascular protection and the minimization of potential adverse effects on glucose metabolism. In the future, the integration of novel biomarkers such as oxidized LDL and advanced lipid particle analyses into routine clinical practice, along with the continuation of large-scale prospective studies, will facilitate a better understanding of this complex relationship and optimize patient care.

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CHAPTER 4

TREATMENT OF ATHEROSCLEROSIS WITH AZITHROMYCIN

AYŞEN ALTINER[1]

Introduction

Atherosclerosis is now firmly established as a chronic inflammatory disease rather than a passive lipid storage disorder. Early conceptual models centered on cholesterol accumulation within the arterial intima; however, decades of mechanistic research have demonstrated that lipid retention is necessary but not sufficient for plaque development. Instead, oxidative stress, endothelial activation, innate immune signaling, and maladaptive cellular metabolism converge to drive lesion initiation, progression, and destabilization. Endothelial dysfunction, oxidative modification of low-density lipoprotein (LDL), monocyte recruitment, macrophage activation, smooth muscle cell proliferation, and extracellular matrix remodeling collectively drive plaque formation and progression (Libby, 2002). Despite aggressive lipid-lowering therapy, substantial residual inflammatory risk persists. This observation has stimulated exploration of adjunctive therapeutic strategies targeting immune pathways (Saikku et al., 1988).

The infectious hypothesis of atherosclerosis emerged in the late twentieth century following epidemiological associations between serological markers of *Chlamydia pneumoniae* infection and coronary artery disease (Saikku et al., 1988). Subsequent identification of microbial DNA and antigenic components within atherosclerotic plaques further strengthened this hypothesis. Given the intracellular tropism of *Chlamydia pneumoniae* and the known capacity of pathogen-associated molecular patterns (PAMPs) to activate innate immune receptors, antimicrobial therapy-particularly with macrolide antibiotics-was proposed as a strategy to attenuate vascular inflammation (Kuo et al., 1993).

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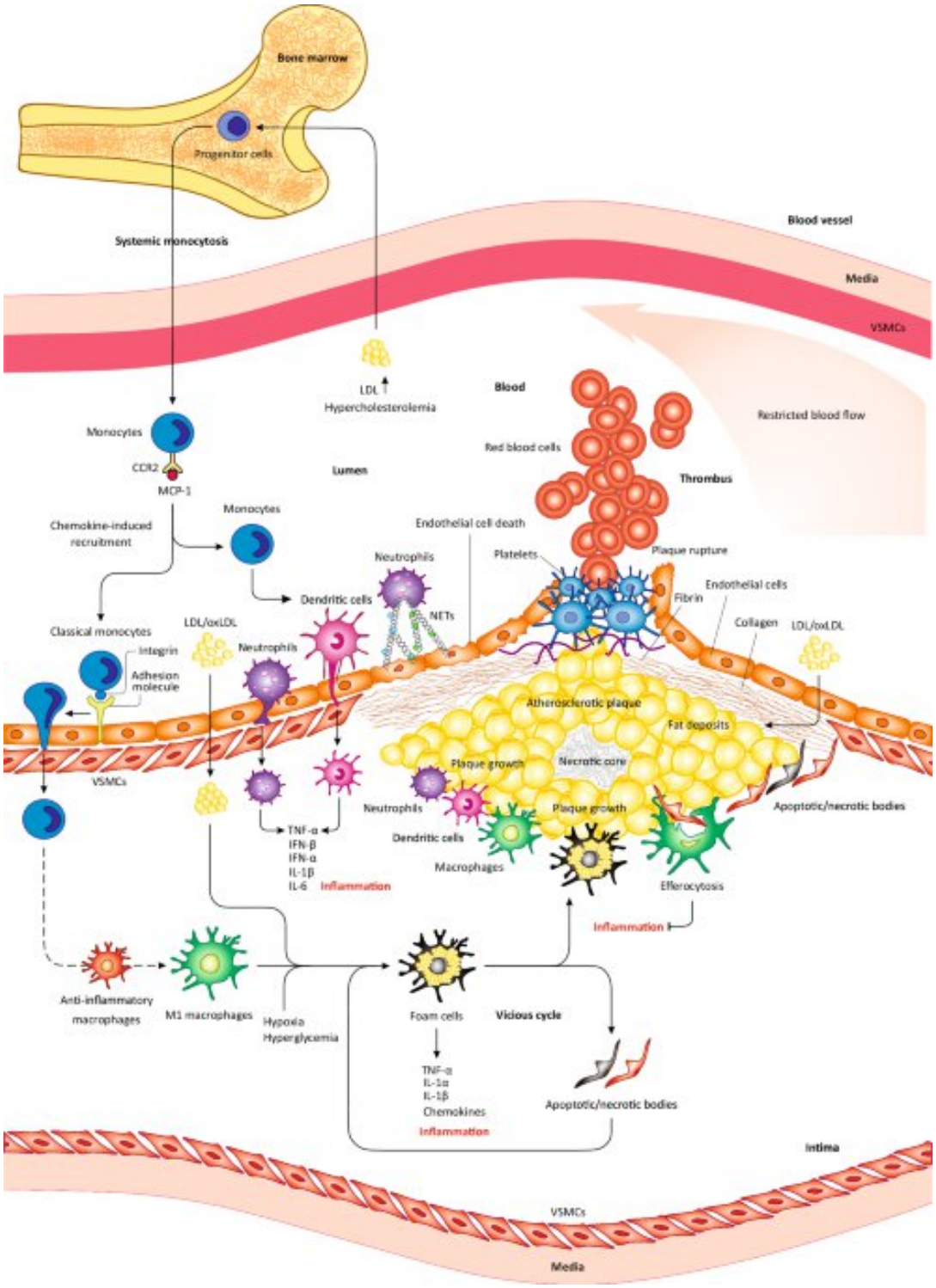
Molecular Biochemistry of Atherogenesis

Experimental studies in hyperlipidemic animal models showed accelerated plaque formation following infection and attenuation of lesion development with antibiotic therapy. Proposed mechanisms include endothelial activation, increased expression of adhesion molecules, toll-like receptor signaling, oxidative stress, and pro-inflammatory cytokine release. However, causal inference remains uncertain. Detection of microbial deoxyribonucleic acid (DNA) does not establish active infection, and immune activation may persist independently of viable pathogens. Moreover, atherosclerosis is multifactorial, involving metabolic, genetic, and environmental contributors that likely outweigh infectious triggers in most patients (Muhlestein et al., 1998).

Retention of apolipoprotein B-containing lipoproteins within the subendothelial space constitutes the initiating event in atherogenesis. Under conditions of endothelial dysfunction, increased permeability permits LDL infiltration into the intima. Reactive oxygen species (ROS) generated by reduced nicotinamide adenine dinucleotide phosphate oxidase isoforms, uncoupled endothelial nitric oxide synthase, xanthine oxidase, and mitochondrial respiratory chain leakage promote oxidative modification of LDL particles. Oxidized LDL interacts with scavenger receptors including fatty acid translocase (CD36) and scavenger receptor A on macrophages, leading to unregulated lipid uptake. Unlike LDL receptor-mediated endocytosis, scavenger receptor pathways lack feedback inhibition, resulting in progressive intracellular cholesterol accumulation and foam cell formation (Libby, 2002).

Intracellular cholesterol crystallization represents a pivotal biochemical transition. Phagocytosed cholesterol crystals destabilize lysosomal membranes, triggering cathepsin release and promoting activation of the nod-like receptor pyrin domain-containing 3 (NLRP3) inflammasome (Duewell et al., 2010). Inflammasome activation requires two coordinated signals: (i) priming via nuclear factor kappa B- (NF-κB)-dependent transcription of pro-interleukin- (IL)-1β and NLRP3, and (ii) activation through ionic flux, ROS generation, or crystalline structures. Engagement of toll-like receptors (TLR2 and TLR4) recruits myeloid differentiation primary response 88, leading to IL-1 receptor-associated kinase phosphorylation, tumor necrosis factor (TNF) receptor associated factor 6 activation, and stimulation of the inhibitor of kappaB (IκB) kinase complex. Degradation of IκB permits nuclear translocation of NF-κB subunits, driving transcription of pro-inflammatory genes including IL-1β and NLRP3. Subsequent assembly of the NLRP3-ASC-caspase-1 complex enables cleavage of pro-caspase-1 into active caspase-1, which processes pro-IL-1β and pro-IL-18 into mature cytokines (Fig. 1) (Ajoolabady et al., 2024).

Figure 1 Inflammation in atherosclerosis.



Reference: (Ajoolabady & al., 2024)

Beyond inflammasome signaling, matrix metalloproteinases (MMP-2, MMP-9) induced by inflammatory pathways degrade extracellular matrix components, contributing to fibrous cap thinning and plaque vulnerability. Activated macrophages within atherosclerotic lesions undergo profound metabolic reprogramming. Pro-inflammatory macrophages display enhanced glycolysis, reduced oxidative phosphorylation, and accumulation of tricarboxylic acid cycle intermediates such as succinate. Succinate stabilizes hypoxia inducible factor-1α, thereby promoting IL-1β transcription and reinforcing inflammatory signaling. Mitochondrial dysfunction further amplifies inflammatory circuits through increased ROS generation and release of mitochondrial DNA, which can serve as endogenous danger-associated molecular patterns (DAMPs) (O'Neill et al., 2016).

Emerging evidence supports the concept of trained immunity in atherosclerosis. Epigenetic modifications including histone acetylation and methylation at pro-inflammatory loci may sustain heightened transcriptional responsiveness even after resolution of initial triggers (Netea et al., 2016). These findings suggest that advanced plaques exhibit features of an entrenched inflammatory memory. *Chlamydia pneumoniae*-derived PAMPs engage TLR2 and TLR4, activating NF-κB–dependent priming pathways. Heat shock protein mimicry and potential intracellular persistence were proposed as mechanisms linking infection to chronic vascular inflammation. However, oxidized phospholipids, cholesterol crystals, and mitochondrial DAMPs activate the same signaling axes. This mechanistic convergence complicates attribution of sustained inflammation in advanced plaques to persistent infection (Saikku et al., 1988).

Pharmacological and Immunomodulatory Profile of Azithromycin

Azithromycin is a second-generation macrolide characterized by extensive tissue penetration and intracellular accumulation within macrophages and neutrophils-key cellular mediators of atherogenesis. Azithromycin accumulates within phagocytes through pH-dependent lysosomal ion trapping, achieving intracellular concentrations that substantially exceed plasma levels. In addition to inhibition of bacterial protein synthesis via binding to the 50S ribosomal subunit, macrolides exhibit immunomodulatory properties (Kanoh & Rubin, 2010).

Independent of antimicrobial activity, azithromycin modulates inflammatory signaling pathways. It suppresses NF-κB activation, reduces production of IL-6 and TNF-α, and influences macrophage polarization toward less inflammatory phenotypes. *In vitro* studies demonstrate attenuation of NF-κB activation, reduced production of IL-6, IL-8, and TNF-α, and modulation of MMP expression. Macrolides may also influence autophagic flux and endosomal TLR signaling by altering lysosomal pH. However, these

effects are generally moderate in magnitude and pleiotropic rather than selectively targeting central inflammatory nodes (Kanoh & Rubin, 2010).

Clinical Trials

In hyperlipidemic animal models infected with *Chlamydia pneumoniae*, antibiotic therapy reduced lesion burden in certain paradigms (Muhlestein et al., 1998). *In vitro* studies demonstrated decreased foam cell formation and attenuation of cytokine secretion in macrophages exposed to oxidized LDL. Although these findings reinforced biological plausibility, preclinical systems frequently rely on acute infection models superimposed on accelerated atherosclerosis. Human disease evolves over decades under complex systemic influences, limiting direct translational extrapolation (Scalia et al., 2024).

Early pilot studies suggested that macrolide therapy might reduce inflammatory markers and recurrent ischemic events in patients with coronary artery disease (Gupta et al., 1997). These preliminary observations led to large randomized controlled trials. O'Connor et al. (2003) evaluated azithromycin in patients with prior myocardial infarction and evidence of *Chlamydia pneumoniae* exposure but found no reduction in recurrent events. Similarly, Grayston et al. (2005) failed to demonstrate benefit in secondary prevention. Subsequent meta-analyses confirmed absence of meaningful reductions in major adverse cardiovascular events with antibiotic therapy. Other trials examining antibiotic therapy in stable coronary disease yielded neutral results.

Advanced atherosclerosis appears predominantly sustained by sterile inflammasome-driven and immunometabolic networks. Redundancy and cross-talk among NF-κB, inflammasome, and metabolic pathways may render partial upstream modulation insufficient to disrupt entrenched inflammatory circuits. Collectively, these data indicate that eradication of presumed infection does not translate into measurable cardiovascular risk reduction. Stage-dependent mismatch further contributes: antimicrobial therapy may influence early lesion initiation but is unlikely to reverse established structural plaque complexity (Duewell et al., 2010).

The negative results of antibiotic trials contrast sharply with the trial of Ridker et al. (2017) which demonstrated that IL-1β inhibition reduces recurrent cardiovascular events independent of lipid lowering. Low-dose colchicine has also shown benefit in secondary prevention settings. These findings underscore that targeted modulation of inflammatory pathways-rather than broad antimicrobial therapy-may represent a more effective strategy. This divergence suggests that inflammation in advanced atherosclerosis may be largely sterile and self-sustaining, limiting the impact of antimicrobial interventions (Tardif et al., 2019).

Azithromycin has been associated with QT interval prolongation and increased risk of cardiovascular death in susceptible individuals. Although absolute risk is small, widespread long-term use in cardiovascular populations warrants caution. Furthermore, indiscriminate antibiotic use raises concerns regarding antimicrobial resistance, a major global public health challenge. Future investigations may focus on identifying subgroups with demonstrable infectious burden or heightened inflammatory activity. Molecular imaging and biomarker-guided stratification could clarify whether localized infection contributes meaningfully in select patients. Development of non-antibiotic macrolide derivatives preserving immunomodulatory properties without antimicrobial effects may represent a potential avenue for research (Ray et al., 2012).

Conclusion

Azithromycin provided important mechanistic insight into the infectious hypothesis of atherosclerosis but has not demonstrated cardiovascular benefit in established disease. Contemporary biochemical evidence supports a sterile inflammasome-driven and immunometabolic model of advanced plaque biology. Advances in cardiovascular therapeutics are more likely to arise from precisely targeted anti-inflammatory strategies grounded in mechanistic clarity. Future therapeutic strategies may therefore benefit from selective targeting of central inflammatory nodes rather than pleiotropic antimicrobial approaches.

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CHAPTER 9

MACHINE LEARNING-BASED PREDICTIVE MODELS IN METABOLİC DISORDERS: A NARRATIVE REVIEW

MUZAFFER KATAR[1]

Introduction

Metabolic disorders, particularly diabetes mellitus and metabolic syndrome, constitute a major and expanding global public health problem due to their increasing prevalence, chronic disease course, and strong association with cardiovascular morbidity and mortality (1,2). Diabetes mellitus is defined as a group of metabolic conditions characterized by persistent hyperglycemia resulting from impairments in insulin secretion, insulin action, or both, and type 2 diabetes accounts for nearly 90% of all diagnosed cases worldwide (1,2). Current global estimates indicate that more than 400 million adults are living with diabetes, with the highest burden observed in low- and middle-income countries (8,9).

Metabolic syndrome is described as a multifactorial clinical condition involving the coexistence of central obesity, dyslipidemia, elevated blood pressure, and abnormalities in glucose metabolism.

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Individuals affected by metabolic syndrome exhibit a substantially higher risk of developing type 2 diabetes and cardiovascular disease compared to metabolically healthy populations (3,10). Epidemiological evidence consistently shows that the clustering of metabolic risk factors leads to synergistic effects, resulting in accelerated progression of cardiometabolic complications (11,12).

The increasing availability of large-scale biomedical data resources, including electronic health records, biobanks, and population-based datasets, has created new opportunities for predictive modeling in metabolic disease research. Traditional statistical methods, although widely applied in clinical studies, generally rely on linear assumptions and limited numbers of predictors, which restrict their ability to capture complex biological interactions (24,25). Consequently, machine learning has emerged as an alternative analytical paradigm capable of identifying nonlinear patterns within high-dimensional data without requiring explicit model specification (4–7).

Machine learning techniques enable the automated extraction of clinically relevant insights from heterogeneous data sources, such as demographic characteristics, anthropometric measurements, biochemical markers, and lifestyle-related variables. These approaches have frequently demonstrated superior predictive performance compared to conventional regression-based models, particularly in tasks related to chronic disease risk prediction, patient stratification, and outcome forecasting (19,26).

Epidemiology of Diabetes and Metabolic Syndrome

The prevalence of diabetes has risen sharply worldwide over the past several decades, largely as a consequence of rapid urbanization, population aging, reduced physical activity, and the widespread adoption of unhealthy dietary patterns. Data reported by the International Diabetes Federation indicate that the global

prevalence of diabetes among adults exceeded 9% in 2019, and projections suggest that this figure will continue to increase substantially in the coming years (8). Large-scale population studies further demonstrate that the number of individuals living with diabetes has increased almost fourfold since 1980 (9).

Metabolic syndrome exhibits a similarly high prevalence and represents a major public health concern in both developed and developing regions. In many industrialized countries, approximately one-third of the adult population meets the diagnostic criteria for metabolic syndrome, while comparable or rising rates are being observed in low- and middle-income countries (10). Epidemiological surveys conducted in South Asian populations, particularly in Bangladesh, have reported prevalence rates ranging from 25% to 37%, depending on the applied diagnostic definitions and the demographic characteristics of the study samples (11,12). These findings emphasize the substantial burden of cardiometabolic risk factors in resource-limited settings.

Advancing age remains one of the most significant determinants of both diabetes and metabolic syndrome. The likelihood of developing metabolic disorders increases progressively with age, reflecting long-term exposure to environmental risk factors, cumulative metabolic stress, and gradual declines in physiological resilience (13). Gender-related differences have also been documented, with several studies indicating higher prevalence rates of metabolic syndrome among women, especially in middle-aged and older age groups (14).

Lifestyle-related behaviors play a central role in the development of metabolic diseases. Insufficient physical activity, unhealthy dietary habits, tobacco use, excessive alcohol consumption, and inadequate sleep have all been consistently associated with increased insulin resistance and the accumulation of abdominal adiposity (15,16). In addition, socioeconomic factors

significantly influence disease distribution, as lower educational levels and limited access to healthcare services are linked to higher rates of undiagnosed and poorly controlled diabetes (17,18).

The multifactorial and heterogeneous nature of metabolic disease epidemiology highlights the limitations of simple risk assessment tools and underlines the necessity for advanced analytical approaches capable of integrating complex risk profiles at both individual and population levels (19).

Traditional Risk Models for Diabetes Prediction

Traditional approaches to diabetes risk prediction have predominantly relied on statistical regression models and clinically derived scoring systems based on data from longitudinal cohort studies. One of the most commonly applied tools is the Framingham Diabetes Risk Score, which integrates variables such as age, body mass index, fasting plasma glucose, lipid profiles, blood pressure, and family history to estimate the probability of developing diabetes over time (20). Similarly, the Finnish Diabetes Risk Score incorporates behavioral and lifestyle-related factors, including physical activity patterns and dietary habits, to assess individual risk (21).

Although these models are relatively simple, interpretable, and easy to apply in clinical practice, their overall predictive performance is often moderate. Meta-analytic evidence suggests that most conventional diabetes risk scores achieve area under the receiver operating characteristic curve values ranging between 0.65 and 0.75, indicating limited discriminative ability across heterogeneous populations (22,23). Moreover, concerns have been raised regarding their external validity, as predictive accuracy frequently declines when these tools are applied to populations with different ethnic, cultural, or socioeconomic backgrounds.

A fundamental limitation of traditional regression-based models lies in their dependence on linear assumptions and predefined interactions between predictors. Such methodological constraints reduce their capacity to represent complex nonlinear relationships and higher-order interactions that characterize biological systems (24). In addition, conventional models struggle to handle high-dimensional datasets and typically require extensive manual feature selection, which may introduce bias and reduce model robustness (25).

These shortcomings have contributed to a gradual shift toward machine learning-based predictive frameworks, which offer greater flexibility, scalability, and potential for improved predictive accuracy. Unlike classical statistical approaches, machine learning algorithms can automatically learn intricate patterns from large datasets and adapt to changing clinical contexts without requiring explicit assumptions about data structure (26).

Machine Learning Approaches in Metabolic Disease Prediction

Machine learning methods have gained substantial attention in metabolic disease research because of their enhanced ability to model nonlinear relationships and process complex, high-dimensional datasets. In contrast to traditional statistical models, machine learning algorithms do not rely on strict assumptions regarding data distributions or variable interactions, allowing them to uncover latent structures directly from empirical data (4–7).

Among supervised learning techniques, support vector machines, random forests, gradient boosting models, and artificial neural networks are the most frequently employed algorithms in studies focusing on diabetes risk prediction. Support vector machines construct optimal decision boundaries and perform particularly well in high-dimensional feature spaces, making them

suitable for biomedical datasets that contain numerous correlated predictors (27,29).

Random forests represent an ensemble learning approach in which multiple decision trees are combined to generate final predictions, thereby improving model stability and reducing the risk of overfitting. Several comparative investigations have shown that random forest models outperform traditional regression-based approaches in metabolic syndrome classification tasks, with reported area under the curve values exceeding 0.85 in certain populations (28,31).

Gradient boosting algorithms, including XGBoost, iteratively optimize weak learners in order to minimize prediction errors and have emerged as state-of-the-art techniques for structured clinical data analysis. These models have demonstrated superior performance in large-scale diabetes prediction studies and are increasingly applied in population health research (32).

Artificial neural networks are capable of representing highly complex nonlinear relationships by simulating interconnected neural structures. Although neural network-based models often achieve high predictive accuracy, their limited interpretability and substantial computational requirements remain significant barriers to routine clinical use (33).

Feature Engineering and Biomarkers

The predictive accuracy of machine learning models is highly dependent on the quality and relevance of the selected input features. In studies focusing on metabolic disease prediction, commonly utilized features include demographic characteristics, anthropometric measurements, biochemical indicators, lifestyle-related variables, and clinical history (34).

Anthropometric indices such as body mass index, waist circumference, and waist-to-height ratio are consistently reported as among the most informative predictors. In particular, the waist-to-height ratio has been shown to outperform body mass index in detecting abdominal obesity and in predicting cardiometabolic risk across diverse population groups (35).

Biochemical parameters, including fasting plasma glucose, triglyceride levels, high-density lipoprotein cholesterol, and total cholesterol, repeatedly emerge as the strongest predictors of both diabetes and metabolic syndrome (36). These biomarkers directly reflect underlying metabolic dysfunction and are routinely measured in standard clinical practice.

Lifestyle-related factors further contribute to model performance. Variables such as smoking status, physical activity, dietary behavior, and sleep duration capture modifiable risk patterns that are not fully represented by biochemical markers alone (15,16,37).

Feature selection techniques play a critical role in reducing dimensionality and improving both model efficiency and interpretability. Approaches such as recursive feature elimination, random forest-based importance ranking, and correlation-based analysis enable the identification of the most informative predictors while minimizing redundancy and noise within the dataset (38).

Recent investigations have also examined the incorporation of emerging biomarkers, including genetic variants and metabolomic profiles. Although these features may enhance predictive performance, their limited accessibility and high associated costs currently restrict their widespread application in routine clinical settings (39).

Model Performance Metrics and Evaluation

The rigorous evaluation of machine learning models is essential to ensure their reliability, generalizability, and potential clinical usefulness. The most frequently reported performance indicators in metabolic disease prediction research include accuracy, sensitivity, specificity, precision, recall, F1-score, and the area under the receiver operating characteristic curve (40).

Accuracy represents the proportion of correctly classified observations but may provide misleading information in imbalanced datasets where disease prevalence is relatively low. In such cases, sensitivity and specificity offer more meaningful insights into model performance (41).

Sensitivity refers to the model's capacity to correctly identify individuals with the disease, whereas specificity reflects the correct classification of disease-free individuals. High sensitivity is particularly important in screening applications, where failure to detect high-risk individuals may delay preventive or therapeutic interventions (42).

The F1-score, calculated as the harmonic mean of precision and recall, provides a balanced measure of performance in classification tasks involving imbalanced data. The area under the curve remains the most widely adopted metric in biomedical machine learning, as it summarizes discriminative ability across all possible classification thresholds (43).

Cross-validation methods, especially k-fold cross-validation, are routinely applied to reduce overfitting and to assess model stability. Ten-fold cross-validation is commonly regarded as a standard approach in biomedical machine learning research (44).

Despite the frequent reporting of high performance metrics, many studies lack external validation using independent datasets.

This methodological limitation raises concerns regarding real-world applicability and highlights the need for more standardized and robust evaluation frameworks (45).

Clinical Implications

The application of machine learning-based predictive models in clinical practice offers significant potential for improving early diagnosis, risk stratification, and personalized management of metabolic disorders. By identifying individuals at increased risk before the manifestation of overt disease, healthcare professionals can implement targeted lifestyle interventions and pharmacological strategies aimed at preventing or delaying disease onset and progression (46,47).

In primary care settings, predictive algorithms may function as clinical decision-support tools, assisting practitioners in screening large populations using routinely collected clinical data. Such systems reduce reliance on invasive diagnostic procedures and support more efficient allocation of healthcare resources (47).

Within specialized clinical environments, machine learning models may facilitate treatment optimization by identifying subgroups of patients who exhibit differential responses to therapeutic interventions. Predictive modeling has been used to classify patients according to their likelihood of achieving glycemic control, maintaining medication adherence, and developing complications such as cardiovascular disease and nephropathy (48).

Despite these promising applications, the integration of machine learning tools into routine clinical practice remains limited. Major obstacles include the absence of standardized validation protocols, technical challenges related to integration with existing health information systems, and the limited interpretability of complex predictive models (49).

Limitations and Ethical Issues

Although machine learning-based models frequently demonstrate high predictive accuracy, several methodological and ethical challenges hinder their widespread adoption in real-world clinical contexts. One of the primary methodological concerns is overfitting, whereby models optimized for training datasets fail to generalize effectively to new populations (50).

Data quality and representativeness also constitute major limitations. A substantial proportion of existing studies rely on datasets derived from single institutions or geographically restricted populations, which limits external validity. Furthermore, missing data, measurement inaccuracies, and class imbalance may distort model performance and compromise reliability (51).

Ethical considerations are equally important and include issues related to data privacy, algorithmic bias, and transparency. Predictive systems trained on biased or unrepresentative datasets may perpetuate existing health inequalities by systematically underperforming in vulnerable or underrepresented populations (52). In addition, black-box models, such as deep neural networks, raise concerns regarding explainability and accountability in clinical decision-making processes (53).

The lack of comprehensive regulatory frameworks governing the use of artificial intelligence in healthcare further complicates clinical implementation and emphasizes the need for multidisciplinary governance structures and ethical oversight mechanisms (54).

Future Directions

Future research should focus on the integration of multi-modal data sources, combining clinical, genetic, imaging, and lifestyle information into unified predictive frameworks. Advances

in wearable technologies and continuous glucose monitoring systems provide new opportunities for real-time risk assessment and dynamic monitoring of disease progression (55).

Explainable artificial intelligence represents a critical area for enhancing clinician trust and regulatory compliance. The development of interpretable models will support transparent clinical decision-making and facilitate broader acceptance of machine learning tools in healthcare environments (56).

Large-scale prospective validation studies are essential to evaluate real-world effectiveness and clinical impact. International data-sharing initiatives may help overcome current limitations related to sample size, population diversity, and reproducibility of predictive models (57,58).

Conclusion

Machine learning-based predictive modeling has emerged as a transformative methodology in metabolic disease research. These approaches extend beyond traditional statistical models by capturing complex nonlinear relationships and effectively handling high-dimensional datasets. When rigorously validated and ethically implemented, machine learning systems have the potential to significantly enhance preventive healthcare by enabling early detection, personalized intervention strategies, and improved long-term outcomes for individuals at risk of diabetes and metabolic syndrome (4–7,26,58).

Kaynakça

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