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Seçkin TUNCER, PhD

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CONTENTS

CHAPTER 1

Na+/K+-ATPase ACTIVITY AND REGULATION IN METABOLIC SYNDROME: BEYOND ION TRANSPORT TO REDOX SIGNALING

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1. Introduction

Metabolic syndrome represents a rapidly expanding global health concern of the modern era, driven largely by profound changes in lifestyle, nutrition, and population demographics (Saklayen, 2018). This multifactorial condition is defined by the coexistence of several metabolic disturbances, including abdominal adiposity, impaired insulin responsiveness, atherogenic lipid profiles, and elevated blood pressure. Collectively, these abnormalities substantially increase the likelihood of developing type 2 diabetes mellitus and cardiovascular complications (Peterseim et al., 2024). The continuous rise in sedentary behavior, excessive caloric intake, and increased life expectancy has intensified the global prevalence of metabolic syndrome, creating a growing burden on healthcare infrastructures and economic systems worldwide. Beyond its clinical presentation, this syndrome reflects a deeper disturbance of systemic homeostasis that involves tightly regulated cellular and molecular networks (Alberti et al., 2005).

At the cellular level, the maintenance of ionic equilibrium is essential for normal physiological activity. A central component of this regulatory system is the Na^+/K^+-ATPase (NKA), a plasma membrane enzyme responsible for sustaining transmembrane electrochemical gradients through the active exchange of sodium and potassium ions. Traditionally, this enzyme has been viewed primarily as a fundamental maintenance factor required for membrane potential stabilization and osmotic balance. However, emerging evidence indicates that its biological significance extends well beyond these classical housekeeping functions. In metabolically active tissues, disturbances in ion transport homeostasis have been associated with impaired glucose utilization, defective insulin signaling, increased oxidative stress, and activation of inflammatory pathways, all of which contribute to the pathophysiology of metabolic syndrome (Contreras et al., 2024).

Over the past decade, the understanding of NKA has evolved considerably, leading to a shift in its conceptual classification. Rather than serving exclusively as an ATP-dependent ion transporter, it is now recognized as an active participant in intracellular signaling networks. This enzyme integrates multiple extracellular and intracellular signals, including metabolic status, hormonal stimuli, and redox-related changes. Of particular importance is its interaction with reactive oxygen species (ROS), which positions NKA as a key molecular interface between oxidative stress and downstream signaling pathways. This expanded functional perspective has redefined its role in cellular physiology, highlighting its contribution not only to ion homeostasis but also to signal transduction processes involved in metabolic regulation (Liu et al., 2018).

The objective of this chapter is to present a detailed and integrative overview of NKA activity and its regulatory mechanisms within the context of metabolic syndrome, with special emphasis on its involvement in redox-dependent signaling pathways. The discussion will begin with the structural organization and functional characteristics of the enzyme, followed by an examination of its regulatory controls under both physiological and pathological conditions. Subsequently, the chapter will explore how metabolic syndrome alters NKA function and how these alterations contribute to systemic metabolic and vascular dysfunction. Finally, attention will be given to the emerging role of redox-mediated signaling mechanisms associated with NKA and their potential relevance for future therapeutic strategies.

2. Structural and Functional Basis of NKA

NKA is a widely distributed membrane-bound enzyme essential for preserving ionic equilibrium, membrane excitability, and cellular metabolic stability. Classically, it functions as an active transporter that exports sodium ions (Na^+) from the cell while importing potassium ions (K^+) into the cytoplasm. However, growing evidence indicates that this enzyme also participates in intracellular signaling, regulation of oxidative stress, and cellular metabolic responses. In metabolic syndrome, characterized by the coexistence of insulin resistance, obesity, dyslipidemia, hypertension, and persistent low-grade inflammation, structural and functional disturbances of NKA are closely associated with impaired cellular homeostasis and redox dysregulation. Consequently, a detailed understanding of the enzyme's molecular structure and ATP-driven transport mechanism is critical for explaining its functions beyond conventional ion exchange (Srikanthan et al., 2016).

2.1. α, β, and FXYD Subunits

NKA is a multisubunit membrane protein complex consisting of catalytic α subunits, regulatory β subunits, and accessory FXYD proteins. The functional coordination of these components is essential for maintaining enzyme activity, membrane localization, structural stability, and transport kinetics (Nguyen et al., 2022).

The α subunit serves as the functional and catalytic center of the enzyme, mediating ATP hydrolysis and transmembrane ion transport. This subunit is a large integral membrane protein composed of ten transmembrane helices along with three principal cytoplasmic domains: the nucleotide-binding (N), phosphorylation (P), and actuator (A) domains. ATP binds to the N domain, while the P domain contains the conserved aspartate residue that becomes transiently phosphorylated during the transport cycle. The A domain facilitates the conformational rearrangements necessary for ion translocation. In addition, the α subunit

possesses binding sites for Na^+, K^+, ATP, and cardiotonic steroids including ouabain. Four distinct α isoforms (α1–α4) have been identified, each displaying tissue-specific distribution patterns. Whereas α1 is broadly expressed in most tissues, α2 and α3 are predominantly localized in cardiac muscle, skeletal muscle, and neuronal tissues. These isoform-dependent differences are closely linked to metabolic regulation and cellular susceptibility to oxidative stress (Clausen et al., 2017).

The β subunit is an essential glycoprotein that supports the proper folding, assembly, and plasma membrane delivery of the α subunit. It contains a single transmembrane region and a highly glycosylated extracellular domain. Beyond its stabilizing role within the enzyme complex, the β subunit also contributes to membrane architecture and intercellular adhesion. Disturbances in β subunit expression or function may disrupt Na^+/K^+-ATPase trafficking and compromise membrane integrity, especially under conditions of metabolic stress (Morth et al., 2011).

FXYD proteins constitute a family of small membrane-associated regulatory proteins that fine-tune Na^+/K^+-ATPase activity in a tissue-specific manner. Characterized by the conserved FXYD amino acid motif, these proteins interact directly with the α/β complex and influence ion affinity, ATP responsiveness, and transport efficiency according to physiological requirements. For instance, FXYD1, also known as phospholemman, is predominantly expressed in cardiac tissue and modulates pump activity during adrenergic stimulation, whereas FXYD2 is highly abundant in the kidney and plays an important role in electrolyte regulation. Abnormal regulation of FXYD proteins has been linked to impaired sodium homeostasis, oxidative stress, and insulin resistance associated with metabolic disorders (Seflova et al., 2025; Geering, 2008).

2.2. ATP-Dependent Ion Transport Mechanism

Na^+/K^+-ATPase functions through an ATP-dependent transport cycle that alternates between two major conformational states, E1 and E2. This cycle enables the active transport of ions against their electrochemical gradients (Cordeiro et al., 2024).

In the E1 conformation, the enzyme has a high affinity for intracellular Na^+ ions. Three Na^+ ions bind to specific cytoplasmic binding sites located within the α subunit. ATP then binds to the nucleotide-binding domain, leading to phosphorylation of a conserved aspartate residue in the P domain. This phosphorylation triggers a conformational change from the E1 to the E2 state (Nielsen et al., 2024).

In the E2-P conformation, the ion-binding sites are exposed to the extracellular space, and the affinity for Na^+ decreases, resulting in the release of three Na^+ ions outside the cell. At

the same time, the enzyme acquires a high affinity for extracellular K^+ ions, allowing two K^+ ions to bind. K^+ binding promotes dephosphorylation of the enzyme and the return to the E1 state. The two K^+ ions are subsequently released into the cytoplasm, completing the transport cycle (Pirahanchi. et al., 2023). The overall reaction can be summarized as:

$$3Na^+(in) + 2K^+(out) + ATP + H_2O \rightarrow 3Na^+(out) + 2K^+(in) + ADP + Pi$$

This transport mechanism is electrogenic, as it exports more positive charges than it imports, thereby contributing to the resting membrane potential. In excitable tissues such as neurons and cardiomyocytes, this function is essential for electrical activity and signal propagation (Dimitrov, 2023).

Under metabolic syndrome conditions, oxidative stress and lipid peroxidation may impair Na^+/K^+-ATPase activity through oxidative modifications of the α subunit. Reduced pump activity leads to intracellular Na^+ accumulation, which secondarily promotes Ca^{2+} overload via Na^+/Ca^{2+} exchangers, mitochondrial dysfunction, and increased reactive oxygen species (ROS) production. Consequently, Na^+/K^+-ATPase dysfunction contributes not only to ionic imbalance but also to redox dysregulation (Bogdanova et al., 2016).

2.3. Membrane Organization and Lipid Raft Association

Na^+/K^+-ATPase is strategically localized within specialized plasma membrane microdomains known as lipid rafts and caveolae. These cholesterol- and sphingolipid-rich regions serve as platforms for the organization of signaling molecules and the facilitation of protein–protein interactions (de Laurentiis et al., 2007).

Localization within lipid rafts enables Na^+/K^+-ATPase to interact with several signaling proteins, including Src kinase, caveolin-1, epidermal growth factor receptor (EGFR), and phosphoinositide-associated signaling complexes. Through these interactions, the enzyme functions not only as an ion transporter but also as an important signaling scaffold (Zhang et al., 2016).

One of the best-characterized signaling mechanisms associated with Na^+/K^+-ATPase involves activation of Src kinase following the binding of cardiotonic steroids such as ouabain. This interaction initiates downstream signaling cascades, including the MAPK/ERK, PI3K/Akt, and NF-κB pathways, which regulate inflammation, oxidative stress, apoptosis, and cellular proliferation. Excessive activation of Na^+/K^+-ATPase-mediated signaling has been implicated in the chronic inflammation and ROS amplification observed in metabolic syndrome (Cai et al., 2023).

Recent studies suggest the presence of a Na^+/K^+-ATPase/Src/ROS amplification loop, in which ROS generated during metabolic stress further stimulate Na^+/K^+-ATPase-dependent

signaling, thereby promoting sustained oxidative damage and inflammatory responses. This mechanism may contribute to obesity-associated insulin resistance, vascular dysfunction, and cardiac remodeling (Marck et al., 2021).

In addition, Na^+/K^+-ATPase interacts with cytoskeletal proteins such as ankyrin and spectrin, which are involved in maintaining membrane polarity and structural integrity. Alterations in membrane lipid composition commonly observed in obesity and dyslipidemia may disrupt lipid raft integrity, thereby impairing Na^+/K^+-ATPase localization and signaling efficiency (Stabach, et al., 2008).

3. Na^+/K^+-ATPase Dysfunction in Metabolic Syndrome

3.1. Insulin Resistance and Na^+/K^+-ATPase Activity

Insulin is an important regulator of Na^+/K^+-ATPase function in multiple tissues such as skeletal muscle, adipose tissue, the heart, and kidneys. Under physiological conditions, insulin enhances Na^+/K^+-ATPase activity by facilitating the movement of pump molecules to the cell membrane and improving ion transport capacity. Through these actions, the enzyme helps preserve membrane potential, maintain electrolyte balance, and support glucose uptake (Wen et al., 2021).

In metabolic syndrome, insulin resistance interferes with these normal regulatory pathways. Defective insulin signaling decreases Na^+/K^+-ATPase stimulation, resulting in reduced pump activity and disturbances in intracellular sodium and potassium levels. Increased intracellular sodium may further disrupt the Na^+/Ca^{2+} exchange system, causing calcium accumulation within cells and contributing to vascular abnormalities, cardiac dysfunction, and altered cellular metabolism (Mou et al., 2025).

The decline in Na^+/K^+-ATPase activity observed during insulin resistance has also been associated with impaired glucose transport, mitochondrial damage, oxidative stress, and persistent low-grade inflammation. In skeletal muscle, reduced pump efficiency can weaken insulin-dependent glucose utilization, thereby worsening hyperglycemia and metabolic dysregulation. Likewise, in cardiovascular tissues, impaired Na^+/K^+-ATPase activity may promote endothelial dysfunction and elevated blood pressure, which are frequently observed in metabolic syndrome (Obradovic et al., 2023).

3.2. Obesity and Adipocyte Membrane Alterations

Obesity is marked by excessive fat accumulation and significant metabolic abnormalities that affect the structure and function of cellular membranes. In adipose tissue, hypertrophy of adipocytes and prolonged exposure to high circulating lipid levels modify

membrane composition, fluidity, and receptor distribution. These changes can negatively affect membrane-bound proteins such as ion channels, transporters, and signaling molecules that are essential for metabolic regulation (Uranga and Allani, 2026).

Alterations in adipocyte membrane characteristics are strongly linked to defective insulin signaling and persistent low-grade inflammation. Elevated concentrations of saturated fatty acids and cholesterol in the plasma membrane may decrease membrane flexibility and disturb lipid raft architecture, leading to impaired receptor-dependent signaling pathways. As a result, insulin resistance, abnormal adipokine release, and disrupted glucose metabolism commonly develop in obesity (Bou Matar et al., 2025).

Obesity is further associated with enhanced oxidative stress and increased production of inflammatory cytokines, both of which promote lipid peroxidation and membrane damage. This oxidative injury weakens membrane integrity and disturbs cellular homeostasis. Moreover, changes in adipocyte membrane behavior may alter Na^+/K^+-ATPase function and other ion transport mechanisms, thereby contributing to electrolyte imbalance and metabolic dysfunction (Manna and Jain, 2015).

3.3. Hypertension and Vascular Tone

Hypertension is a central feature of metabolic syndrome and is strongly linked to impaired regulation of vascular tone. Under normal conditions, vascular tone is controlled by a balance between vasoconstrictive and vasodilatory processes involving endothelial cells, vascular smooth muscle cells, ion transport mechanisms, and multiple signaling pathways. Disruption of these regulatory networks contributes to increased peripheral vascular resistance and persistent elevation of blood pressure (Martinez-Quinones et al., 2018).

In metabolic syndrome, factors such as insulin resistance, oxidative stress, and chronic inflammation negatively affect endothelial function and decrease the availability of nitric oxide. As an important vasodilator, nitric oxide supports vascular relaxation and preserves normal vessel function. Reduced nitric oxide levels, together with enhanced vasoconstrictor responses, promote vascular rigidity and impaired vasodilation (Mendizabal et al., 2013).

Abnormalities in ion transport systems, especially impaired Na^+/K^+-ATPase activity, also play an important role in altered vascular tone. Decreased activity of this enzyme can elevate intracellular sodium concentrations in vascular smooth muscle cells, which may subsequently increase intracellular calcium accumulation via the Na^+/Ca^{2+} exchanger. Elevated calcium levels stimulate smooth muscle contraction, enhance vascular resistance, and contribute to the development of hypertension (Staehr et al., 2023).

Additionally, increased activation of the renin–angiotensin–aldosterone system and the sympathetic nervous system further intensifies vasoconstriction and sodium retention in metabolic syndrome. Together, these pathological mechanisms support the persistence of hypertension and elevate the risk of cardiovascular complications Thethi et al., 2012).

3.4. Dyslipidemia and Membrane Lipid Composition

Dyslipidemia, which is characterized by high triglyceride levels, elevated low-density lipoprotein (LDL) cholesterol, and decreased high-density lipoprotein (HDL) cholesterol, represents a key component of metabolic syndrome. Disturbances in lipid metabolism have major effects on the composition of cellular membranes and the functions associated with these structures (Pappan et al., 2024).

Cell membranes mainly consist of phospholipids, cholesterol, and proteins, and their proper structural arrangement is necessary for preserving membrane fluidity and efficient signaling processes. Under dyslipidemic conditions, the excessive accumulation of cholesterol and saturated fatty acids within the membrane can disrupt membrane organization, reduce fluidity, and impair the activity of membrane proteins, receptors, and ion transport systems (Cui et al., 2025).

Altered membrane lipid composition may also disturb lipid raft structure and negatively influence insulin receptor signaling, glucose transport, and ion exchange mechanisms. These membrane-related abnormalities contribute to insulin resistance, endothelial dysfunction, and defective intercellular communication. In addition, changes in the lipid environment surrounding Na^+/K^+-ATPase may impair the activity of this enzyme and reduce ion transport efficiency (Warda et al., 2025).

Furthermore, elevated lipid concentrations in circulation increase oxidative stress and stimulate lipid peroxidation, leading to structural damage of membrane lipids and weakening membrane stability. These pathological changes promote chronic inflammation, vascular damage, and the progression of cardiovascular complications associated with metabolic syndrome (Ascaso et al., 2007).

4. Association with Systemic Inflammation

Systemic inflammation represents a central pathological component of metabolic syndrome and is strongly associated with both metabolic disturbances and cardiovascular impairment. It is typically defined by a persistent low-grade inflammatory state, accompanied by elevated levels of circulating pro-inflammatory mediators, including TNF-α, IL-6, and CRP.

This ongoing inflammatory condition plays a significant role in the development of insulin resistance, endothelial dysfunction, and abnormal lipid metabolism (Domingo et al., 2024).

Within metabolic syndrome, adipose tissue—especially visceral fat—functions as an active endocrine organ that secretes various adipokines and inflammatory factors, thereby intensifying systemic inflammatory responses. This pro-inflammatory environment enhances oxidative stress, disrupts intracellular signaling mechanisms, and contributes to progressive cardiovascular injury, such as atherosclerotic changes and adverse cardiac remodeling (Jung and Choi 2014).

Furthermore, systemic inflammation may interfere with membrane-associated enzymes, including Na^+/K^+-ATPase, potentially impairing their normal regulatory and signaling roles. Accordingly, modulation of inflammatory pathways is considered a key therapeutic approach for mitigating metabolic abnormalities and improving cardiovascular outcomes (Pratt et al., 2020).

5. Therapeutic Potential and Clinical Implications

5.1 Na^+/K^+-ATPase–Targeted Drug Strategies

Na^+/K^+-ATPase functions not only as a fundamental ion transporter but also as an important signaling hub involved in intercellular communication. Through its localization in specialized membrane microdomains such as lipid rafts and caveolae, it interacts with signaling proteins including Src kinase and participates in multiple intracellular signaling cascades. Consequently, pharmacological approaches aimed at both the transport and signaling roles of Na^+/K^+-ATPase may offer innovative therapeutic avenues for regulating oxidative stress, inflammation, and pathological cardiac remodeling in metabolic syndrome and cardiovascular disorders (Huang et al., 2024).

5.2. Reassessment of Cardiac Glycosides

Cardiac glycosides, including digitalis derivatives, have traditionally been used in the management of heart failure due to their positive inotropic effects (Omole et al., 2025). Nevertheless, recent findings indicate that their biological actions extend beyond contractility enhancement, involving regulation of Na^+/K^+-ATPase–mediated intracellular signaling pathways. At lower doses, these compounds may exert anti-inflammatory and anti-proliferative effects, which has renewed interest in their therapeutic potential, particularly in metabolic syndrome–related cardiac structural alterations (Skubnik et al., 2021).

5.3. Antioxidant-Based Therapeutic Approaches

Oxidative stress, driven by excessive reactive oxygen species generation, lipid peroxidation, and mitochondrial dysfunction, represents a key pathological mechanism in metabolic syndrome. Antioxidant interventions, such as polyphenols, flavonoids, and compounds that support endogenous antioxidant defense systems, may help mitigate cellular injury. These strategies are especially relevant for maintaining endothelial integrity and reducing inflammatory activity within cardiac tissues (Ross and Benjamin 2025).

5.4. Combination Treatment Strategies in Metabolic Syndrome

Due to the complex and multifactorial nature of metabolic syndrome, monotherapy approaches are often inadequate. Therapeutic strategies combining agents that target insulin resistance, dyslipidemia, hypertension, and systemic inflammation may yield more effective clinical outcomes. The integrated use of antioxidants, insulin sensitizers, and lipid-lowering drugs may contribute to slowing disease progression and limiting end-organ damage (Lillich et al., 2021).

5.5. Potential of Clinical Biomarkers

Biological parameters such as Na^+/K^+-ATPase activity, oxidative stress indicators, inflammatory cytokines, and cardiac enzymes (including CK, CK-MB, AST, and LDH) hold significant potential as biomarkers for early diagnosis and disease monitoring in metabolic syndrome. These markers may facilitate the detection of subclinical pathology and support the development of individualized therapeutic strategies (Dhama et al., 2019).

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CHAPTER 2

NEUROPATHIC PAIN: A BIOPHYSICAL PERSPECTIVE

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1. Introduction

Pain is a protective biological response to stimuli that threaten the integrity of the organism and has important physiological functions for the continuation of life. However, pain is not simply a sensory perception originating from peripheral tissues; it is a complex process resulting from the interaction of the nervous system, the immune system, and various psychosocial factors. According to the definition updated in 2020 by the International Association for the Study of Pain (IASP), pain is defined as an unpleasant experience associated with or resembling actual or potential tissue damage (Raja et al., 2020). This definition shows that pain is not only a biological event but is also shaped by an individual's past experiences, environmental conditions, and psychological state. Pain is generally classified into three main groups: nociceptive, neuropathic, and nociplastic pain. Among these pain types, neuropathic pain holds particular importance due to its complex mechanisms, tendency to become chronic, and resistance to treatment. The IASP defines neuropathic pain as pain resulting from a lesion or disease affecting the somatosensory nervous system (Finnerup et al., 2016; Finnerup et al., 2021). Unlike other types of pain, neuropathic pain originates from structural or functional disorders of the nervous system and often persists even after the initial damage has been resolved. Neuropathic pain manifests clinically with various symptoms such as burning, stinging, tingling, electric shock sensations, numbness, and prickling. Sensory disturbances, such as pain caused by non-painful stimuli (allodynia) or increased response to painful stimuli (hyperalgesia), are also frequently observed (Colloca et al., 2017). These symptoms not only cause physical discomfort but can also significantly affect individuals' daily life activities, sleep patterns, work performance, and social relationships. The higher incidence of additional problems such as depression, anxiety, and decreased quality of life in individuals with chronic neuropathic pain increases the individual and societal burden of this disease (Smith, 2023). Epidemiological studies show that neuropathic pain is a significant health problem worldwide. Its prevalence in the general population is reported to be approximately 5–10% (Colloca et al., 2017; Finnerup et al., 2021). Diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia, peripheral nerve injuries, spinal cord injuries, multiple sclerosis, and chemotherapy-induced peripheral neuropathy are among the most common causes of neuropathic pain. The increasing incidence of peripheral neuropathies, particularly those caused by chemotherapeutic agents used in cancer treatment, has further increased the clinical importance of neuropathic pain (Yogi et al., 2025). Neuropathic pain can originate from peripheral or central sources. Peripheral neuropathic pain results from involvement of peripheral nerves or dorsal root ganglia, while central neuropathic pain develops due to damage to somatosensory pathways at the brain and

spinal cord level (Colloca et al., 2017). Although clinical symptoms are similar, the underlying mechanisms and responses to treatment can differ. Therefore, understanding the processes involved in the development of neuropathic pain is crucial for developing effective treatment strategies.

Recent studies have shown that neuropathic pain is not merely a direct result of nerve damage. Today, neuropathic pain is considered a disease closely related to changes in the electrical properties of nerve cells. From a biophysical perspective, information transmission in the nervous system occurs through electrical signals generated across the cell membrane. In this process, fundamental biophysical events such as membrane potential, ion distribution, action potential generation, and synaptic transmission play a critical role. Therefore, understanding neuropathic pain requires not only an examination of clinical symptoms or histopathological changes, but also of the mechanisms that determine the electrical behavior of nerve cells (Alles & Smith, 2021; Finnerup et al., 2021; Smith, 2023). The electrical properties of nerve cells are largely regulated by ion channels located in the cell membrane. Voltage-gated sodium, potassium, and calcium channels, along with various cation channels, play a fundamental role in the generation and propagation of action potentials. Recent studies have shown that changes in the expression or function of these channels may play a significant role in the generation and transmission of pain signals (Alles & Smith, 2021; Devor, 2006; Yogi et al., 2025). In particular, voltage-gated sodium channels such as Nav1.7, Nav1.8, and Nav1.9 are among the molecules that are intensively investigated in understanding pain mechanisms and identifying new therapeutic targets. From a biophysical perspective, neuropathic pain can be considered a clinical manifestation of changes in the electrical behavior and information transmission processes of nerve cells. Therefore, membrane biophysics, ion channels, electrical excitability, cellular signal transduction, and the functional properties of neuronal networks have become fundamental research areas in understanding neuropathic pain. Today, thanks to advanced electrophysiological methods, molecular biology techniques, and experimental animal models, these mechanisms can be studied in more detail, contributing to the development of new treatment approaches (Dib-Hajj & Waxman, 2019; Liu & Wood, 2011; Yogi et al., 2025). This section will address current information on neuropathic pain from a biophysical perspective; the classification of neuropathic pain, its basic biophysical and cellular mechanisms, experimental models, evaluation methods, and current treatment approaches will be examined in light of the current literature.

2. Classification of Neuropathic Pain

Neuropathic pain is a heterogeneous clinical condition resulting from lesions or diseases affecting the somatosensory nervous system. Numerous diseases with different etiologies, anatomical locations, and pathophysiological mechanisms can cause neuropathic pain. Therefore, the classification of neuropathic pain is important not only from a diagnostic point of view but also for determining appropriate treatment strategies and understanding the underlying biological mechanisms (Ahmadi et al., 2024; Finnerup et al., 2021). Currently, the most common classification is the distinction between peripheral and central neuropathic pain based on the location of the lesion. Peripheral neuropathic pain develops as a result of damage to peripheral nerves, nerve roots, or dorsal root ganglia. Diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia, peripheral nerve injuries, sciatic nerve damage, and chemotherapy-induced peripheral neuropathy are included in this group (Colloca et al., 2017) (Wang & Backonja, 2024). Peripheral neuropathies, especially those caused by chemotherapeutic agents such as paclitaxel, oxaliplatin, and cisplatin, have become an important area of research in recent years. Central neuropathic pain results from the involvement of somatosensory pathways at the brain or spinal cord level. Stroke, multiple sclerosis, spinal cord injuries, and certain neurodegenerative diseases are among the most important causes of central neuropathic pain (Scholz et al., 2019; Smith, 2023). Central neuropathic pain generally has a more widespread distribution and may be more resistant to current treatments.

Neuropathic pain can be classified as diabetic neuropathy, traumatic neuropathy, compression neuropathies, infection-related neuropathies, chemotherapy-induced peripheral neuropathy, and metabolic neuropathies (Rugnath et al., 2024). Although these diseases have different clinical features, there are certain similarities among the basic mechanisms involved in pain development. In recent years, the view that neuropathic pain can be classified not only according to anatomical location but also according to dominant biological mechanisms has gained importance. In particular, mechanisms such as changes in ion channel function, increased neuronal excitability, neuroimmune interactions, and synaptic plasticity show common features in many neuropathic pain syndromes (Alles & Smith, 2021; Finnerup et al., 2021). Therefore, mechanism-based approaches are becoming increasingly important in the development of personalized treatment strategies. From a biophysical perspective, although there are clinical differences among neuropathic pain types, changes in the electrical properties of nerve cells stand out as a common feature. Changes in ion channels involved in membrane potential regulation affect action potential generation and synaptic transmission processes,

contributing to the emergence of pain signals. Therefore, understanding the biophysical changes observed in different types of neuropathic pain is as important as classifying neuropathic pain itself (Alles & Smith, 2021; Devor, 2006).

Currently, the most common classification is the distinction between peripheral and central neuropathic pain based on the location of the lesion (Colloca et al., 2017).

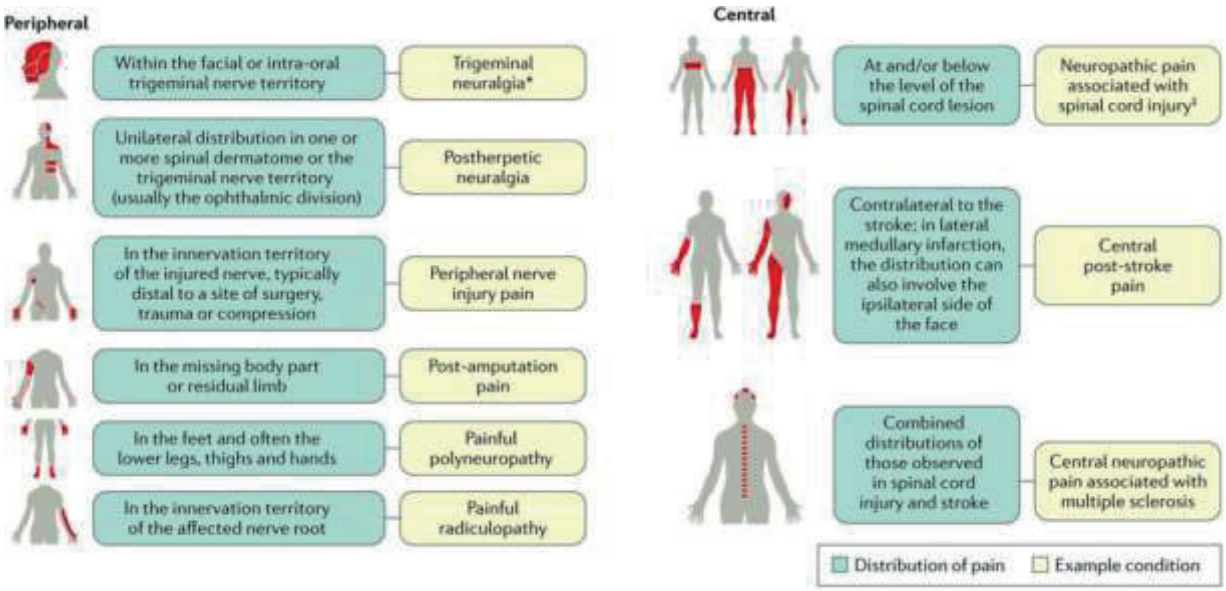

Figure 1. Neuroanatomical distribution of pain symptoms and sensory findings in widespread peripheral and central neuropathic pain. In peripheral neuropathic pain, symptoms are generally limited to the innervation area of the affected nerve, nerve root, or dermatome; while in central neuropathic pain, the distribution varies depending on the location of the central nervous system lesion. The figure shows examples of peripheral neuropathic pain such as trigeminal neuralgia, postherpetic neuralgia, peripheral nerve injury, post-amputation pain, painful polyneuropathy, and radiculopathy, as well as examples of central neuropathic pain associated with spinal cord injury, post-stroke central pain, and multiple sclerosis (Colloca et al., 2017).

3. Biophysical Foundations of Neuropathic Pain

Neuropathic pain is a complex clinical condition resulting from lesions or diseases of the somatosensory nervous system, characterized by significant changes in the normal electrical function of nerve cells. Currently, it is accepted that not only structural nerve damage but also changes in neuronal excitability play a significant role in the development of neuropathic pain (Finnerup et al., 2021; Smith, 2023). In the nervous system, information transmission occurs through electrical signals generated by the controlled movement of ions across cell membranes. Biophysical mechanisms such as membrane potential, ion channels, action potential generation,

and synaptic transmission are key determinants in this process. Changes in these mechanisms following peripheral nerve injury affect the electrical properties of neurons responsible for pain transmission, contributing to the amplification and chronicity of pain signals (Alles & Smith, 2021). The biophysical basis of neuropathic pain involves changes in the function of ion channels, increased membrane excitability, remodeling of synaptic transmission, and alterations in the functional organization of neural networks. These processes can occur at different levels in peripheral nerves, dorsal root ganglia, dorsal horn of the spinal cord, and supraspinal centers. Recent studies have shown that voltage-gated sodium, potassium, and calcium channels, as well as transient receptor potential (TRP) channels, play a critical role in the development of neuropathic pain (Alles & Smith, 2021; Devor, 2006; Yogi et al., 2025).

3.1. Ion Channels and Neuronal Excitability

The electrical activity of nerve cells results from the coordinated operation of ion channels located in the cell membrane. These channels regulate the movement of specific ions into or out of the cell, maintaining the membrane potential, generating action potentials, and enabling synaptic transmission. Neuronal excitability, defined as a neuron's capacity to generate an action potential in response to a stimulus, largely depends on the functional properties of these ion channels (Alles & Smith, 2021; Devor, 2006). In neuropathic pain, significant changes occur in the expression, distribution, and function of ion channels following peripheral nerve injury or diseases affecting the nervous system. These changes lower the excitation threshold of neurons, facilitating the generation and transmission of pain signals. As a result, stimuli that do not normally cause pain may be perceived as painful, or the existing pain response may be significantly increased (Finnerup et al., 2021; Smith, 2023).

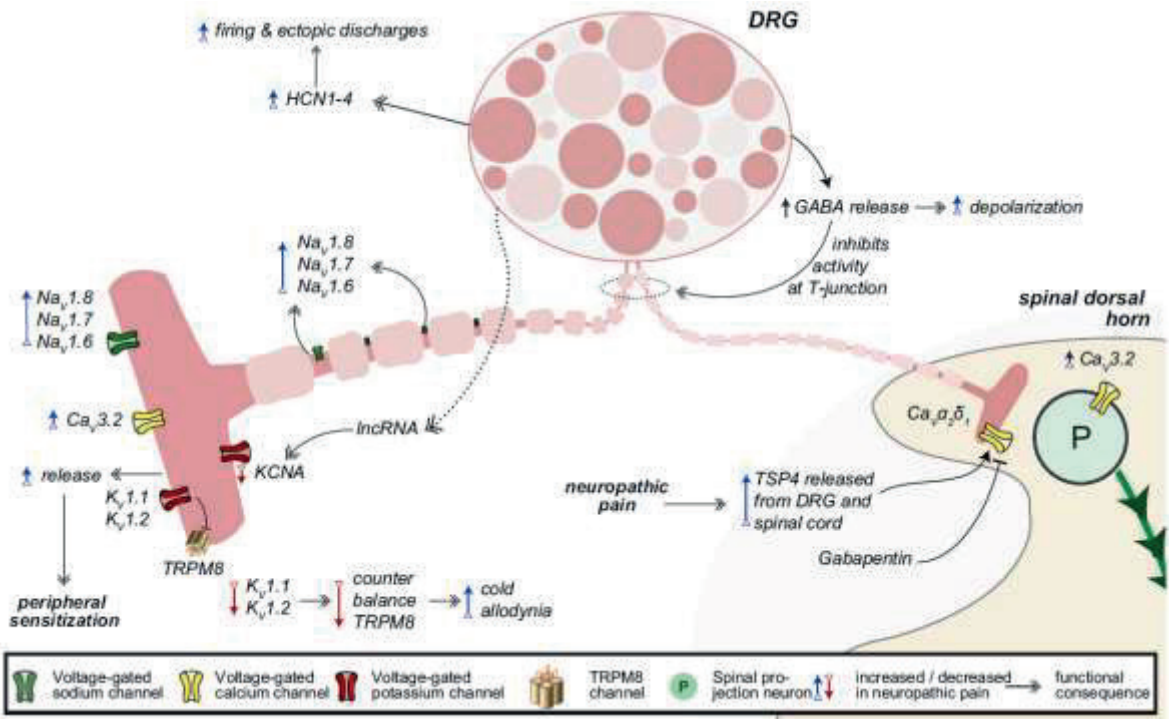

Figure 2. Schematic representation of ion channel dysregulations observed in sensory-spinal circuits in neuropathic pain. Changes in the expression and function of voltage-gated sodium (Nav), calcium (Cav), potassium (Kv), HCN, and TRPM8 channels following peripheral nerve damage affect neuronal excitability, contributing to the generation and transmission of pain signals. DRG: dorsal root ganglion (Finnerup et al., 2021).

Voltage-gated sodium channels (Nav) are membrane proteins that play a fundamental role in initiating and propagating action potentials. Specifically, subtypes Nav1.7, Nav1.8, and Nav1.9 have been shown to play important roles in pain transmission. Changes in the expression of these channels following peripheral nerve injury can lead to easier stimulation of sensory neurons and increased pain signals. In recent years, the development of selective inhibitors targeting the Nav1.7 channel has created a significant area of research for new analgesic treatment strategies (Dib-Hajj & Waxman, 2019; Yogi et al., 2025). Voltage-gated calcium channels (Cav) are involved in regulating neurotransmitter release at synaptic terminals. In particular, channels belonging to the Cav2 and Cav3 families are reported to play a significant role in pain transmission. Calcium influx through these channels facilitates the release of excitatory neurotransmitters, contributing to the transmission of pain signals to the central nervous system. The fact that commonly used drugs such as gabapentin and pregabalin exert their effects via the α2δ subunit of calcium channels highlights the clinical importance of these channels (Alles & Smith, 2021; Finnerup et al., 2021). Potassium channels (Kv) are involved

in stabilizing membrane potentials and facilitating repolarization after action potentials. In neuropathic pain, a decrease in the expression of some potassium channels has been reported, resulting in increased neuronal excitability. These changes in potassium channels may contribute to the development of long-term functional changes in pain transmission pathways (Alles & Smith, 2021; Devor, 2006). In recent years, transient receptor potential (TRP) channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels have also attracted attention in neuropathic pain research. While channels belonging to the TRP family are involved in the perception of temperature, mechanical stimuli, and chemical signals, HCN channels play a role in the regulation of neuronal rhythmic activity and membrane excitability. Functional changes occurring in these channels are thought to contribute to the maintenance of pain perception (Alles & Smith, 2021; Rugnath et al., 2024). Consequently, neuropathic pain is affected not only by structural changes due to nerve damage, but also by an electrical restructuring process resulting from changes in the function of ion channels. Therefore, ion channels hold a central position in understanding the biophysical mechanisms of neuropathic pain and identifying new therapeutic targets.

3.2. Formation of Membrane Potential and Action Potential

In the nervous system, information transmission relies on the ability of neurons to generate and transmit electrical signals. This electrical activity is fundamentally based on the electrical potential difference between the two sides of the cell membrane, i.e., the membrane potential. In a neuron at rest, the intracellular environment has a more negative electrical charge than the extracellular environment. This potential difference is mainly due to the asymmetric distribution of sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}) ions inside and outside the cell, as well as selective membrane permeability (Bean, 2007). Potassium ions play a crucial role in maintaining the resting membrane potential. Due to the high permeability of the cell membrane to potassium, K^+ ions tend to move out of the cell along the concentration gradient. Conversely, negatively charged proteins inside the cell contribute to maintaining a negative intracellular environment. The sodium-potassium ATPase pump plays an important role in maintaining membrane potential by ensuring the maintenance of ion gradients (Hall and Hall, 2021). When a neuron encounters a sufficiently large stimulus, the membrane potential reaches a threshold value, and an action potential is generated. This process begins with the opening of voltage-gated sodium channels. Rapid influx of Na^+ into the cell causes depolarization of the membrane, creating the rising phase of the action potential. Subsequently, sodium channels are inactivated while voltage-gated potassium channels open, and

repolarization occurs with the outflow of K^+ ions. After a short hyperpolarization phase, the membrane returns to its resting potential (Bean, 2007).

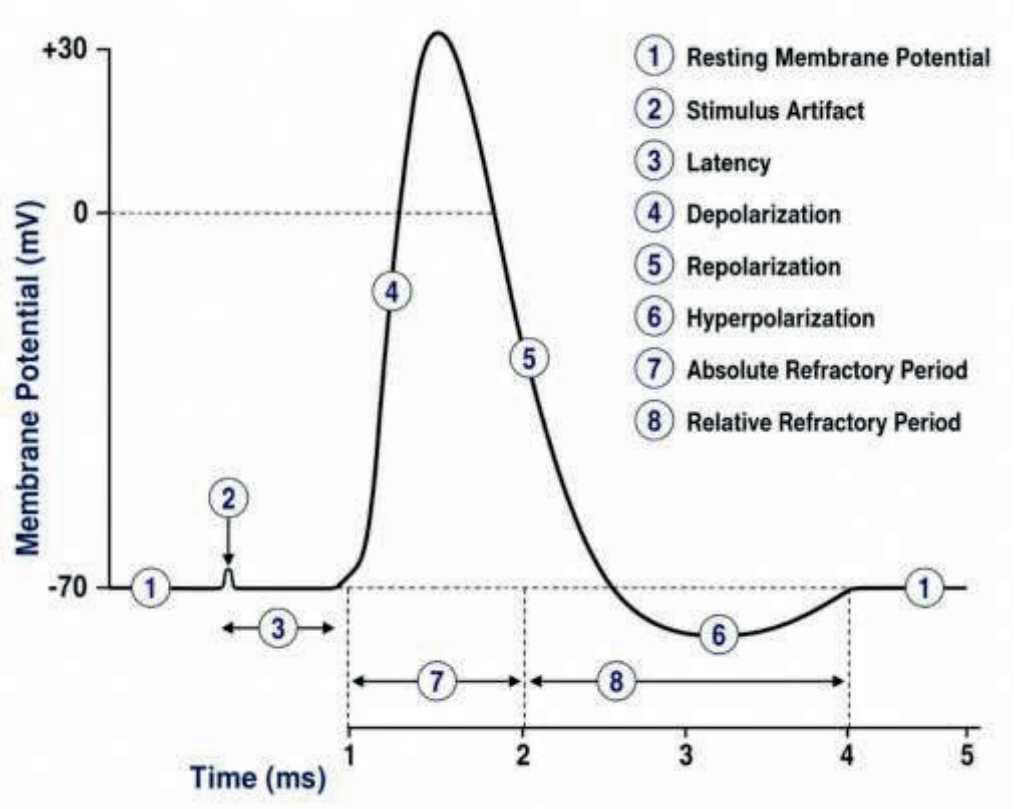

Figure 3. Schematic representation of the phases of an action potential in a neuron. An action potential consists of resting membrane potential, depolarization, repolarization, and hyperpolarization phases. During depolarization, voltage-gated sodium channels open, leading to Na^+ influx, while during the repolarization phase, voltage-gated potassium channels open, resulting in K^+ outflow. Absolute and relative refractory periods play an important role in regulating neuronal excitability (Hall, 2017).

In neuropathic pain, various changes occur in these electrical processes following peripheral nerve injury. In particular, increased expression of voltage-gated sodium channels, decreased potassium channels, and changes in the activity of some calcium channels can increase neuronal excitability. This leads to a decrease in the threshold required for the generation of action potentials and causes sensory neurons to be activated more easily than normal (Alles & Smith, 2021; Devor, 2006). Disruptions in the regulation of membrane potentials are not limited to peripheral nerves. Electrical changes occurring in dorsal root ganglia and spinal dorsal horn neurons also contribute to the amplification of pain signals. Consequently, neuropathic pain is considered not only a result of anatomical nerve damage but also a reflection of changes in the electrical properties of neuronal membranes (Finnerup et al., 2021; Smith, 2023). Therefore,

the generation of membrane potentials, the occurrence of action potentials, and the regulation of ion channels are fundamental concepts in understanding the biophysical mechanisms of neuropathic pain.

3.3. Neuronal Plasticity and Sensitization in Neuropathic Pain

One of the key mechanisms involved in the onset and chronicity of neuropathic pain is peripheral and central sensitization processes. Sensitization is defined as the activation of neurons involved in pain transmission pathways at lower thresholds than under normal conditions, resulting in a stronger response to the same stimuli. These processes occur as a result of biochemical, cellular, and electrophysiological changes that develop after nerve injury (Finnerup et al., 2016; Ji et al., 2018). Peripheral sensitization is characterized by functional changes occurring in damaged nerve fibers and nociceptors. Following nerve injury, changes in the expression and function of ion channels located in the cell membrane affect the electrical properties of the neuronal membrane, increasing excitability. This leads to a decrease in the threshold required for action potential generation and makes it easier for sensory neurons to be activated. As a result, stimuli that do not normally cause pain can cause pain, or the response to painful stimuli can be significantly increased (Kocot-Kepska et al., 2021). Central sensitization refers to long-term functional changes occurring in the dorsal horn of the spinal cord and the supraspinal centers involved in pain processing. Intense or repetitive stimuli from peripheral nerves increase synaptic activity in neuronal circuits in the central nervous system, altering the way pain signals are processed. During this process, excitatory synaptic transmission is strengthened while inhibitory mechanisms may weaken, thus increasing the sensitivity of pain-related neural networks (Ji et al., 2018; Ma et al., 2024). One of the key features of central sensitization is synaptic plasticity. Permanent changes in synaptic connections can lead to stronger perception of pain signals by the central nervous system. This is considered one of the mechanisms explaining the persistence of pain even after healing from peripheral damage. Furthermore, glutamatergic transmission, NMDA receptor activation, and intracellular signaling pathways are reported to play significant roles in the development of the central sensitization process (Quesada et al., 2021). Recent studies have shown that sensitization processes are not limited to neurons alone. Neuroinflammatory mechanisms regulated by microglia, astrocytes, and other glial cells contribute to the maintenance of functional changes in pain transmission pathways. Therefore, neuropathic pain is now considered not only as a direct result of nerve damage, but also as a product of multifaceted cellular and molecular remodeling processes occurring in the peripheral and central nervous systems (Ji et al., 2018;

Ma et al., 2024). From a biophysical perspective, peripheral and central sensitization can be considered a clinical manifestation of changes in the electrical properties of neuronal membranes. Alterations in ion channel function, reorganizations in synaptic transmission, and increased excitability of neuronal networks play a fundamental role in the development and persistence of neuropathic pain. Comparison of the key features of peripheral and central sensitization processes in neuropathic pain. Although both mechanisms lead to increased excitability in pain transmission pathways, they differ in terms of their locations, cellular mechanisms, and clinical outcomes (Table 1) (Ji et al., 2018; Kocot-Kepska et al., 2021). Recent studies have shown that sensitization processes cannot be explained solely by neuronal mechanisms. Glial cells such as microglia and astrocytes in the central nervous system play a significant role in the development and maintenance of neuropathic pain. Microglia, activated following peripheral nerve injury, can affect neuronal excitability and increase sensitivity in pain transmission pathways by releasing various cytokines, chemokines, and growth factors. Similarly, astrocytes contribute to the remodeling of pain-related neuronal circuits by participating in the regulation of the synaptic microenvironment (Kocot-Kepska et al., 2021). These processes are generally considered under the concept of neuroinflammation. Neuroinflammation refers to inflammatory responses occurring within the nervous system and is considered one of the important mechanisms in the chronicity of neuropathic pain. The neuroinflammatory environment resulting from microglia and astrocyte activation can contribute to the strengthening of synaptic transmission and the development of permanent functional changes in pain processing pathways. Therefore, neuroinflammation is currently emerging as an important research area not only in understanding the pathophysiology of neuropathic pain but also in identifying new treatment targets (Ji et al., 2018; Ma et al., 2024).

Table 1. Comparison of the Main Characteristics of Peripheral and Central Sensitization

Characteristic	Peripheral Sensitization	Central Sensitization
Site of occurrence	Peripheral nerve endings and dorsal root ganglia	Spinal dorsal horn and supraspinal centers
Triggering factors	Nerve injury, inflammation, tissue damage	Prolonged or repetitive nociceptive input
Primary mechanism	Increased nociceptor excitability	Enhanced synaptic activity within the central nervous system
Ion channel alterations	Functional changes in Nav, Cav, and TRP channels	Alterations in synaptic receptor and ion channel activity
Neuronal effect	Reduced activation threshold	Increased responsiveness of pain-processing circuits
Clinical manifestations	Primary hyperalgesia, allodynia	Secondary hyperalgesia, chronic pain
Reversibility	Greater in the early stages	More limited in long-term conditions
Therapeutic targets	Peripheral nerves and ion channels	Central pain pathways and neuromodulation approaches

4. Experimental Neuropathic Pain Models

Experimental animal models play a significant role in understanding the biophysical, cellular, and behavioral mechanisms of neuropathic pain. While these models may not perfectly reflect all the characteristics of neuropathic pain seen in humans, they allow for the investigation of mechanical allodynia, thermal hyperalgesia, changes in neuronal excitability, neuroinflammation, and nerve conduction disorders that develop after nerve damage. The correct selection of experimental models is crucial for understanding the study's objective, targeted mechanism, and evaluation method (Benamar, 2024; Casaril et al., 2024). Experimental neuropathic pain models can generally be classified as traumatic nerve injury models, metabolic neuropathy models, and chemotherapy-induced peripheral neuropathy models. Among traumatic nerve injury models, chronic constriction injury, spared nerve injury, and spinal ligation are the most commonly used. Current assessments report that spinal ligation, spared nerve injury, and chronic constriction injury models are among the most frequently used mononeuropathic models in preclinical neuropathic pain studies (Sadler et al., 2022). Sciatic nerve-based models are particularly important in peripheral neuropathic pain research. In these

models, mechanical sensitivity, thermal pain response, motor function changes, and nerve conduction characteristics can be evaluated by creating controlled damage to the sciatic nerve. The spared nerve injury model is frequently preferred because it produces long-lasting and significant mechanical allodynia. The behavioral, biochemical, and electrophysiological characteristics of this model have been described in detail in preclinical studies (Guida et al., 2020). Chemotherapy-induced peripheral neuropathy models have also gained importance in recent years. Models created with antineoplastic agents such as paclitaxel, cisplatin, and oxaliplatin allow for the experimental investigation of neuropathic pain seen after cancer treatment in clinical practice. In these models, mechanical allodynia, changes in thermal sensitivity, peripheral nerve damage, mitochondrial dysfunction, and neuroinflammatory responses can be evaluated. Studies comparing chemotherapy-induced peripheral neuropathy models have reported that paclitaxel and cisplatin models are suitable for experimental research when created with application routes similar to clinical practice (Gadgil et al., 2019; Lv et al., 2023). The neuropathic pain model induced by paclitaxel is widely used, particularly to assess mechanical allodynia and signs of peripheral nerve damage. In this model, dose, duration of administration, animal species, sex, and time of assessment can affect the results. A systematic review of paclitaxel-induced neuropathic pain reported that most studies used male Sprague-Dawley rats, preferred low-dose paclitaxel protocols, and highlighted mechanical nociceptive tests in behavioral assessments (Bacalhau et al., 2023). Experimental neuropathic pain models allow for the combined use of behavioral tests and biochemical, histological, and electrophysiological assessments. This enables the evaluation not only of pain behavior but also of structural changes in nerve tissue, inflammatory markers, oxidative stress parameters, and nerve conduction characteristics. However, it is important to remember that animal models reflect human neuropathic pain only to a limited extent. Therefore, when selecting an experimental model, the primary objective of the research, the targeted mechanism, and its clinical relevance should be carefully considered.

5. Assessment of Neuropathic Pain

The assessment of neuropathic pain is a multidimensional process requiring the combined use of clinical findings, behavioral tests, and auxiliary laboratory methods. Due to the subjective nature of pain, a definitive assessment cannot be made using a single method. Therefore, different assessment tools are used in both clinical practice and experimental studies (Finnerup et al., 2021). In clinical assessment, a detailed patient history and neurological examination form the basic step. While evaluating the localization, duration, intensity, and character of the

pain, symptoms such as sensory deficits, paresthesia, dysesthesia, and allodynia are also investigated. Currently, diagnostic algorithms recommended by the International Association for the Study of Pain (IASP) and the Neuropathic Pain Special Interest Group (NeuPSIG) are widely used in the diagnosis of neuropathic pain. These approaches are based on the combined evaluation of clinical history, neuroanatomical distribution, sensory examination findings, and confirmatory tests (Colloca et al., 2017; Finnerup et al., 2021). Behavioral assessment methods play a significant role in experimental studies. Mechanical allodynia is frequently assessed using von Frey filaments, while thermal pain responses are examined using hot plate and tail-flick tests. These tests allow for the objective evaluation of pain behaviors developing in models of peripheral nerve injury or chemotherapy-induced neuropathy (Bacalhau et al., 2023). Electrophysiological methods provide important information in the assessment of neuropathic pain. Electromyography and nerve conduction studies are widely used in the evaluation of peripheral nerve function. In particular, decreased nerve conduction velocity, amplitude changes, and conduction blocks can provide information about the degree of nerve damage. In addition, electrophysiological methods offer significant advantages in evaluating the effects of experimental treatments on nerve function (Smith, 2023). In addition to behavioral and electrophysiological assessments, histopathological and biochemical analyses are also commonly used. Histological examinations allow for the evaluation of degeneration, demyelination, and inflammatory changes in nerve fibers, while biochemical analyses provide information about oxidative stress markers, inflammatory cytokines, and cell death mechanisms. The combined use of these methods contributes to a more comprehensive understanding of the underlying mechanisms of neuropathic pain (Ji et al., 2018; Kocot-Kepska et al., 2021). In conclusion, the evaluation of neuropathic pain requires a multidisciplinary approach involving a combination of clinical observation, behavioral analysis, electrophysiological measurements, and laboratory methods.

Table 2. Common Behavioral Tests Used in Experimental Neuropathic Pain Studies

Test	Parameter Assessed	Advantages	Limitations
von Frey Test	Mechanical allodynia	Widely accepted standard method; sensitive for detecting changes in mechanical pain threshold	Results may be influenced by operator experience and animal behavior
Hot Plate Test	Thermal hyperalgesia and nociceptive response	Simple and rapid assessment of thermal pain sensitivity	May be affected by motor impairment and learning effects
Tail-Flick Test	Spinal nociceptive reflex response	Rapid, objective, and easy to perform	Primarily evaluates spinal reflexes rather than supraspinal pain processing
Acetone Test	Cold allodynia	Useful for assessing cold hypersensitivity, particularly in chemotherapy-induced neuropathy models	Standardization may be challenging
Rotarod Test	Motor coordination and balance	Useful for distinguishing analgesic effects from motor deficits	Does not directly measure pain perception

Table 2. Common behavioral tests used in experimental neuropathic pain research. These tests are widely employed to evaluate mechanical allodynia, thermal hyperalgesia, cold hypersensitivity, and motor performance in animal models of neuropathic pain. The table was prepared based on information synthesized from the literature (Bacalhau et al., 2023; Sadler et al., 2022).

6. Current Treatment Approaches and Future Perspectives

Treatment of neuropathic pain presents significant clinical challenges due to the fact that eliminating the underlying cause is not always possible and the pain mechanisms are quite complex. Current treatment strategies aim to reduce pain, preserve functional capacity, and improve quality of life. However, current treatment options do not demonstrate sufficient

efficacy in all patients, and treatment success may be limited in some patients due to side effects (Finnerup et al., 2021). In pharmacological treatment, gabapentinoids (gabapentin and pregabalin), serotonin-norepinephrine reuptake inhibitors (especially duloxetine), and tricyclic antidepressants are among the first-line options for treating neuropathic pain. These drugs reduce neuronal excitability and modulate the processing of pain signals by acting on voltage-gated calcium channels, monoaminergic pain modulation systems, and synaptic transmission mechanisms. However, since neuropathic pain is a heterogeneous clinical picture, the response to treatment can vary significantly among individuals. This has increased interest in developing patient-specific treatment approaches and combination therapy strategies in recent years (Bates et al., 2019; Fornasari, 2017; Sadegh et al., 2024). In recent years, therapies targeting ion channels have gained significant importance in neuropathic pain research. In particular, novel agents developed targeting voltage-gated sodium, calcium, and transient receptor potential channels aim to reduce abnormal electrical activity in pain transmission pathways. Furthermore, treatment strategies targeting neuroinflammatory processes and neuroprotective approaches are also showing promising results in experimental studies (Smith, 2023). Among non-pharmacological approaches, neuromodulation methods are gaining increasing importance. Techniques such as transcranial magnetic stimulation, spinal cord stimulation, and peripheral nerve stimulation aim to reorganize the electrical activity associated with pain in the nervous system. These methods are reported to be able to reduce pain intensity and improve quality of life, especially in cases of treatment-resistant neuropathic pain (Blackmore et al., 2019). In addition, new generation neuromodulation methods such as low-intensity focused ultrasound are attracting attention due to their non-invasive properties and potential to affect neuronal activity. It has been shown that ultrasound can modulate neuronal membrane properties and synaptic activity through its mechanical and neurophysiological effects, and this approach is considered one of the promising methods for the treatment of neuropathic pain in the future (Rabut et al., 2020). Neuropathic pain is a complex clinical condition resulting not only from nerve damage but also from the interaction of ion channel functions, membrane potential changes, neuronal plasticity, sensitization processes, and neuroinflammatory mechanisms. Therefore, a better understanding of neuropathic pain is of great importance not only for basic science research but also for the development of new treatment strategies. The increased knowledge of pain biology in recent years has enabled the development of more selective pharmacological targets and novel neuromodulation approaches (Yekkirala et al., 2017).

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CHAPTER 3

OXIDATIVE STRESS-MEDIATED CELLULAR DAMAGE IN METABOLIC SYNDROME

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1. Introduction

Metabolic disorders, such as metabolic syndrome, obesity, and type 2 diabetes mellitus (T2DM), represent a major global health burden characterized by progressive metabolic dysregulation and multi-organ involvement. Although these conditions are traditionally defined by disturbances in glucose and lipid metabolism, accumulating evidence indicates that their pathogenesis extends far beyond classical metabolic pathways. In particular, disturbances in cellular redox balance and oxidative stress have emerged as key factors driving the onset and progression of metabolic dysfunction (Roberts et al., 2009).

Reactive oxygen species (ROS) are constantly produced as normal byproducts of aerobic metabolism, mainly through the mitochondrial electron transport chain and enzymatic pathways, including nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs). Under physiological conditions, ROS act as essential signaling mediators involved in the regulation of gene expression, immune function, insulin signaling, and vascular homeostasis. This controlled redox signaling is essential for maintaining cellular integrity and metabolic flexibility (Divvela et al., 2025). However, when ROS generation surpasses the capacity of antioxidant defense mechanisms, oxidative stress develops, resulting in damage to lipids, proteins, and nucleic acids, as well as impairment of normal cellular functions.

To counteract oxidative stress, cells are equipped with highly organized antioxidant defense mechanisms consisting of non-enzymatic and enzymatic components, as well as repair mechanisms that restore oxidatively damaged biomolecules. Despite the efficiency of these systems, persistent metabolic overload, chronic inflammation, and mitochondrial dysfunction can overwhelm redox homeostasis. This imbalance is particularly evident in metabolic syndrome, where excess nutrient availability, hyperglycemia, and elevated free fatty acids promote sustained ROS generation and impair antioxidant capacity (Masenga et al., 2023).

An increasing body of evidence indicates that redox imbalance is not simply a downstream effect of metabolic disease, but instead a core pathogenic mechanism (Manzoor et al., 2022). Oxidative stress has been demonstrated to interfere with insulin signaling pathways, promote lipid peroxidation, and enhance the formation of advanced glycation end products (AGEs), collectively contributing to the progressive development of insulin resistance. Furthermore, reactive lipid-derived aldehydes and oxidative modifications of signaling proteins amplify cellular dysfunction and establish self-perpetuating cycles of metabolic and inflammatory stress (Masenga et al., 2023).

Within this context, lipid peroxidation represents a critical downstream effect of oxidative stress, causing both functional disruption and structural damage of cellular

membranes. The resulting accumulation of reactive aldehydes further exacerbates insulin signaling defects and mitochondrial dysfunction. Together, these processes converge to drive systemic metabolic impairment and organ dysfunction characteristic of metabolic syndrome (Zheng et al., 2024; Gaschler et al., 2017).

Insulin resistance, therefore, can be understood as the integrated outcome of multiple interconnected pathological processes, including oxidative stress, inflammation, lipid toxicity, and impaired antioxidant defenses. Rather than arising from a single molecular defect, it reflects a complex network of dysregulated signaling pathways that progressively compromise metabolic homeostasis (Ordóñez-Díaz et al., 2021).

This chapter is intended to provide a thorough overview of ROS biology, antioxidant defense systems, and the contribution of redox imbalance to metabolic syndrome. By integrating current knowledge on oxidative stress-mediated mechanisms, lipid peroxidation, and insulin resistance, it highlights redox dysregulation as a central treatment target in metabolic diseases and related complications.

2. ROS and Redox Homeostasis

An individual oxygen atom carries a single unpaired electron in its outer valence shell, while molecular oxygen has two unpaired electrons, classifying it as a biradical. Under normal conditions, the mitochondrial electron transport chain facilitates the full four-electron reduction of oxygen to water. Nevertheless, oxygen can also undergo partial (one-electron) reduction, resulting in the generation of reactive intermediates. Due to the reductive characteristics of the intracellular environment, such incomplete reductions occur relatively frequently. As a result, reactive molecules-including the highly reactive hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and superoxide anion ($O_2^{\bullet-}$) are continuously formed as natural byproducts of oxidative reactions and aerobic metabolism (Hernansanz-Agustín et al., 2021; Palma et al., 2024).

ROS encompass both radical and non-radical forms, such as $O_2^{\bullet-}$, H_2O_2, •OH, and peroxyl radicals. These species are primarily generated in mitochondria during oxidative phosphorylation and by membrane-associated NOXsAt physiological levels, ROS serve as important signaling mediators that help maintain cellular homeostasis, gene expression, immune responses, and metabolic pathways (Quilaqueo-Millaqueo et al., 2024; Forrester et al., 2018).

In contrast, an imbalance caused by impaired antioxidant defenses or excessive ROS generation results in oxidative stress, defined as a disturbance in redox homeostasis. Elevated ROS levels are harmful, as they promote oxidative injury to essential cellular macromolecules including proteins, DNA, and lipids. This damage can impair cellular function, induce cell cycle

arrest, and trigger cell death. Increasing evidence highlights the involvement of oxidative stress in the development of various diseases, including cancer, atherosclerosis, neurodegenerative conditions, cardiovascular disorders (e.g., hypertension), acute respiratory distress syndrome, diabetes mellitus, idiopathic pulmonary fibrosis, chronic inflammatory diseases, asthma, and chronic obstructive pulmonary disease (Gao et al., 2025; Ginckels et al., 2022).

2.1 Antioxidant defense systems

Antioxidant defense systems are vital for preserving redox equilibrium in biological organisms by inhibiting or slowing the oxidation of cellular components. In living cells, antioxidant defenses are highly structured, comprising both non-enzymatic and enzymatic elements that function in an integrated and hierarchical fashion (Halliwell, 2024).

Among ROS and peroxynitrite ($ONOO^-$), reactive nitrogen species (RNS), and •OH are especially damaging because of their exceptionally high reactivity with cellular macromolecules. These species react at, or very close to, diffusion-limited rates, which makes their direct neutralization by low-molecular-weight antioxidants largely ineffective. Consequently, the primary protective approach focuses on preventing their generation rather than scavenging them after formation. This is mainly accomplished through enzymatic detoxification of upstream precursors such as $O_2^{\bullet-}$ and H_2O_2, mediated by antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Moreover, reducing superoxide levels also limits the formation of $ONOO^-$, which is generated through the rapid interaction between $O_2^{\bullet-}$ and nitric oxide (NO), thereby further diminishing subsequent oxidative and nitrosative injury (Birben et al., 2012; Altanam et al., 2025).

Antioxidant defenses are typically divided into three main lines. The first line consists of enzymatic antioxidants that directly neutralize reactive species or block their generation. Major enzymes in this category include GPx, CAT, and SOD. SODs serve as the principal defense against superoxide radicals and exist in several isoforms with specific cellular distributions. Cu,Zn-SOD is mainly localized in the cytosol and nucleus, Mn-SOD is found within the mitochondrial matrix, and extracellular SOD functions outside the cell. This compartmentalization enables precise regulation of redox balance across different cellular regions. CAT, largely located in peroxisomes, rapidly decomposes H_2O_2 into water and oxygen via a heme-dependent mechanism. GPx, characterized by the presence of selenocysteine in their active sites, reduce both H_2O_2 and lipid hydroperoxides using reduced glutathione (GSH). The regeneration of oxidized glutathione to GSH is catalyzed by glutathione reductase (GR). in a process dependent on NADPH, thereby linking antioxidant capacity to cellular metabolism (Averill-Bates, 2023).

Beyond these core enzymes, additional redox-active proteins such as thioredoxins (TRX), glutaredoxins, and heme oxygenase-1 also play significant roles in sustaining cellular redox homeostasis. These systems are especially critical in organs exposed to high oxidative burdens, such as the lungs, where antioxidant defenses are robustly expressed to counter continuous environmental and metabolic stressors (Kansal et al., 2023; Hanschmann et al., 2013).

The second tier of defense consists of non-enzymatic, small-molecule antioxidants including flavonoids, vitamin C, carotenoids, vitamin E, and various dietary compounds. Although less efficient than enzymatic systems in directly scavenging radicals, these molecules contribute by interrupting chain reactions, stabilizing oxidized species, and regenerating other antioxidants (Carocho et al., 2013; Ndhlala et al., 2010).

The third line of defense involves mechanisms responsible for the repair and removal of oxidatively damaged biomolecules. These processes include degradation of oxidized proteins, DNA repair pathways, and lipid remodeling. Notably, oxidative stress itself can stimulate the expression of enzymes involved in these repair systems, reflecting an adaptive cellular response (Cadet et al., 2017).

2.2 Redox imbalance in metabolic syndrome

Redox homeostasis is a fundamental requirement for preserving cellular integrity, metabolic flexibility, and precise signal transduction in mammalian systems. It is maintained through a tightly regulated balance between the production of RNS and ROS and their removal by endogenous antioxidant protection networks. In metabolic syndrome, this equilibrium becomes chronically disrupted, leading to a sustained shift toward oxidative and nitrosative stress. Rather than representing a secondary consequence of metabolic dysfunction, redox imbalance is now widely recognized as a central pathogenic mechanism linking obesity, insulin resistance, cardiovascular disease, T2DM (Zhou et al., 2025; Wilkinson et al., 2025).

Within the context of metabolic syndrome, this regulatory system is further destabilized by persistent metabolic overload. Elevated levels of free fatty acids and glucose enhance the activity of the mitochondrial electron transport chain, which promotes electron leakage and subsequent ROS formation. Concurrently, pro-inflammatory cytokines stimulate enzymatic sources of ROS, particularly NOX complexes, thereby intensifying intracellular oxidative burden (Bhatti et al., 2017; Masenga et al., 2023).

As ROS accumulation progresses, endogenous antioxidant defense mechanisms become increasingly exhausted and functionally compromised. This shift drives the cellular

environment toward a persistent pro-oxidant state, resulting in oxidative injury and triggering of stress-responsive internal cell signaling cascades (Liu et al., 2026).

Beyond dysfunction of mitochondria, chronic hyperglycemia activates multiple alternative glucose metabolism pathways, including the polyol, hexosamine, and AGE pathways. These metabolic routes further exacerbate oxidative stress while also promoting carbonyl stress and glycation-associated molecular damage (Luo et al., 2016).

A key consequence of these alterations is the accumulation of reactive dicarbonyl species such as methylglyoxal, which chemically modify proteins and facilitate AGE formation. Binding of AGEs to their receptor enhances ROS generation and triggers pro-inflammatory signaling cascades, thereby establishing a self-amplifying loop of metabolic dysfunction and oxidative stress. Increased AGE burden is closely linked to vascular complications and insulin resistance (Cepas et al., 2020; Song et al., 2012).

Furthermore, excessive intracellular glucose flux inhibits glyceraldehyde-3-phosphate dehydrogenase, causing accumulation of glycolytic intermediates upstream in the pathway and diversion into alternative oxidative pathways. This metabolic bottleneck further aggravates oxidative stress and disrupts cellular function, particularly in insulin-sensitive tissues (Yan, 2014).

Consistently elevated concentrations of oxidative stress biomarkers including 8-oxo-2'-deoxyguanosine, F2-isoprostanes, and malondialdehyde (MDA) in obesity, prediabetes, and T2DM reflect extensive oxidative injury to nucleic acids, proteins, and lipids. These biomarkers correlate strongly with insulin resistance, inflammatory burden, and alterations in body composition (Monzo-Beltran et al., 2017; Ma et al., 2017).

3. Impairment of Antioxidant Defense Systems

Impairment of antioxidant defense systems is a major pathological process underlying the onset and development of oxidative stress-associated disorders, especially metabolic syndrome and related metabolic diseases. Under normal physiological conditions, redox balance is preserved by a coordinated antioxidant network consisting of both non-enzymatic and enzymatic components. Enzymatic defenses include SOD, CAT, and GPx, whereas non-enzymatic antioxidants such as glutathione, vitamin C, and vitamin E play essential roles in neutralizing ROS. (Sharebiani et al., 2024; Varra et al., 2025).

When ROS generation surpasses the capacity of these protective mechanisms, oxidative stress develops, leading to progressive cellular dysfunction and tissue damage. In metabolic syndrome, this imbalance is intensified by multiple interconnected metabolic abnormalities. Hyperglycemia is a major initiating factor, as excess glucose undergoes autoxidation and

directly contributes to ROS formation. AGEs further enhance oxidative stress by stimulating additional ROS generation through processes such as metal-catalyzed oxidation reactions, thereby establishing a self-amplifying oxidative cycle. This sustained pro-oxidant state plays a major role in damaging cellular structures across multiple organ systems (Chen et al., 2013; Zhang et al., 2019).

In this setting, antioxidant defense impairment occurs through several overlapping mechanisms, including decreased expression of antioxidant enzymes, oxidative modification and inactivation of antioxidant proteins, depletion of glutathione stores, and defective regeneration of redox cofactors. These changes collectively diminish cellular resistance to oxidative insults and promote persistent redox disequilibrium. Notably, both experimental and clinical data indicate that such impairments are not limited to advanced disease stages but are already detectable in early metabolic alterations such as obesity and prediabetes, highlighting antioxidant dysfunction as an early and progressively worsening feature of disease development (Furukawa et al., 2004; Vávrová et al., 2013).

At the molecular level, experimental studies further emphasize the importance of intact redox regulatory pathways. A study demonstrated that caspase-2 deficiency impairs antioxidant defense mechanisms and promotes excessive ROS accumulation, indicating that specific intracellular signaling proteins are critical for maintaining redox homeostasis (Shalini et al., 2012). This supports the idea that antioxidant dysfunction may arise not only from increased ROS production but also from intrinsic defects in regulatory networks governing cellular redox control.

Clinical findings also strongly link antioxidant impairment to metabolic disease. A study observed that adolescents with type 1 diabetes and children and concurrent metabolic syndrome exhibit markedly reduced antioxidant capacity together with elevated oxidative stress markers (Grabia et al., 2023). This imbalance is associated with faster metabolic deterioration and a higher risk of long-term complications.

4. Lipid Peroxidation and Cellular Membrane Injury

Lipid peroxidation is a prominent downstream outcome of oxidative stress and serves as a fundamental mechanism of cellular membrane damage, especially in the context of metabolic syndrome. It primarily affects polyunsaturated fatty acids located in cellular and organelle membranes, owing to their heightened vulnerability to free radical-mediated oxidation. In metabolic syndrome, multiple interconnected factors - including mitochondrial dysfunction, activation of NOXs, hyperglycemia, elevated circulating free fatty acids, and

persistent low-grade inflammation - contribute to excessive ROS generation, thereby sustaining a pro-oxidative environment that strongly drives lipid peroxidation (Martemucci et al., 2023).

This process proceeds as a self-propagating radical chain reaction initiated when ROS, particularly hydroxyl radicals, extract hydrogen atoms from membrane lipids, forming lipid radicals. Once triggered, lipid peroxidation can spread extensively across membrane structures and is difficult to contain due to the limited efficiency of endogenous antioxidant and detoxification systems in eliminating lipid-derived reactive intermediates. Termination occurs when radical species interact with each other or are neutralized by antioxidant defenses, resulting in relatively stable oxidized lipid end products (Porter et al., 1995).

A critical consequence of this process is the generation of reactive lipid-derived aldehydes, including 4-hydroxy-2-hexenal (4-HHE), 4-oxo-2-nonenal (4-ONE), 4-hydroxy-2-nonenal (4-HNE), and MDA. These highly electrophilic compounds readily form covalent bonds with proteins, nucleic acids, and lipids, thereby extending oxidative damage well beyond the initial membrane site of injury. While MDA is frequently employed as an indicator of oxidative stress, 4-HNE and related aldehydes also function as bioactive mediators capable of altering cellular signaling pathways (Grimsrud et al., 2008).

From a structural perspective, lipid peroxidation disrupts membrane integrity by decreasing fluidity, increasing permeability, and disturbing ion homeostasis. It also compromises the activity of membrane-bound proteins, including receptors, transporters, enzymes, and ion channels, leading to impaired cellular communication, metabolic imbalance, and mitochondrial dysfunction. Under more severe oxidative conditions, secondary protein oxidation - such as modification of sulfhydryl groups and protein cross-linking - further intensifies enzyme inactivation and cellular dysfunction (Stadtman et al., 2000; Murphy, 2009).

Lipid peroxidation products are also closely linked to metabolic disturbances. Increased levels of 4-HNE - protein adducts are associated with obesity-related indicators including waist circumference, body mass index, and the severity of insulin resistance. At the molecular level, 4-HNE can directly modify critical components of insulin signaling pathways, including Akt, thereby diminishing insulin-stimulated glucose uptake. Similarly, elevated 4-HHE disrupts insulin signaling, suppresses glucose transporter type 4 (GLUT4) translocation, and depletes intracellular GSH, thereby weakening antioxidant defense capacity. Notably, replenishment of GSH has been shown to reverse these effects, emphasizing the importance of redox buffering systems in preserving insulin responsiveness (Pillon et al., 2012; Murdolo et al., 2023).

The process is further exacerbated by organelle-level damage. Disruption of lysosomal membranes can lead to the release of hydrolytic enzymes such as phospholipases, which

accelerate lipid breakdown and intensify membrane injury. This establishes a self-reinforcing cycle in which oxidative stress promotes membrane damage, while membrane damage in turn enhances susceptibility to further oxidative insult. Even at sub-lethal levels, lipid peroxidation products can alter membrane transport dynamics and lipid bilayer organization, thereby affecting intracellular signaling and metabolic regulation (Yin et al., 2011; Kagan et al., 2005).

5. Insulin Resistance as an Integrated Pathological Outcome

Rather than resulting from a single molecular abnormality, insulin resistance arises from the cumulative interaction of multiple interconnected disturbances such as oxidative stress, lipid excess, mitochondrial impairment. Accordingly, insulin resistance should be considered not an isolated defect, but instead as the ultimate manifestation of systemic metabolic dysregulation (Szendroedi et al., 2011).

Under normal physiological conditions, insulin exerts its effects through a tightly regulated signaling cascade initiated by insulin receptor activation, followed by phosphorylation of insulin receptor substrates (IRS), and subsequent stimulation of the PI3K/Akt pathway. This signaling axis ultimately drives the translocation of GLUT4 to the plasma membrane, thereby facilitating glucose uptake in insulin-sensitive tissues, including adipose tissue and skeletal muscle. However, under conditions of metabolic stress, this signaling network becomes progressively compromised at several regulatory checkpoints (van Gerwen et al., 2023; Lee et al., 2022).

A primary upstream determinant of insulin resistance is oxidative stress, impairing insulin signaling through multiple mechanisms. Overproduction of ROS impairs IRS phosphorylation and downstream Akt signaling. In addition, ROS activate NF-κB, a key regulator of inflammatory gene expression, thereby intensifying inflammatory responses and further suppressing insulin action. Lipid-derived metabolites, including diacylglycerols and ceramides, also contribute by directly inhibiting key components of insulin signaling pathways. Collectively, these processes converge to diminish cellular insulin responsiveness (Lee et al., 2022; Ly et al., 2017).

Sustained metabolic excess, particularly in obesity, further worsens this dysfunction. When the capacity of adipose tissue to store lipids is overwhelmed, lipids accumulate ectopically in non-adipose organs, including the liver. This lipid spillover induces lipotoxic stress and significantly contributes to insulin resistance. Importantly, metabolic impairment is not exclusively defined by body mass index (BMI), but rather by the functional capacity of adipose tissue. As such, individuals with normal BMI may still exhibit marked insulin resistance (“metabolically obese”), while some individuals with obesity maintain preserved

insulin sensitivity (“metabolically healthy obese”), emphasizing the critical role of adipose tissue functionality (Longo et al., 2019).

At the cellular level, impaired antioxidant defenses within adipose tissue further promote insulin resistance. Decreased levels of glutathione, MnSOD, and CAT have been identified in visceral fat of metabolically compromised individuals, alongside increased NOX expression, which collectively amplifies oxidative stress and disrupts insulin signaling. Moreover, elevated concentrations of asymmetric dimethylarginine, reduce NO availability, thereby contributing to vascular and metabolic dysfunction in insulin-resistant states (Taherkhani et al., 2021).

Inflammatory processes serve as a major amplifying mechanism in this context. Lipid accumulation and oxidative stress activate inflammatory signaling pathways, establishing a self-perpetuating cycle in which inflammation both impairs insulin signaling and enhances ROS production. This feed-forward interaction sustains chronic metabolic inflammation, a hallmark strongly associated with progression toward T2DM (Shoelson et al., 2006).

Insulin sensitivity is also critically influenced by regulatory pathways that control oxidative stress, particularly the nuclear factor erythroid 2–related factor 2 (NRF2) signaling axis. Pharmacological activation of NRF2 using compounds such as 1-[2-cyano-3, 12-dioxooleana-1, 9(11)-dien-28-oyl] imidazole and oltipraz has been shown to reduce weight gain, limit white adipose tissue expansion. In contrast, NRF2 deficiency yields complex, tissue-dependent effects: while myeloid-specific loss may impair insulin sensitivity, global or adipocyte-specific NRF2 deletion in leptin-deficient models can reduce fat mass but simultaneously exacerbate systemic metabolic disturbances, including hyperglycemia and hypertriglyceridemia. These findings underscore the context-dependent nature of NRF2 in metabolic regulation (Yu et al., 2011).

6. Conclusion

Metabolic syndrome is a multifaceted and progressive metabolic disorder in which oxidative stress and disturbances in redox homeostasis serve as central, unifying pathogenic mechanisms. Rather than being a downstream consequence, substantial evidence indicates that abnormal ROS generation, weakened antioxidant defense capacity, and impaired maintenance of redox equilibrium are primary contributors to cellular dysfunction across diverse tissues.

In this context, mitochondrial activity and NOX systems constitute major sources of excessive ROS production, particularly under conditions of caloric excess, persistent hyperglycemia, and chronic low-grade inflammation. When antioxidant systems become insufficient or dysfunctional, oxidative stress ensues. Over time, these alterations progressively

undermine membrane stability, enzymatic function, and gene expression control, thereby exacerbating metabolic derangements.

A critical outcome of prolonged oxidative stress is the establishment of self-amplifying pathological loops. Reactive lipid-derived aldehydes generated during lipid peroxidation, AGEs, and pro-inflammatory mediators collectively enhance ROS production and further destabilize redox balance. These interconnected mechanisms directly contribute to impaired insulin signaling, mitochondrial dysfunction, and vascular damage, ultimately forming a widespread network of metabolic impairment.

Accordingly, insulin resistance - the defining characteristic of metabolic syndrome - should be viewed as the cumulative result of multiple interacting processes, including oxidative stress, lipid accumulation, inflammation, and defective antioxidant regulation. This conceptual framework broadens the understanding of metabolic syndrome beyond a purely glucose-centered disorder, framing it instead as a systemic redox-metabolic disease in which cellular stress responses play a central role.

Importantly, current evidence underscores the therapeutic significance of restoring redox balance. Interventions aimed at enhancing antioxidant defenses, limiting excessive ROS formation, improving mitochondrial efficiency, and modulation redox-sensitive pathways may offer promising strategies to re-establish metabolic homeostasis and slow disease progression.

In summary, oxidative stress and redox imbalance represent fundamental mechanistic links between metabolic overload and cellular injury, driving systemic metabolic dysfunction. A more detailed understanding of these processes not only deepens insight into the pathophysiology of metabolic syndrome but also supports the development of targeted redox-based therapeutic approaches for metabolic disorders (Figure 1).

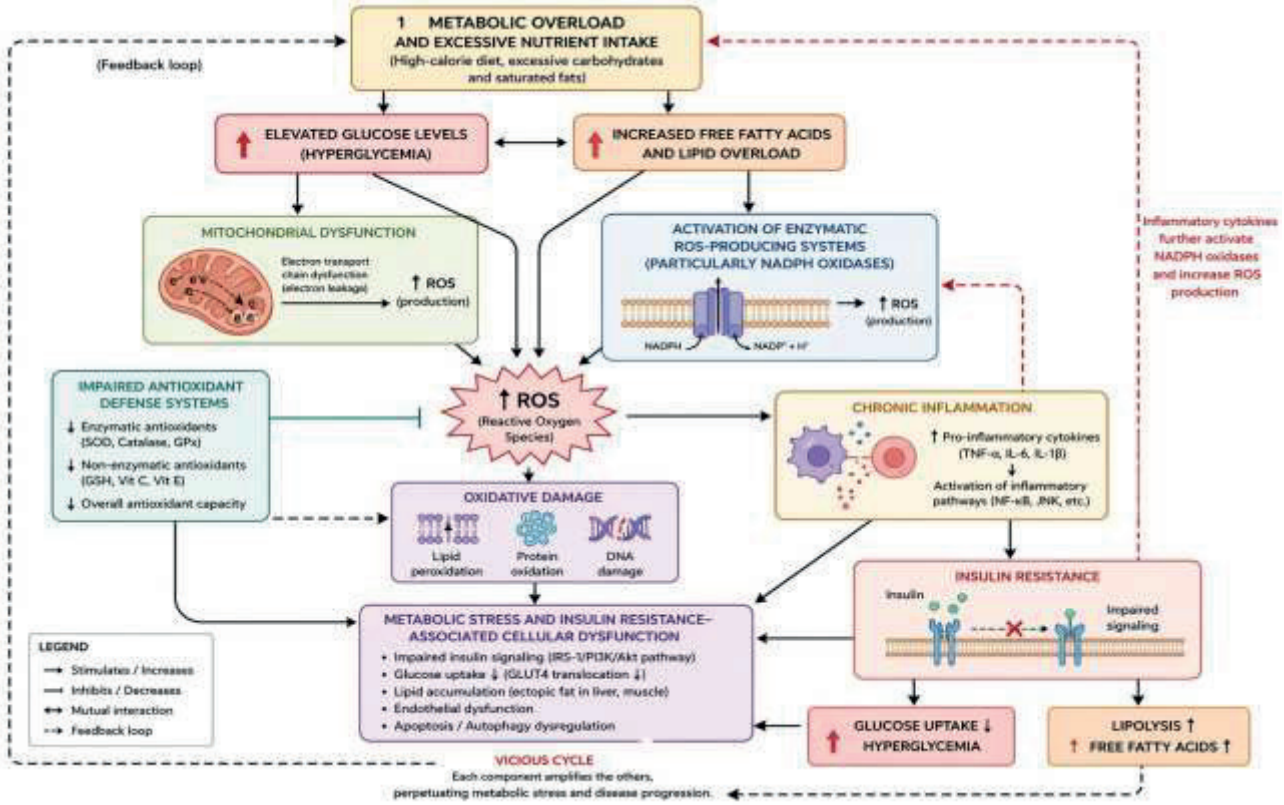

Figure1: ROS Production and ROS-Driven Pathophysiological Mechanisms

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