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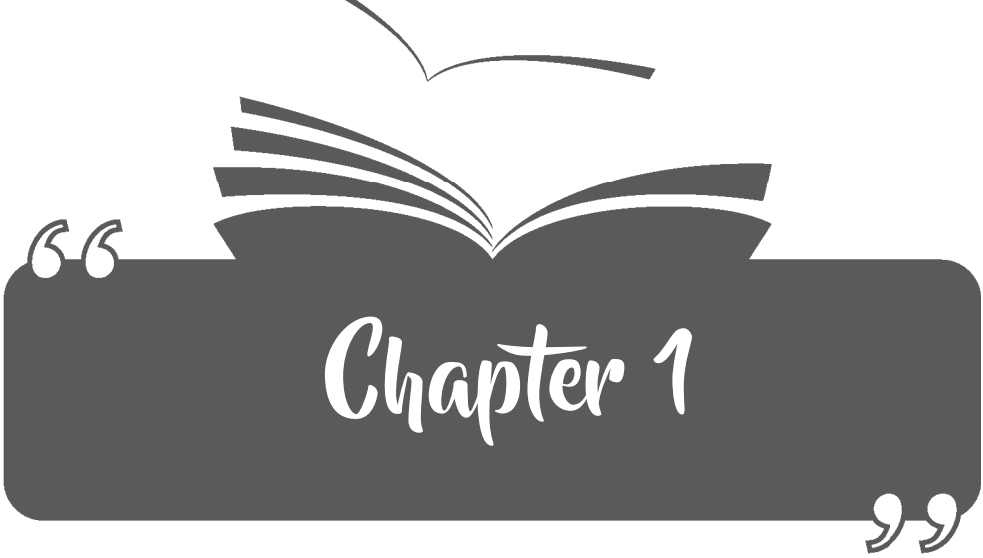

PATHOPHYSIOLOGICAL ROLE OF CENTRAL AND PERIPHERAL SOMATOSENSORY SYSTEMS IN PAINFUL DIABETIC NEUROPATHY

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Introduction

Neuropathic pain is one of the common complications of diabetes and is detected in approximately 24.7% of patients with type 2 diabetes (Bouhassira 2013). Neuropathic pain is an important clinical problem due to the development of tolerance to strong opioid analgesics such as morphine used in its treatment (Ozdemir et al., 2023; Avcı et al., 2022; Courteix et al, 1998). Classic symptoms such as allodynia, spontaneous pain, hyperalgesia, and sensory loss are observed in peripheral neuropathy (Scholz et al., 2019). Neuropathy often shows different pain characteristics and is often chronic and severe. Patients' quality of life is low and psychosocial and health care costs are high (Bates et al., 2019). Neuropathic pain refers to pain that occurs as a result of various diseases or injuries affecting the somatosensory nervous system (Jensen et al., 2011). A wide variety of central or peripheral pathophysiological conditions can cause this condition, including toxic substances (chemotherapy agents), viral infection, surgical nerve compression or tumor infiltration, multiple sclerosis, and stroke.

Neuropathic pain is often associated with depression and anxiety and has significant detrimental effects on quality of life (Ding et al., 2019; Damanik 2021). One of the most important factors responsible for the development of neuropathic pain is diabetes. In addition, obese patients without diabetes may also be a risk factor for peripheral neuropathy, and features of diabetic neuropathy can be seen in people with prediabetes (Stino and Smith, 2017; Jang and Oh, 2023). Peripheral diabetic neuropathy is a condition that occurs as a result of an abnormal function in the peripheral somatosensory system (Tesfaye et al., 2010). In the neurological system, glucose uptake by the Schwann cell is impaired due to insulin resistance, which prevents adequate energy uptake in the axon (Elafros et al., 2022). Neuropathic pain is a condition that occurs when the central and peripheral nervous system are stimulated by noxious stimuli as a result of trauma, mechanical injury, or chronic inflammatory diseases such as diabetes. Central sensitization processes result in increased neuropathic pain hypersensitivity (Ji et al., 2018).

Neuropathic pain is divided into central or peripheral neuropathic pain depending on the affected area (Rumora et al., 2019). Peripheral neuropathic pain is caused by damage or disease affecting the peripheral nervous system. Peripheral neuropathic pain is associated with a chronic disease and is dysfunctional, such as spontaneous pain (pins and needles) and increased sensitivity to mechanical and thermal stimuli (Loeser and Treede, 2008).

The pathogenesis of diabetic peripheral neuropathy is complex, and although persistent hyperglycemia plays a central role in the development of diabetic peripheral neuropathy, tight glycemic control does not eliminate the risk of diabetic peripheral neuropathy. This suggests the need to understand the role of the central nervous system in the development of diabetic peripheral neuropathy in order to adjust treatment regimens accordingly (Zhang et al., 2023). Therefore, this article will focus on the mechanisms of central changes in diabetic peripheral neuropathy, especially painful diabetic neuropathy, and related imaging evidence.

The pathophysiological mechanisms underlying peripheral and central neuropathy extend from primary terminal afferents in the skin (Feldman et al., 2017) to central regions in the spinal cord and brain that process nociceptive information (Colloca et al., 2017). This review focuses on the central and peripheral mechanisms that trigger diabetic neuropathy and examines therapeutic targets that can eliminate diabetic neuropathic pain.

Neuropathic pain classification

Neuropathic pain is defined as peripheral neuropathic pain (PNP) or central neuropathic pain (CNP) according to the International Classification of Diseases-11 (ICD-11) (Du et al., 2023) (Table 1).

Table 1. *Classification of chronic neuropathic pain (ICD-11)*

Peripheral neuropathic pain (PNP)	**Central neuropathic pain (CNP)**
Painful polyneuropathy - Painful diabetic neuropathy - Chemotherapy-induced neuropathic pain	Contains central neuropathic pain associated with spinal cord injury
Postherpetic neuralgia	Central neuropathic pain associated with brain injury
Painful radiculopathy (include Sciatica)	Central post-stroke pain
Postherpetic neuralgia neuropathic pain after peripheral nerve injury	Central neuropathic pain caused by multiple sclerosis
Trigeminal neuralgia	Other specified and unspecified chronic central neuropathic pain
Other specified and unspecified chronic peripheral neuropathic pain	

Pathophysiological Mechanisms in Diabetic Neuropathy

The mechanisms underlying diabetic neuropathy are not yet fully understood. However, it is generally assumed that it is associated with a series of pathophysiological changes, including lipid metabolism disorders, hyperglycemia, and hexosamine pathway, glycolytic pathway, polyol pathway, protein kinase C (PKC) pathway, and toll-like receptor 4 (TLR4) signaling pathway (Zhang et al., 2023). These pathophysiological changes result in endoplasmic reticulum (ER) stress, DNA damage, mitochondrial dysfunction, and advanced inflammatory responses. Furthermore, impaired diabetic microcirculation and insulin signaling, irreversible deterioration of glial cells and neurons, also lead to the progression of diabetic neuropathy. At the same time, central and peripheral sensitization play an important role in the mechanisms proposed for the causes of pain in patients with diabetic neuropathy (Feldman et al., 2017). In diabetic neuropathy, metabolic abnormalities such as dyslipidemia, obesity, and hypertension are also important risk factors (**Figure 1**).

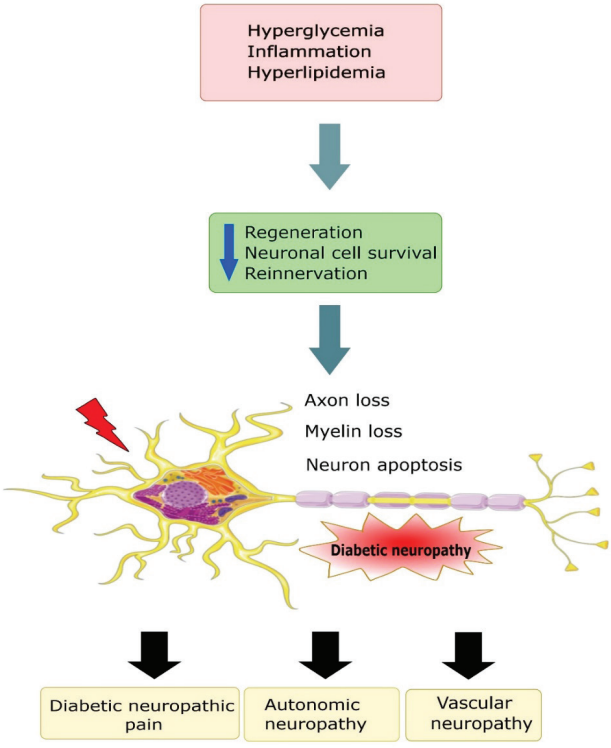

Figure 1. *Pathophysiological mechanism of diabetic neuropathy (Callaghan et al., 2016).*

Obesity and visceral fat accumulation in the body increase the development of diabetic neuropathy, and these symptoms observed in type II diabetes with insulin resistance have an important place in the pathophysiology (Oh et al., 2019). In the nervous system, insulin resistance can impair glucose uptake by the Schwann cell, which can create an energy deficit in the cell (Elafros et al., 2022).

Peripheral Sensitization Mechanism

Peripheral sensitization occurs as a result of decreased activation thresholds, hyperexcitability of primary afferent neurons, and increased sensitivity of sensory neurons to noxious afferent signals. Ectopic discharges following nerve injury occur as a result of stimulation of inflammatory responses and increased pain signals (Gao et al., 2024). Transient receptor potential vanilloid type-1 (TRPV1) is widely found in dorsal root ganglion (DRG) neurons and is a receptor closely associated with the perception of noxious stimuli and the production of nociception (Düzova et al., 2021).

Oxidative stress increases TRPV1 expression in DRG and upregulates voltage-gated calcium channels (VDCC) via TRPV1 activation, resulting in Ca2+ influx. Use of TRPV1 channel antagonists reduces Ca2+ concentration and apoptosis in DRG, thereby reducing nociceptive signaling. However, stimulation of TRPV1 receptors results in increased secretion of substance P (SP) and calcitonin gene-related peptide (CGRP). This in turn increases the release of inflammatory factors by activating various immune cells. Inflammatory factors initiate a positive feedback loop by further increasing TRPV1 activation (Julius et al, 2013).

Pregabalin and gabapentin, which are used for their anticonvulsant effects, selectively inhibit voltage-dependent channels containing α2δ-1 subunits, disrupting the inward flow of Ca2+ and thus reducing the release of glutamate, norepinephrine (NE) and SP, thus preventing nociception. Clinical studies provide evidence that gabapentin and pregabalin have a role in the treatment of diabetic neuropathic pain (Price et al., 2022).

The distal part of the injured peripheral nociceptive afferent fibers undergoes Wallerian degeneration, and the proximal axons and cytosol of the neuron cell begin to produce ectopic discharges autonomously. It has been determined that increased expression of voltage-gated sodium channels (VGSC) plays a critical role in the generation of ectopic discharges. Numerous VGSC channels are localized on the DRG neuronal cell surface, and among them, those related to pain are concentrated in Nav1.3, Nav1.7, and Nav1.8 (Wood et al., 2004). One of the important mechanisms leading to abnormal neuronal firing is the continuous sodium in-

flux caused by NaV1.8. Inhibition of Nav1.8 expression in a spinal nerve ligation neuropathic rat model reduced pain sensitivity symptoms (Lai et al., 2002). Nav1.7 plays important roles in increasing nociceptive perception, and mutations in Nav1.7 lead to decreased pain perception in patients (Goldberg et al., 2007). It was found that lacosamide administration to patients with nerve fiber lesions related to Nav1.7 caused a significant decrease in pain. These results showed that therapeutic agents targeted for Nav1.7 could be effective analgesic drugs (de Greef et al., 2019). In studies related to peripheral neuropathy, sodium channel blockers have been shown to reduce diabetic neuropathic pain (Price et al., 2022). In addition, inflammatory factors cause an increase in channel expression via TRPV1 receptor activation. This causes Ca2+ ions to increase intracellularly. As a result, Ca2+ accumulated intracellularly induces ectopic stimulations to occur more frequently in neurons.

Pathophysiological Mechanism of Central Sensitization

The most important feature of central sensitization is the plasticity changes in synaptic transmission between neurons. Changes in central neuroplasticity, known to be triggered by noxious stimuli, are seen in the spinal cord, brainstem, thalamus, and different cerebral cortex areas. Peripheral nerve damage leads to high secretion of excitatory neurotransmitters SP and glutamate from the axon terminals of afferent neurons that transmit pain. Glutamate interacts with AMPA and N-methyl-d-aspartate (NMDA) receptors on the surface of spinal dorsal horn neurons, causing an increase in synaptic activity. This allows the influx of Ca2+ into the cell, initiates central sensitization, and stimulates intracellular signaling pathways (Zhang et al., 2023). SP activates PKC by stimulating neurokinin 1 (NK-1) receptors, which then depolarizes postsynaptic projection neurons via NMDA receptors. This depolarization removes the Mg2+ blockade of NMDA receptors, initiating a greater rate of intracellular current. Furthermore, PKC also inhibits γ-aminobutyric acid (GABA) receptors, weakening their binding to GABA, thus suppressing the activity of GABA inhibitory interneurons in the dorsal horn of the spinal cord (Seybold et al., 2009).

After peripheral nerve injury, apoptosis of GABA inhibitory interneurons in layers I and II of the dorsal horn of the spinal cord has been detected. The prevailing view is that this apoptosis seen in interneurons is due to a large Ca2+ influx that initiates a neurotoxic response by activation of NMDA receptors (Zeilhofer et al., 2012). Therefore, ketamine, which antagonizes NMDA receptors, has a strong analgesic effect and is used as an effective drug for various chronic pains in the clinic (Gao et al., 2016).

Microglial cells play important roles in the central sensitization process. During peripheral nerve injury, stimulation of the chemokine fractalkine receptor activates microglia. In the next step, activation of the p38 mitogen-activated protein kinase (p38MAPK) signaling pathway leads to the release of proinflammatory mediators such as interleukin 1β (IL-1β). In addition, microglia activation activates the tyrosine kinase B (TrkB) receptor and releases brain-derived neurotrophic factor (BDNF) (Clark et al., 2014).

Activation of the BDNF-TrkB pathway causes a decrease in the effect of inhibitory interneurons together with the disruption of spinal Cl^- homeostasis. All these effects result in the intense perception of neuropathic pain (Tender et al., 2010). The periaqueductal gray matter (PAG), which is the source of inhibitory signals for pain, receives nociceptive information from higher centers such as the thalamus, frontal lobe, amygdala and insula cortex. The PAG then suppresses the pain response by secreting neurotransmitters such as NE and 5-hydroxytryptamine (5-HT) to the dorsal horn of the spinal cord. It specifically achieves this effect by stimulating inhibitory neurons in the dorsal horn.

Under normal physiological conditions, the downstream inhibitory effect is dominant and pain transmission is inhibited. However, after neurological injury affecting sensory nerves, the function of the downstream inhibitory system may be impaired. Most likely, changes in some neurotransmitter release during neurological injury inactivate the downstream inhibitory pathways of 5-HT and NE, thus sensitizing the dorsal horn (Rahman et al., 2008). Serotonin and NE reuptake inhibitor drugs enhance the inhibitory effect of the downstream inhibitory pathway, leading to increased analgesic efficacy and a reduction in diabetic neuropathic pain (Ormseth et al., 2011). Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, exert significant analgesic effects through the downstream inhibitory pathway (Ozdemir et al., 2011).

Parts of the central nervous system, such as the cerebral cortex and thalamus, receive pain information from afferent neurons and actively participate in the process of interpreting pain information and generating the necessary responses. During nerve injury, there is an increase in the inward Ca2+ flow in dendritic endings in the anterior cingulate cortex, which enhances the long-term potentiation (LTP) of cingulate cortex synaptic transmission. Stimulation of AMPA receptors increases postsynaptic currents by activation of extracellular signal-regulated kinase (ERK) (Li et al., 2010).

Ascending sensory pain pathways from the spinal cord stop at the ventroposterior lateral nucleus of the thalamus. Initial analysis and integration of information occurs in the thalamus, and the modulated information is then transmitted to the somatosensory cortex. Therefore, it is particularly important to evaluate the thalamus functionally, which functions as a sensory gateway to the sensory cortex. Several MRI perfusion-weighted imaging studies have found increased thalamic perfusion in patients with painful diabetic neuropathy compared to patients with painless diabetic neuropathy (Selvarajah et al., 2006). The reason for the increase in perfusion in the thalamus in painful diabetic neuropathy is not yet known for sure. However, it is suggested that it may be related to increased excitability and abnormal discharge activity in the thalamus. In the future, it will be possible to clarify the relevant mechanisms by developing designed animal experiments.

In diabetic neuropathy, synaptic transmission in sensory neurons in the spinal cord is significantly increased by amplifying signals from active nociceptor receptors (Woolf, 2011). Nociceptive signals accumulate temporally and spatially in sensory neurons, resulting in further amplification of signals. In diabetic neuropathic individuals, increased release of proinflammatory factors TNF-α, interleukin (IL)-6, IL-1β, and BDNF by microglial cells is observed. This further increases nociceptive signal transmission in the spinal dorsal horn. Activation of microglia in the diabetic mouse spinal cord also causes activation of astrocytes (Liu M et al., 2019). Hypersensitivity to pain has been developed in spinal nociceptive neurons of animals with a diabetes model through ERK activation (Xu et al., 2014). Furthermore, peripheral inflammation that occurs with chronic painful stimulation causes an increase in the release of certain neurotransmitters (such as BDNF, substance P, glutamate, calcitonin gene-related peptide, and CGRP) from sensory fibers to the spinal dorsal horn. Excessive increases in these substances result in increased excitability of spinal and supraspinal sensory neurons. This condition is often referred to as central sensitivity to pain.

Autonomic Neuropathy in Diabetes

Autonomic neuropathy is a serious complication due to the high mortality rate seen in diabetic neuropathy. In some cases, it can lead to spontaneous respiratory arrest and sudden death of unknown cause. Various lines of evidence suggest that the areas that make up the central autonomic network consist of the limbic system (amygdala), prefrontal cortex (ventral medial cortex, anterior cingulate cortex, orbitofrontal and insula) and brainstem regions (PAG and ventral medial medulla) (Benarroch et al. 1993).

The limbic system, known as the visceral brain, has important effects on brain activity. The hypothalamus, a component of the limbic system, is one of the most important subcortical regulators of visceral activity and plays a critical role in the regulation of mood swings. The reflex activity of autonomic nerves is carried out mostly in the medulla oblongata and has a significant effect on the body's instinctive behavioral and emotional responses. There are important relationships between the central autonomic nervous system and diabetic neuropathy. Patients with autonomic neuropathy have been found to perform worse on cognitive function tests related to visual memory than patients with nonautonomic neuropathy (Zaslavsky et al., 1995).

Role of Neuroinflammation in Diabetic Neuropathy

Numerous evidences suggest that neuroinflammation is closely associated with chronic pain (Borghi et al., 2019; Fang et al., 2022; An et al., 2025). Central and peripheral nervous system inflammation produce different results. First, the permeability of the blood-brain barrier (BBB) increases. Then, leukocyte infiltration occurs as a result of increased vascular permeability. Finally, the secretion of proinflammatory mediators and activation of glial cells are detected (Ellis and Bennett, 2013). Chronic pain is presumed to result from neuronal plasticity in pain signaling pathways. Neuronal plasticity occurs through either central sensitization or peripheral sensitization (Luo et al., 2014). Neuroinflammation, in particular, plays a crucial role in central and peripheral sensitization.

The most important effect of neuroinflammation in peripheral sensitization of nociceptors is a decrease in the threshold of stimulation and an increase in the spontaneous excitatory response (Rosenberger et al., 2020). Hyperexcitability of sensory neurons in diabetic patients or animal models results in an altered stimulus-response function (Kim et al., 2012). This abnormally increased activity is necessary for the development of diabetic neuropathy pain. Many studies show that Schwann cells, which surround peripheral nerve fibers in the setting of chronic hyperglycemia, are damaged due to demyelination in diabetic neuropathy (**Figure 2**).

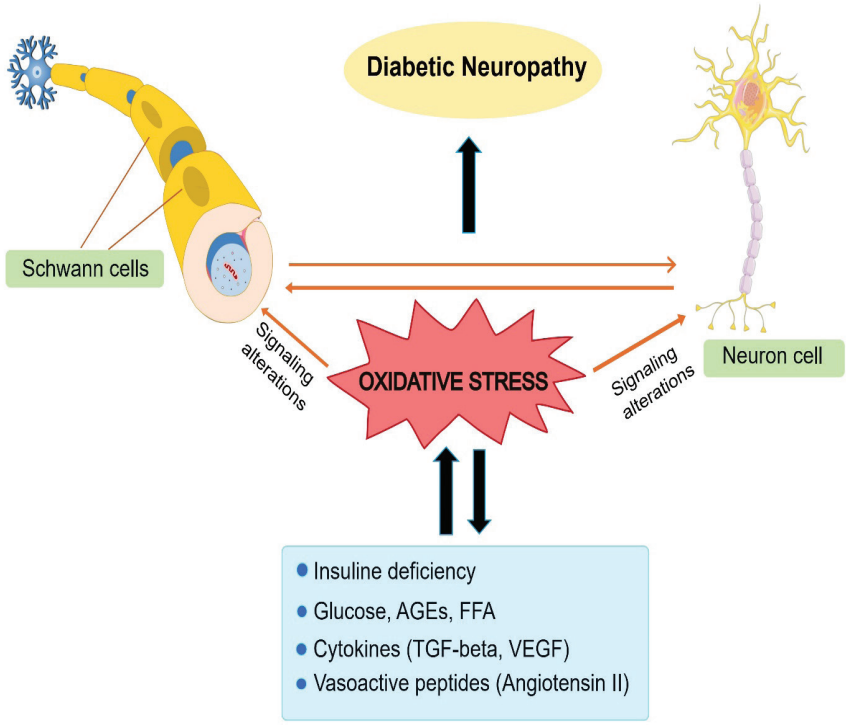

Figure 2. *Mechanisms of oxidative stress-induced diabetic neuropatic pain in injured neuronal cells. AGE$_s$, advanced glycation end products; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; FFA, free fatty acid (Dunnigan ve ark., 2013).*

These cells, which contain neurotrophins, have receptors for these mediators on their surfaces. In diabetic patients or animal models of diabetes, decreased neurotrophin levels in Schwann cells prevent the regeneration of nerve fibers (Richner et al., 2014). In animals with streptozotocin (STZ)-induced diabetes, decreased levels of ciliary neurotrophic factor, a neurotrophic factor released from Schwann cells, were observed (Calcutt et al., 1992). In addition, important interactions between Schwann cells and T cells have been identified in diabetes.

Several lines of evidence have shown that satellite glial cells in ganglia are important for the functionality of the peripheral nervous system and activation of glia. Under pathological conditions, disruption of this communication between satellite cells leads to abnormal pain signaling (Huang et al., 2013). In diabetic rats, excessive secretion of glial fibrillary acidic protein (GFAP) from satellite glial cells is considered as activation of SGCs, which has been shown to be associated with the induction of

neuropathic pain (Hanani et al., 2014). In diabetic rats, increased activation of satellite cells via purinergic signaling leads to increased release of tumor necrosis factor alpha (TNF-α) from these cells. This results in increased pain sensitivity via enhanced excitability of dorsal root ganglion (DRG) neurons (Gonçalves et al., 2018).

The activity status of ion channels in afferent neurons is important in the transmission of pain signals (Waxman and Zamponi, 2014). Voltage-gated sodium channels (Nav), calcium channels (Cav), potassium channels, and transient receptor potential channels (TRP) participate in the generation of action potentials (Waxman and Zamponi, 2014). Three important Nav isoforms (Nav1.7, Nav1.8, and Nav1.9) have been identified in sensory neurons (Hameed, 2019). Nav1.7 and Nav1.9 play an important role in modulating nerve terminal excitability, while Nav1.8 participates in the generation of action potentials in nociceptors. Conversely, potassium channels reduce the excitability of sensory neurons and play a role in inhibition. Furthermore, T-type Ca2+ channels significantly contribute to the transmission of diabetic neuropathic pain by modulating the excitability of nociceptors in the subthreshold range. Cav3.2 channel activity is increased in diabetes, causing DRG neurons to become hyperexcitable (Orestes et al., 2013). One of the molecules that plays a critical role in the peripheral sensitivity of chronic pain is the TRPV1 ion channel. These ion channels are frequently detected in nociceptive DRG sensory neurons (Moore et al., 2018; Gao et al., 2024). In one study, it was determined that activation of A-type DRG neurons directly activated the TRPV1 channel, increasing diabetic neuropathic pain and creating rapid currents with calcium increases in DRG neurons (Xie et al., 2022). One of the important mechanisms in central sensitization is the activation of NMDAR channels located on postsynaptic membrane surfaces. In a diabetic animal model, activation of astrocytes in the spinal cord significantly increases IL-1β expression. This strongly accelerates pain signaling via NMDAR phosphorylation, especially in db/db mice (Liao et al., 2011).

Pharmacological Treatments for Painful Diabetic Neuropathy

Pain in diabetic neuropathy is a chronic pain that occurs as a result of a direct effect of a lesion or disease in the sensory nerves and can occur without tissue damage. Therefore, neuropathic pain medications are used to treat painful diabetic neuropathy. The drugs used for painful diabetic neuropathy are pregabalin, duloxetine, and tapentadol, which were approved by the US Food and Drug Administration (FDA) and later added capsaicin to this treatment (Yang et al., 2019). Gabapentinoids reduce diabetic neuropathic pain by inhibiting sensory neuron sensitivity in the spinal cord dorsal horn. Their mechanism of action is to suppress excit-

atory neurotransmitter release by binding to the α2δ subunit of calcium voltage-gated channels (**Table 2**).

Table 2. Medications used for painful diabetic neuropathy

Drugs	Mechanisms	Effects	Adverse effects	Refs.
Gabapentin	Inhibition of neurotransmitter release through binding to the α2δ subunit of calcium voltage gated channels	Pain relief in painful diabetic neuropathy, postherpetic neuralgia	Dizziness, somnolence, peripheral	Backonja et al. (1998)
Pregabalin	Inhibition of neurotransmitter release through binding to the α2δ subunit of calcium voltage gated channels	Pain relief in painful diabetic neuropathy, postherpetic neuralgia, fibromyalgia, and spinal cord injury	Dizziness, somnolence, dry mouth, edema, blurred vision, weight gain	Rosenstock et al. (2004)
Duloxcetine	Inhibition of reuptake of serotonin and norepinephri	Pain relief in painful diabetic neuropathy, fibromyalgia and musculoskeletal pain	Nausea, dry mouth, somnolence, fatigue, constipation, hyperhidrosis	Goldstein et al. (2005)
Amitriptyline	Inhibition of reuptake of NE and 5-HT in presynaptic neurons and antagonizing NMDA receptors	Pain relief in painful diabetic neuropathy, neuropathic pain and fibromyalgia	Dry mouth, somnolence, dizziness, constipation, weight gain	Max et al. (1987)
Alphalipoic acid	Antioxidants	Pain relief in painful diabetic neuropathy	Headache, hearburn, nausea, vomiting	Ziegler et al. (1995)

Carbamazepine	Inhibition of the secretion of neurotransmitters by blocking presynaptic voltage-sensitive sodium channels	Pain relief in neuropathic pain	Dizziness, somnolence, unsteadiness, nausea, vomiting	Razazian et al. (2014)
Capsaicin 8% patch	Removal of substance P from vanilloid nerve receptor	Relief of neuropathic pain associated with postherpetic neuralgia and diabetic neuropathy	Site erythema, application site pain, application site pruritus	Simpson et al. (2017)

In addition, gabapentinoids induce inhibition via the descending serotoninergic pathway and produce anti-inflammatory effects (Chincholkar, 2018). Pregabalin is a potent inhibitor of the α2δ subunit of the voltage-gated calcium channel. It is a drug approved in the United States for the treatment of diabetic pain and spinal cord injury (28). In addition, pregabalin relieves severe pain associated with diabetic neuropathy and improves sleep disturbance and mood states in diabetic patients (Rosenstock et al., 2004).

Serotonin (5-HT) and norepinephrine (NE) are two important mediators in the descending inhibitory nociceptive pathway in the dorsal column. Serotonin-norepinephrine reuptake inhibitors (SNRIs) enhance inhibition in the descending pathway and reduce diabetic neuropathic pain (Bates et al., 2019; Mokhtar et al., 2023). Among these drugs, duloxetine is used for the symptomatic treatment of painful diabetic neuropathy. In an experimental study, duloxetine showed a significant effect in improving neuropathic pain (Iyengar et al., 2004). In a clinical study on patients with major depressive disorder, duloxetine reduced pain associated with depression (Detke et al., 2002).

Tricyclic antidepressants (TCAs) reduce pain by inhibiting NE and 5-HT reuptake in presynaptic neurons and by blocking NMDA receptors, which mediate hyperalgesia (Boulton, 2005). TCAs are low-cost drugs but can have significant side effects such as constipation, dry mouth, orthostatic hypotension, and urinary retention. At the same time, TCAs should be used with caution in patients with cardiac arrhythmias and glaucoma (Benbouzid et al., 2008).

It has been reported that increased oxidative stress due to antioxidant defense disorders caused by hyperglycemia in diabetes leads to neural hypoxia and nerve dysfunction, which contribute to diabetic neuropathic pain (Low et al., 1997). Experimental studies have shown that alpha-lipoic acid, an antioxidant substance, reduces neurovascular abnormalities associated with diabetic neuropathy (Nagamatsu et al., 1995). Clinical studies have also shown that intravenous alpha-lipoic acid administration to patients with symptomatic diabetic neuropathy significantly reduces diabetic neuropathic pain (Ziegler et al., 1995). Carbamazepine, which is frequently used for the treatment of epilepsy and generalized convulsions, blocks the secretion of glutamine, an excitatory neurotransmitter, particularly by inhibiting presynaptic voltage-sensitive Na+ channels of central neurons (Spiller et al., 2002). Evidence suggests that oxcarbazepine, a derivative of carbamazepine, is effective in diabetic neuropathic pain (Dogra et al., 2005).

Other agents used for the treatment of diabetic neuropathic pain include opioid drugs such as tapentadol and tramadol. On the other hand, opioid drugs, which have a strong effect against pain, have a high rate of side effects after long-term and high-dose administration. Therefore, drugs in this group are not preferred for diabetic neuropathy pain (Price et al., 2022; Jang et al., 2023). However, it seems possible that combined drug administration with opioid drugs will reduce these side effects. An experimental study has shown that neuropathic pain was significantly reduced by administering low-dose morphine and metformin, an antidiabetic drug, to rats with painful diabetic neuropathy (Avcı et al., 2022). Similarly, when aspirin, an anti-inflammatory drug, was injected together with morphine into rats with diabetic neuropathy, a significant improvement in neuropathic pain was achieved (Ozdemir et al., 2023; Ferreira et al., 2025).

Conclusion

The pathophysiology of diabetic neuropathy is quite complex and persistent hyperglycemia plays an important role in the development of diabetic peripheral neuropathy. In order to organize the treatment of neuropathic pain, the role of the central and peripheral nervous systems in the development of diabetic neuropathy must be well understood. Effective treatment of diabetic neuropathy is of critical importance. Because it causes significant deterioration in the quality of life of patients with diabetic neuropathy and causes loss of labor. Drugs such as serotonin-norepinephrine inhibitors, gabapentinoids, sodium channel blockers, alpha-lipoic acid and opioids are used in the treatment of painful diabetic neuropathy. The most beneficial therapeutic approach in painful diabetic

neuropathy that does not respond to classical drug therapy may be combination therapy with opioids or capsaicin patch. Considering the inadequacy of existing drugs developed considering the pathophysiology of diabetic neuropathy, new studies are needed for the treatment of diabetic neuropathy pain.

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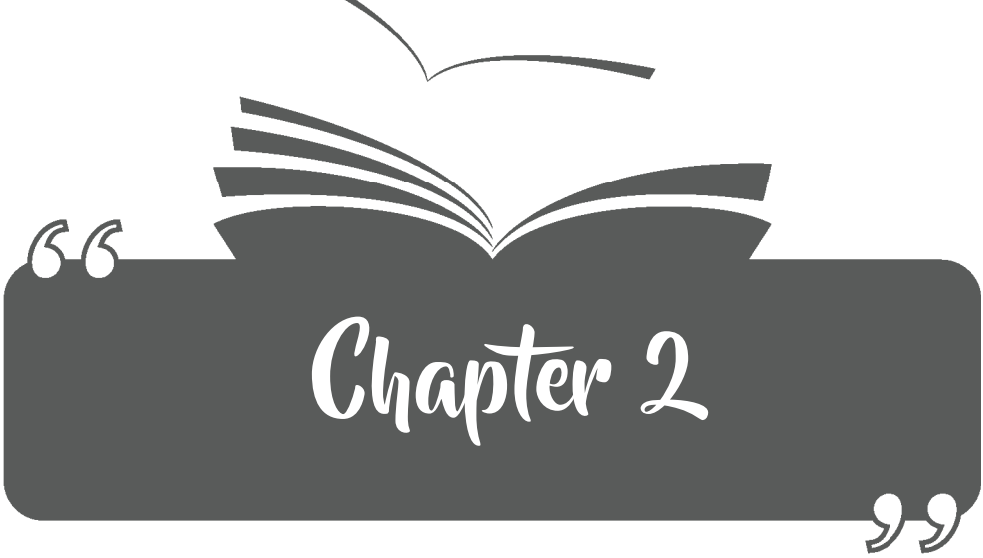

ENDOVASCULAR TREATMENT OF ABDOMINAL AORTIC ANEURYSM

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Aortoiliac Anatomy, Aneurysm Localisation

An abdominal aortic aneurysm is defined as a 50 percent enlargement of the normal aortic diameter in the segment where dilation is detected (Johnston et al., 1991). The normal aortic diameter at the level of the renal artery is about 2 centimeters (1.4-3.0). An aortic diameter greater than 3 centimetres is considered an aneurysm in most individuals.

The abdominal aorta is a retroperitoneal structure starting from the diaphragmatic hiatus and extending to the common iliac arteries with its bifurcation at the level of the 4th lumbar vertebra. It is located slightly left of the midline next to the vena cava. The branches of the aorta are, from top to bottom, left and right inferior phrenic, left and right suprarenal, celiac trunk, superior mesenteric, left and right renal, possible accessory renal, left and right gonadal, inferior mesenteric, left and right common iliac, middle sacral and L1-L4 lumbar pairs. The common iliac arteries divide into the external and internal iliac arteries at the entrance to the pelvis. The internal iliac artery (also known as the hypogastric artery) splits into anterior and posterior branches, which supply the pelvic organs and muscle groups, while the external iliac arteries turn into the common femoral arteries after passing under the inguinal ligament (Sandhu & Pipinos, 2005).

Abdominal aortic aneurysms (AAA) are classified based on the level of the renal and visceral vessels involved. If the aneurysm originates below the renal artery, it is infrarenal; if above, it is juxtarenal; and if it involves the area above the renal artery, it is suprarenal. AAA typically forms between the renal artery and the inferior mesenteric artery, but in about 5% of cases, the visceral and renal arteries are affected. Most endovascular treatments are for infrarenal aneurysms, while specialized endografts and techniques are used for juxtarenal and suprarenal aneurysms. Several terms help with anatomical feasibility and endograft size selection, including aortic neck diameter, aortic neck length, aortic neck angle, and infrarenal aortic length (Schanzer et al., 2011).

Renal artery anomalies; accessory renal artery is found in 30% of the population and closure of this artery during endograft placement may lead to partial infarcts in the kidneys. However, in most patients with horseshoe kidney and its variants resulting from ectopic and fusion anomalies, renal arteries have been observed to arise from the aneurysm.

Aortoiliac Imaging

Before evaluating endovascular aneurysm repair, aortoiliac imaging is required to define the anatomy, determine the feasibility of endovascular

repair, and select the size and configuration of endograft components. Computed Tomography (CT) is usually used for elective abdominal aortic aneurysm (AAA) repair, but in emergency conditions, endograft feasibility and size can be determined by intraoperative arteriography.

CT angiography provides a three-dimensional (3D) reconstruction of the abdomen and pelvis with slices smaller than 2.5 mm. Although two-dimensional (2D) CT images can be used, measurement errors (aortic diameter, aortic length) may occur due to volume averaging. Furthermore, aortic diameter measurements will be overestimated if the aorta is angulated and the longitudinal axis is not perpendicular to the imaging plane. CT angiography with 3D reconstruction allows measurements to be made perpendicular to the true axis of the aorta (Higashiura et al., 2009).

Magnetic resonance (MR) angiography can be used for preoperative endograft planning; however, gadolinium administration is a relative contraindication in renal dysfunction.

The use of DSA (Digital Subtraction Arteriography) is limited due to parallax and magnification problems in aortic measurements. Since the inner lumen and not the aortic wall is visualised, DSA cannot assess the true luminal diameter, size of thrombus, plaque or degree of calcification. Errors in length measurement can occur when using intraluminal catheters that follow the shortest distance around the aortic folds. In treating a ruptured AAA with endovascular aneurysm repair(EVAR), arteriography may be used in emergencies to estimate the proximal aorta diameter and iliac graft landing zones, but it is not recommended as a routine pre-EVAR imaging method

Superficial duplex ultrasound is not an adequate imaging modality to determine feasibility or plan EVAR. Intravascular ultrasound (IVUS) provides accurate intraoperative diameter and length measurements, making it preferred over other methods in some cases, especially for renal failure patients. However, its use is limited due to its invasive nature and the need for considerable technical expertise.

Indications for Repair

Abdominal aortic aneurysm (AAA) repair is indicated in symptomatic patients (tenderness or abdominal or back pain, evidence of embolisation, rupture) with AAA ≥5.5 cm or AAA enlarged by more than 0.5 cm within 6 months.

Anatomic compatibility is the key factor for long-term success in EVAR. In early endograft designs, approximately 50 per cent of patients were not candidates for EVAR due to unsuitable aneurysm location, extent, morphology or access vessels. The availability of devices with shorter proximal closure zones and a lower profile has expanded the use of EVAR to almost two-thirds of patients with infrarenal AAA. To exclude blood flow from the aneurysm sac, the endograft must create an adequate seal at the landing zones: proximally at the aortic neck and distally at each iliac artery. Since endografts do not have any suture-mediated stability, the safety of the repair relies solely on the radial force generated by the graft at the landing zones. Therefore, some anatomical criteria must be fulfilled to perform EVAR (Howell, Villareal, & Krajcer, 2001).

The required endograft diameter is determined by measuring the aortic neck diameter (e.g. 20 mm) and adding an additional 15 to 20 per cent (20 mm + 3 to 4 mm = 23 to 24 mm) to the aortic neck diameter. A small endograft diameter can result in inadequate closure and failure to exclude the aneurysm. Oversizing the endograft by 15 to 20 percent of the measured aortic neck diameter ensures sufficient radial force to prevent device displacement. Commercially available devices have endograft diameters as large as 36 mm, allowing endovascular repair of aneurysms up to a maximum aortic neck diameter of 32 mm (Oliveira et al., 2017).

A case series evaluated the two-year clinical and morphological outcomes of 188 patients two years after EVAR for AAA with a wide (≥28 mm) infrarenal neck. Such wide aortic necks have been associated with enlargement of the infrarenal aortic neck, high risk of proximal type I leakage and proximal neck-related reintervention (Gargiulo et al., 2017).

For these reasons, some sources have recommended keeping the oversize above 15% or suprarenal fixation in this patient group. However, the risks of folding of the graft as a result of excessive oversize and the related risks of endoleak and thrombus predisposition should not be forgotten (Mohan, Laheij, & Harris, 2001).

The aortic neck length should be at least 10 to 15 mm to ensure adequate endograft fixation. The proximal aorta should ideally be normal, without significant thrombus or calcification. While not an absolute contraindication for EVAR, large thrombus or calcification can affect graft fixation and increase the risk of migration or type I leak.

Ideally, the aortic neck angle should be less than 60º.Larger angles lead to potential difficulties with implantation, kinking, endoleak and distal migration of the device. Severe angulation (>60°) is generally considered a contraindication for EVAR, although newer more compliant devices in

development may be useful in such anatomical situations. However, the ability to place a device into aneurysms with a significant angle to the neck depends on the suitability and delivery characteristics of the specific device.

Iliac arteries should have minimal calcification and tortuosity, with distal graft landing sites free of significant stenosis or mural thrombus. To maintain pelvic perfusion, hypogastric embolization may be needed before endograft placement to prevent bleeding into the aneurysm sac. However, if there is severe stenosis at the hypogastric artery origin, this may not be necessary, as the origin could be thrombosed by the stent graft. The need for hypogastric embolization can be reduced with the use of iliac branch devices that protect one or both hypogastric arteries during EVAR.

Minimal external iliac artery diameter of 7 mm is needed for safe passage of most endograft delivery sheaths, though low-profile devices can be used through access cups with diameters <6 mm. To ensure an adequate seal, the main iliac artery diameter should be between 8 and 22 mm, with at least 15 to 20 mm of normal-diameter artery length for endograft limb fixation. Atherosclerotic narrowing and slight angulation can be managed with standard techniques, while extensive narrowing or marked calcification poses more challenges. If the device cannot pass, an iliac conduit can be created by retroperitoneal approach. Aneurysmatic iliac segments (>22 mm) should be excluded with endograft.

In abdominal aortic aneurysm patients, the inferior mesenteric artery is often occluded by thrombus, making its exclusion by endograft unimportant. If the inferior mesenteric artery is open and there is significant narrowing of the superior mesenteric artery, it may provide crucial collateral blood flow to the intestines. In such situations, covering the origin of a patent inferior mesenteric artery with an endograft could result in intestinal ischemia (Abu-Ghaida et al., 2002). In these patients, lower mesenteric artery revascularisation can be achieved with open surgery while endovascular intervention to the aneurysm is performed with a hybrid procedure.

EVAR is contraindicated in patients who do not fulfil anatomical criteria. EVAR is relatively contraindicated in patients who may have difficulty with follow-up compliance. It is also controversial whether younger patients (<60 years) who are not at high risk for open surgery should opt for EVAR over open surgical repair. Long-term surveillance exposes patients to higher levels of cumulative radiation, and EVAR does not fully eliminate the risk of future aortic rupture. Guidelines from the major me-

dical and surgical societies emphasise an individualised approach when choosing endovascular repair, taking into account the patient's age and risk factors for perioperative morbidity and mortality (Chaikof et al., 2009).

Operation Preparation

Patients undergoing AAA repair (whether endovascular or open surgery) are at moderate to high risk for deep vein thrombosis, and thromboprophylaxis is recommended. In a study of 193 patients undergoing AAA repair, the incidence of thromboembolism was lower for endovascular aneurysm repair compared with open surgery; however, the incidence of deep vein thrombosis after EVAR is 5.3%, despite the use of pharmacological thromboprophylaxis (de Maistre et al., 2009).

Delayed initiation of pharmacological prophylaxis is linked to an increased incidence of deep vein thrombosis. Before endograft placement, antibiotic prophylaxis is recommended within 30 minutes after skin incision. Cefazolin is administered 2 g IV/day if the patient is <120 kg. If the patient is ≥120 kg, 3 g IV/day, vancomycin or clindamycin can be used in case of penicillin allergy (Bratzler et al., 2013).

EVAR increases the risk of renal complications, mainly due to the administration of intravenous contrast agents, but potentially also associated with dislodgement of embolic debris by manipulation of catheters and wires near renal arteries or compression of the renal ostium by the graft in those using suprarenal fixation. When EVAR is performed in a patient with preexisting renal failure, strategies that reduce the risk of contrast-induced nephropathy should be used (Chaikof et al., 2002).

The need to place a stent-graft near the origin of stenotic renal arteries is not uncommon. Although supra-renal fixation grafts do not appear to affect renal function in patients with normal renal arteries, it is unclear whether this applies in cases of severe renal artery stenosis and pre-existing renal failure. The need for prophylactic renal artery stenting in such situations remains uncertain. Even after adjusting for risk factors that may contribute to postoperative renal dysfunction, concurrent RAAS was significantly linked to adverse renal outcomes after EVAR for infrarenal AAA. When a device with suprarenal fixation is required, the potential compromise of renal artery flow due to suprarenal struts should be considered against the risks and potential benefits of prophylactic renal artery stenting (Nejim et al., 2017).

During endovascular repair of aortic aneurysms involving the distal main iliac artery and/or the hypogastric artery (i.e., internal iliac artery),

embolization of the hypogastric artery (i.e., internal iliac artery) may be necessary to prevent endoleaks from the hypogastric artery. When unilateral hypogastric embolisation is required, it is usually performed preoperatively, but can also be performed immediately before endovascular stent-graft placement. Patients with bilateral iliac artery aneurysms usually undergo a staged approach. Hypogastric embolisation increases the proportion of patients with anatomy suitable for endovascular aneurysm repair.

Between 13 and 60 per cent of patients experience a transient acute flu-like inflammatory syndrome following aortic endograft placement, which can often delay rapid recovery. The Preoperative Methylprednisolone EndoVascular Aortic Repair (POMEVAR) study addressed whether to provide specific preoperative prophylaxis to prevent this syndrome in 153 patients randomly selected to receive 30 mg/kg intravenous methylprednisone infusion or placebo before endovascular aortic repair. Markers of inflammation (maximal plasma interleukin, C-reactive protein, interleukin 8, soluble tumour necrosis factor) were lower in the methylprednisone group. Postoperative narcotic requirements were significantly reduced and fulfilment of discharge criteria occurred one day earlier for the treated group versus the placebo group (three days versus two postoperative days) with no difference in perioperative medical or surgical morbidity (de la Motte et al., 2014).

Larger studies are needed to confirm these promising results and to determine optimal dosing, potential long-term side effects (e.g. wound complications, leakage), as well as the subgroup of patients who may benefit (or be harmed) by such prophylaxis. A comparison of prophylactic versus purely symptomatic treatment for postimplantation syndrome is also needed. Therefore, such prophylactic treatment is not included in the guidelines.

Endograft Placement

The selected device and its components should be available in the operating theatre before the procedure begins and additional device components, guidewires and sheaths should be immediately available to manage any technical issues that may arise.

Centres treating AAA using EVAR should ideally be equipped with a dedicated endovascular operating theatre (hybrid operating theatre) where conversion to open repair can be efficiently performed if required. EVAR requires portable C-arm fluoroscopic devices or fixed imaging systems. Although fixed imaging systems provide better image quality and include simultaneous computed tomography (CT) and three-dimensio-

nal imaging, the new generation of portable fluoroscopic systems provide sufficient quality for routine EVAR.

Once the patient is anaesthetised, endovascular aneurysm repair is performed in an orderly sequence including gaining vascular access, placement of arterial guidewires and sheaths, imaging to confirm aortoiliac anatomy, main trunk placement, gate cannulation (bifurcated graft), iliac leg placement, graft ballooning if necessary after imaging.

EVAR can be performed under general anaesthesia or regional anaesthesia, total intravenous anaesthesia (TIVA) or local anaesthesia with conscious sedation. The type of anaesthesia used is usually one of the surgeon's preferences, but numerous studies have shown the benefit of limiting the use of general anaesthesia whenever possible (Harky et al., 2020).

Local or regional anaesthesia is linked to shorter operative times and reduced hospital stays, with no significant difference in complications or perioperative mortality when compared to general anaesthesia. If the patient is expected to have poor co-operation during the procedure, general anaesthesia may be preferred to limit patient movement for accurate graft positioning.

Bilateral femoral access is required to implant endografts. Endovascular repair of AAA can be performed via surgical femoral cutdown or percutaneously. The cutdown approach is similar to that used for femoral embolectomy or femoral graft placement; however, in patients with limited atherosclerotic disease and no evidence of femoral aneurysm, exposure may be limited to the area of the main femoral artery to be punctured. The incision may be longitudinal or transverse, but if patch repair of the main femoral artery is anticipated, a longitudinal incision is preferred. Open access can be more difficult in obese patients or those who have undergone previous groin surgery. Arteriotomy repair with a percutaneous suture device is possible even after the use of large diameter introducers (Howell et al., 2001).

Various factors play a key role in assessing whether an artery is appropriate for use with a percutaneous artery closure device, including the size of the femoral artery, previous groin surgery or use of a closure device, significant arterial occlusive disease, and extensive femoral aneurysm. Once the main femoral artery is accessed, placement of the sheath in the anterior main femoral artery is critical. Proximal holes through the inguinal ligament can complicate proper knot tying, and achieving correct pressure application for hemostasis may become more challenging. More distal accesses (e.g. superficial femoral artery) may lead to vessel thrombosis.

In percutaneous access, standard guidewire access is used followed by progressive dilators to place the endograft sheath rather than direct exposure of the access vessel. After endograft placement, closure of the hole in the vessel is usually performed using specialised vascular closure devices (Nelson et al., 2014).

A fascia suturing method has also been outlined, where sutures are inserted into the cribriform fascia around the sheath following the enlargement of the skin puncture incision (Larzon, Geijer, Gruber, Popek, & Norgren, 2006).

The reported success rates of percutaneous EVAR are between 90 and 100 per cent (Howell et al., 2001). When the factors in the success of the procedure are analysed, the size of the sheath, the degree of femoral artery calcification and the operator's experience with the technique are considered (Torsello et al., 2003).

In the PEVAR study, procedural technical success was 98% for the open femoral approach and 94 and 88% in the ProGlide and Prostar groups, respectively. The percutaneous approach was associated with shorter times for haemostasis and completion of the procedure, less blood loss, less groin pain and better quality of life. The results were sustained for up to six months, with no instances of aneurysm rupture, conversion to open surgery, device migration, or stent graft blockage. The findings of this trial align with results from multiple institutions where percutaneous EVAR is routinely performed using various endografts with different profiles (Nelson et al., 2014).

Small diameter access vessels increase the technical difficulty of EVAR, especially in the setting of vessel calcification and tortuosity. Severe vascular stenoses or occlusions of the iliac arteries (TASC C and D lesions) increase the risk of major iliac vessel complications and are an independent predictor of procedure failure (Jean-Baptiste et al., 2009).

Iliac rupture is a potentially life-threatening complication that occurs more frequently in women, possibly due to the generally smaller caliber of the iliac artery (Fernandez, Craig, Garrett, Burgar, & Bush, 2009).

Small-calibre iliac vessels can be managed in a stepwise manner. The first approach is to dilate the artery using graduated dilators or focal balloon angioplasty as needed. If these manoeuvres are not successful, the stent-graft can be delivered through an iliac condit, a graft (e.g., Dakron, expanded polytetrafluoroethylene [ePTFE]) sutured to the common iliac artery or distal aorta. The need for an iliac conduit should be reviewed based on preoperative imaging. After ensuring adequate exposure of the

pelvic vasculature through a low retroperitoneal incision, the sheath conduit graft is placed after anastomosis of the iliac conduit. The conduit graft can be pulled under the inguinal ligament to the groin area to reduce the angle while advancing the endograft.

An alternative technique is called internal endoconduitis. A covered stent is placed in the iliac artery and then the endovascular sheath is passed through the main femoral artery access site; an adequately sized femoral artery is a prerequisite for this technique. Any disruption of the iliac artery during the passage of the sheath is controlled by the covered stent. Unexplained haemodynamic instability may indicate avulsion of the iliac artery. This technique may be useful in patients with a hostile retroperitoneum or colostomy, which may complicate placement of an open iliac conduit (Peterson & Matsumura, 2008).

Prior to device placement, systemic anticoagulation is initiated, typically using an intravenous heparin dose of 80 to 100 units/kg. The target is an active clotting time (ACT) of 200 seconds or longer. After device placement, anticoagulation can be reversed at the surgeon's discretion. Anticoagulation is not routinely neutralised unless indicated due to bleeding unresponsive to routine measures or possibly leakage.

Once vascular access has been established and landmarks for device positioning have been obtained by aortography, the main body is positioned with particular attention to the position of the opening for the contralateral iliac leg. A slight craniocaudal and left anterior oblique angulation may improve visualisation of the renal ostia in the aortic neck. Once the proximal radiopaque markers of the graft are appropriately positioned below the lowest renal artery, the graft body is positioned at the site of the contralateral gate.

Guidewire is advanced from the contralateral access site to the contralateral gate. Gate cannulation is confirmed by placing a mesh catheter over the guidewire into the main section of the graft body, removing the guidewire and confirming that the mesh catheter rotates freely within the main portion of the graft body; if not, the catheter is assumed to be within the aneurysm sac. Once the contralateral guidewire is placed within the main body of the endograft, placement of the endograft into the neck of the aneurysm is completed, followed by placement of the contralateral, then ipsilateral iliac artery legs (depending on graft type). After placement of the endograft components, gentle angioplasty is performed with a compliant or semi-compliant balloon at the junctions and endograft connections, if necessary.

Completion aortography is performed to assess patency of the renal arteries and endoleak. In aortography performed without removal of the main trunk, filling defects may be observed in the vascular structures on the side where the main trunk is advanced since the device remains in the vessel.

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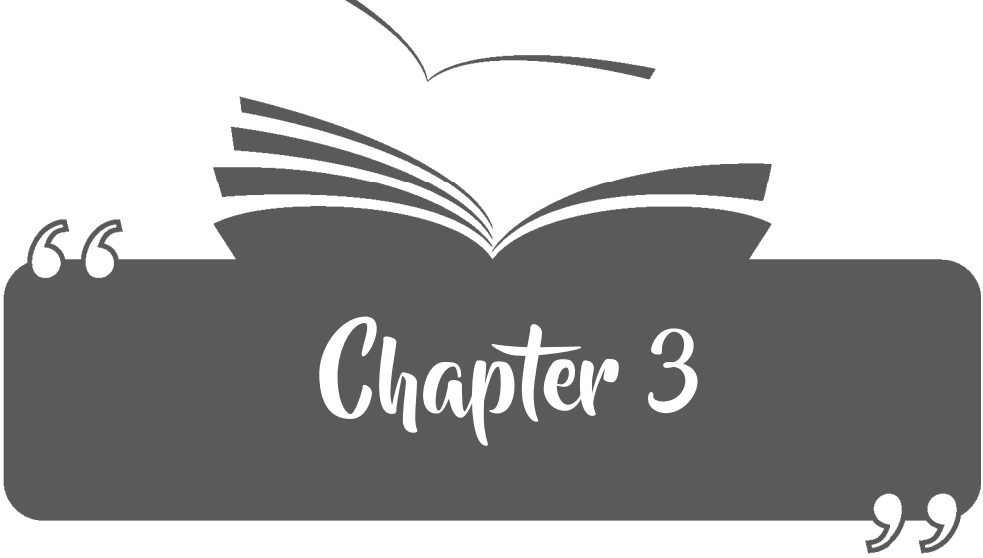

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN AGE-RELATED MACULAR DEGENERATION

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1. Oxidative Stress and Mitochondria

1.1. Oxidative Stress

Oxidative stress arises from an imbalance between two types of molecules in the body, free radicals and antioxidants. Specifically, it occurs when there is an excess of free radicals and insufficient quantity of antioxidants. Consequently, the surplus of free radicals causes significant damage to cells and tissues. Moreover, they impair the functionality of cellular components such as lipids and proteins. Oxidative stress is implicated in the etiology of numerous chronic and degenerative diseases, including cancer, cardiovascular diseases, kidney diseases, neurological diseases, respiratory diseases, rheumatoid arthritis, and eye disorders (Houldsworth, 2024). Oxidative stress can increase cancer risk by damaging DNA in healthy cells. It can also induce plaque accumulation in arteries, leading to coronary artery disease, myocardial infarction, and other complications. Sustained oxidative stress can result in scar tissue formation in kidneys, potentially causing renal failure and requiring dialysis. Researchers have associated oxidative stress with neurological diseases such as Alzheimer's, Parkinson's, and multiple sclerosis (Peoples et al., 2024). Excessive free radical levels can lead to neuronal loss and progressive dementia (Ekundayo et al., 2024). Oxidative stress and associated inflammation can also contribute to respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Taylor-Blair et al., 2024). Free radicals contribute to chronic inflammation in rheumatoid arthritis (Chandimali et al., 2025). Oxidative stress also causes visual diseases such as retinopathy and AMD, which severely affect visual function (Zhang et al., 2024).

Redox signaling involves the transfer of electrons and participation of free radicals, redox-active metals, or reducing equivalents to encode cellular processes (Li et al., 2025). The modification of specific protein residues, such as cysteine, is crucial for cellular function. This process involves changes like S-nitrosylation, sulfenylation, disulfide bridge formation, and S-glutathionylation. These alterations are specific, reversible, and distinct, playing a vital role in redox signaling. Proteins that participate in redox signaling encompass cellular networks of kinases, phosphatases, ion channels, and mechanisms leading to apoptosis, which can significantly impact cellular transcriptional activity (Sies et al., 2024). The primary free radical groups involved in cellular redox signaling processes are reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Rauf et al., 2023). Under normal conditions, ROS production is confined to the mitochondria to protect other cellular organelles from oxidative damage via enzymatic and non-enzymatic processes (An et al., 2024).

Nonetheless, when ROS are produced in excess, they can trigger oxidative stress, where the harmful impact of ROS surpasses the cell's antioxidant defenses. In the absence of a strong antioxidant system in mitochondria, the large quantities of free radicals generated within these organelles can harm proteins, DNA, and membrane lipids, potentially impairing cellular functions. Oxidative stress can also interfere with the normal physiological roles of redox signaling, resulting in irreversible chemical alterations (Chahla et al., 2024). Biomarkers of oxidative stress are classified into two categories: molecules that are modified by interactions with ROS in the microenvironment, and antioxidant system molecules that are altered in response to increased redox stress (Kong et al., 2024). Examples of molecules that can be modified by excess ROS in vivo include nucleic acids, lipids (phospholipids), proteins, and carbohydrates. Some of these modifications have direct effects on the function of the molecule (e.g., inhibition of enzyme function), while others merely reflect the degree of oxidative stress in the microenvironment.

1.2. Mitochondria

The molecular architecture of mitochondria is characterized by a dual-membrane system delineating distinct compartments. The inner membrane, notably protein-rich, exhibits a complex organization with mitochondrial cristae and extensive invaginations that increase the surface area for oxidative phosphorylation. The outer membrane, less intricate in structure, is adjacent to the inner border membrane. This arrangement is pivotal for various mitochondrial functions, including energy generation, calcium regulation, and synthesis of crucial molecules (Ouyang et al., 2024). It is important to recognize that mitochondrial structure exhibits significant variability across different cell types and physiological conditions. Additionally, mitochondrial inclusions, which may manifest as granular, tubular, or filamentous structures, constitute a part of the molecular framework and are predominantly localized within the matrix. The dynamic nature of the mitochondrial structure is further evidenced by its capacity to alter its composition and architecture in response to metabolic requirements (Tábara et al., 2024). Consequently, the molecular structure of mitochondria is both intricate and adaptable, featuring a double-membrane system that facilitates diverse functions and structural modifications to meet cellular needs. Although the outer membrane serves as an interface with the cellular environment, the complex topology of the inner membrane, particularly the cristae, is essential for optimal organelle performance. The observed variability in the mitochondrial structure across different cellular contexts underscores the adaptability of the organelle and its integral role in cellular metabolism and homeostasis (Liang et al., 2024). Mitochondria, the fundamental organelles in eukaryotic cells, are

renowned for their pivotal role in adenosine triphosphate (ATP) production, earning them the moniker "cellular powerhouses." However, these organelles possess multifaceted functionalities that extend beyond energy generation. They are intricately involved in diverse cellular processes including signal transduction, differentiation, cell cycle regulation, growth, apoptosis, and redox equilibrium. Mitochondria exhibit remarkable plasticity and undergo morphological alterations through mitophagy, fusion, and fission (Muench et al., 2021). Additionally, they contribute to reactive oxygen species (ROS) production and calcium signaling pathways. Mitochondrial functionality is influenced by both intracellular conditions and external stimuli, including environmental toxins, which can potentially lead to mitochondrial degradation and various pathological states (Zhong et al., 2024). Notably, certain bacterial pathogens have evolved mechanisms to target mitochondria and manipulate host cell death and immune responses through effector molecule secretion. This underscores mitochondria's critical role in maintaining cellular health and mounting defenses against pathogenic challenges. Mitochondria's significance extends beyond ATP synthesis. These organelles are integral to cell death mechanisms, immune system functionality, and cellular adaptation to environmental stressors.

Mitochondrial dysfunction has been implicated in the aging process and in a spectrum of age-related disorders, as well as specific mitochondrial diseases resulting from genetic mutations affecting respiratory chain function (Bartman et al., 2024). Growth differentiation factor 15 (GDF15) has emerged as a potential biomarker for mitochondrial diseases, and is associated with adverse outcomes in aging and age-related conditions (Conte et al., 2022). Furthermore, mitochondrial impairment has been linked to endothelial dysfunction and vascular diseases including atherosclerosis and hypertension. It also plays a role in the pathogenesis of neurodegenerative disorders, metabolic abnormalities, and ischemic events. The intricate involvement of mitochondria in these diverse physiological and pathological processes highlights their central importance in cellular functions and overall organismal health (Chen et al., 2023). Therapeutic strategies to enhance mitochondrial function include mitochondrial-targeted antioxidants, caloric restriction, and pharmacological agents such as metformin, aspirin, and polyphenols. Mitochondrial transfer from mesenchymal stem cells (MSCs) has the ability to restore cellular bioenergetics in cells with mitochondrial DNA defects, suggesting a potential therapeutic approach for diseases associated with mitochondrial dysfunction (Tan et al., 2022). The significance of regular physical activity in promoting mitochondrial biogenesis and function is well established, and may ameliorate metabolic diseases associated with aging. Identifying

biomarkers, elucidating disease mechanisms, and developing therapeutic strategies targeting mitochondrial dysfunction remains a critical area of ongoing research. In this context, the role of regular physical activity in promoting mitochondrial biogenesis and function has been recognized, which may mitigate metabolic diseases associated with aging.

1.3. Anti-Oxidant Compounds

Given their capacity to shield biomacromolecules from the oxidative stress induced by free radicals, the pivotal role of antioxidants in human health cannot be overstated. These compounds exhibit varied functions contingent on the specific radical they target and the molecules they safeguard. Antioxidants play a crucial role in reducing the risk of degenerative disorders by stabilizing highly reactive species (Pisoschi et al., 2021). Although some antioxidants are synthesized endogenously, others must be acquired through dietary sources or supplementation. Antioxidants encompass several categories, including enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase), hydrophilic (e.g., urate, ascorbate, glutathione, and flavonoids), and lipophilic (e.g., tocopherols, carotenoids, and ubiquinol) (Albano et al., 2022). Beyond their physiological significance, antioxidants are used to prolong the shelf life of various products, including foodstuffs, supplements, and pharmaceuticals. These compounds can be classified as primary or secondary based on their mode of action, with the former eliminating radical species through hydrogen atom or electron transfer and the latter inactivating pro-oxidant catalysts. Notably, certain synthetic antioxidants have demonstrated toxic and carcinogenic properties at specific concentrations. Antioxidants can be further categorized into three subgroups according to their defense mechanisms: preventive, radical scavenging, and enzyme restorative (Hassan et al., 2024). Plant-derived antioxidants are predominantly hydrophilic, whereas lipophilic antioxidants, such as tocopherols, vitamin K, and carotenoids, exhibit hydrophobic properties. Some antioxidants, including uric acid and ascorbic acid, are characterized by their water soluble.

Human organisms have evolved a diverse array of antioxidant defence mechanisms to combat the detrimental effects of oxidative stress. These protective systems include antioxidants that inhibit or retard oxidation processes, thereby mitigating or eliminating the consequences of oxidative stress through inhibition, blockade, and repair. The principal antioxidant defense network of the body can be categorized into two distinct classes: enzymatic and non-enzymatic. Enzymatic antioxidants, including SOD, catalase, GPx, and coenzyme Q10, have been widely acknowledged for their capacity to protect against oxidative stress (Roy et al., 2023). These enzymes and lipid-soluble antioxidants exhibit a high affinity for ROS,

thus providing robust protection. SOD, a metalloenzyme, is one of the most efficacious enzymatic antioxidants present in all subcellular compartments (Rosa et al., 2021). It functions as a protective agent against the noxious effects of elevated ROS concentration by facilitating the decomposition of superoxide into oxygen and hydrogen peroxide. Three variants of SOD enzymes exist in the human body and are classified according to their metal cofactors: cytosolic CuZn-SOD, mitochondrial Mn-SOD, and Fe-SOD (Zheng et al., 2023). These enzymes contribute to the prevention of hydroxyl radical formation catalyzed by transition metals, which can be detrimental to cellular integrity. Glutathione-S-transferase primarily catalyzes the reaction between the tripeptide glutathione and electrophilic xenobiotic substrates (Mazari et al., 2023). Furthermore, it participates in the conversion of toxic compounds, such as hydroxypiperoxide and pesticides, into non-toxic metabolites, thereby aiding the maintenance of hormonal homeostasis. Non-enzymatic antioxidants, including Vitamin E, carotenoids, polyphenols, and ascorbic acid, are crucial for maintaining fundamental physiological functions in the human body (Chaudhary et al., 2023). Vitamin E manifests in eight distinct forms, with α-tocopherol being the most prevalent form (Wallert et al., 2021). Coenzyme Q10, synthesized through the combination of a benzoquinone ring and isoprenoid chain, can be supplemented via dietary intake to enhance the body's energy production through ATP (Gasmi et al., 2024). Nutritional intake and dietary composition play significant roles in the body's defense mechanisms against oxidative stress. Glutathione, uric acid, and bilirubin are low-molecular-weight antioxidants present in human bodily fluids. Vitamins C and E, in conjunction with β-carotene (a provitamin A carotenoid), are classified as antioxidant vitamins that contribute to the prevention of diseases induced by oxidative damage (Didier et al., 2023). Although supplementation with these antioxidants can be beneficial, it is imperative to note that excessive consumption may lead to adverse health effects.

1.4. Oxidative Stress Related Diseases

Oxidative stress is a significant factor in the etiology of numerous pathological conditions, including diabetes, obesity, and diabetes-induced microvascular diseases, such as diabetic retinopathy, macular degeneration, end-stage renal disease, atherosclerosis, and cardiovascular disease (Yang et al., 2024). This phenomenon occurs when there is an excessive accumulation of reactive free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), in cells. Although ROS and RNS are integral to normal cellular function and contribute to the body's defense against pathogens, an excess can lead to various conditions including neurological diseases, cancer, diabetes, retinopathy, and age-re-

lated macular degeneration. Free radicals are highly reactive molecules with unpaired electrons that can act as oxidants or reductants, formed when oxygen interacts with certain molecules. RNS are produced by all aerobic cells and pose a significant risk in aging and age-related diseases. In addition to their deleterious effects, RNS production is involved in energy extraction, immune defense, and signaling processes. Aging is characterized by a decline in tissue and organ function over time (Guo et al., 2022). The free radical theory of aging posits that this decline is caused by the accumulation of oxidative damage to macromolecules (such as lipids, DNA, and proteins) by RNS. Although the precise mechanism of oxidative stress-induced aging has not yet been fully elucidated, it has been hypothesized that increased levels of RNS lead to cellular senescence, a physiological process that halts cellular proliferation in response to damage that occurs during replication.

The proliferation of free radicals, including ROS, RNS, and reactive carbonyl species, is responsible for the manifestation of oxidative stress in the body (Leyane et al., 202). Free radicals, primarily generated through metabolic or environmental processes, with ROS being the most significant, compete for shared electrons of intracellular molecules. This results in lipid peroxidation, protein modification, and damage to chromosomal and mitochondrial DNA (mtDNA). These alterations can disrupt information transfer and gene expression, potentially leading to autophagy, apoptosis, and necrosis, causing tissue and organ dysfunction. Oxidative stress also plays a crucial role in degenerative ocular diseases by inducing tissue damage, structural and functional changes, increased vascular permeability, microvascular abnormalities, and neovascularization (Zhao et al., 2023). These pathological changes can manifest as corneal, conjunctival, and optic nerve lesions; lens crystalline denaturation; elevated intraocular pressure; and retinal degeneration. The retina's high metabolic demand for generating and transmitting visual-evoked potential signals results in substantial ROS production within the mitochondria. Consequently, retinal cells experience greater oxidative stress compared to other tissues. Retinal pigment epithelium (RPE) cells, which are essential for maintaining normal retinal function, are particularly susceptible to oxidative stress-induced pro-inflammatory responses, autophagic cell death, and apoptosis.

2. Age Related Macular Degeneration (AMD) and Pathophysiology

Age-related macular degeneration (AMD) is a disease that affects the macular region of the retina and causes progressive central vision loss (Coleman et al, 2008; Lim et al, 2012; Bourne et al, 2013). Early AMD includes clinical signs, such as drusen and abnormalities in the retinal pig-

ment epithelium (Fleckenstein et al., 2021). Late AMD can occur in two forms: neovascular (also known as wet or exudative) and non-neovascular (known as atrophic, dry, or exudate-free). Late AMD causes loss of central visual acuity, leading to severe and permanent visual impairment, with a major impact on quality of life and visual functioning (Flores et al., 2021). AMD is a common cause of blindness in older adults and is characterized by progressive macular degeneration. This complex condition involves a variety of metabolic, functional, genetic, and environmental factors. AMD is characterized by significant abnormalities, such as photoreceptor loss, RPE cell degeneration, Bruch's membrane thickening, and choroidal thinning. In addition, oxidative stress may contribute to the development of AMD by disrupting RPE-extracellular matrix interactions. This disease can be classified into two types: dry and wet. Dry AMD is characterized by RPE degeneration and the subsequent loss of photoreceptors in the macular area, leading to extracellular deposits between the RPE and Bruch's membrane. This loss of protective properties of RPE is detrimental to the retina. In contrast, wet AMD is associated with choroidal neovascularization, which can damage the RPE and retina through exudation, hemorrhage, inflammation, and scar tissue formation.

The underlying mechanisms of AMD are primarily associated with the progressive degeneration of retinal pigment epithelium (RPE) cells, leading to the subsequent death of photoreceptors and resulting in impaired vision (Vitillo et al., 2019). Given their essential role in cellular energy production and homeostasis, mitochondria play a crucial role in stem cell-based interventions designed to regenerate or repair damaged RPE cells in AMD (Kaarniranta et al., 2019; Tong et al., 2022). Recent studies highlight the involvement of innate immune responses—including the activation of the complement system, microglial function, and disruptions in the blood-retinal barrier—in the pathogenesis of AMD (Ascunce et al., 2023). Despite originating from distinct genetic backgrounds, AMD and Alzheimer's disease share similar aging-related cellular dysfunctions, including oxidative stress and chronic inflammation (Kaarniranta et al., 2011). Genetic predisposition has also been implicated, particularly variations in inflammation-associated genes, such as complement Factor H polymorphisms, which have been linked to an increased risk of developing AMD (Telander, 2011; Wang and Zhao, 2015). Additionally, transcriptomic analyses of AMD-affected tissues have provided valuable insights into molecular alterations that may guide future research directions (Whitmore and Mullins, 2012). Mitochondrial dysfunction is another crucial factor contributing to the progression of AMD (Chu et al., 2024). Electron microscopy studies have demonstrated that RPE cells in elderly individuals, particularly those with AMD, exhibit a reduction in mito-

chondrial size and number, coupled with significant ultrastructural abnormalities and cytoplasmic displacement (Bianchi et al., 2013). However, it remains uncertain whether these mitochondrial alterations are a cause or a consequence of disease progression. Prior investigations have reported extensive mitochondrial DNA (mtDNA) damage in the RPE cells of AMD patients, suggesting that mtDNA integrity deteriorates with disease severity (Terluk et al., 2015). Interestingly, these mtDNA lesions appear to be exclusive to RPE cells and are absent in other retinal regions (Qu et al., 2024). Earlier findings indicate that disruptions in mitochondrial DNA stability may contribute to oxidative stress by impairing mtDNA transcription and replication, both of which are critical for mitochondrial biogenesis and cellular function (Blasiak et al., 2013; Lenin et al., 2024). While these discoveries highlight the importance of mitochondrial health in AMD, further research is necessary to determine whether these abnormalities serve as initiators or accelerators of disease pathology.

The global prevalence of age-related macular degeneration (AMD) was estimated at 200 million patients in 2021, with projections suggesting an increase to 300 million by 2040. This trend underscores AMD's status as a major public health challenge with considerable socioeconomic ramifications. Despite being ranked the third leading cause of irreversible severe vision loss worldwide, various experimental and clinical investigations targeting genetic, physiological, and molecular biological factors have shown promise in reducing the incidence of visual impairment. Nevertheless, a comprehensive and definitive treatment protocol remains elusive, with ongoing research efforts (Coleman et al., 2008; Lim et al., 2012; Bourn et al., 2013). Historically, AMD diagnosis relied on clinical examinations or color fundus photograph evaluations. In recent decades, spectral-domain optical coherence tomography (SD-OCT) and fundus autofluorescence imaging have been adopted for enhanced lesion detection. Fluorescein angiography has proven valuable in identifying choroidal neovascularization, confirming neovascular AMD, and determining the histological location and activity of AMD through dye leakage patterns. Optical coherence tomography angiography, a recent noninvasive diagnostic approach, eliminates the need for dye administration while detecting choroidal vascular networks associated with choroidal neovascularization. Multimodal imaging techniques contribute to AMD diagnosis by providing complementary information. AMD patient classification systems vary (Klein et al., 2014). Population-based studies typically categorize patients into early and late stages, while clinic-based studies and trials often use the Age-Related Eye Disease Study (AREDS) severity scale. The AREDS scale assigns risk factors based on large drusen, pigment abnormalities, or moderate drusen in both eyes. Early AMD is generally as-

ymptomatic, with some patients experiencing mild central distortion and reduced reading ability under low light. Late AMD affects central vision, with neovascular forms progressing rapidly (weeks to months) and atrophic forms advancing slowly (years to decades). Initial AMD symptoms include impaired vision during reading, driving, or television viewing and a central scotoma that hinders facial recognition. Unilateral AMD may remain unnoticed until the affected eye becomes isolated.

Numerous risk factors have been identified for AMD, the leading cause of vision loss worldwide. Age remains the strongest determinant, with almost all late-stage AMD cases occurring in individuals aged > 60 years. Aging is associated with declining RPE function, oxidative stress accumulation, and mitochondrial dysfunction, contributing to AMD pathogenesis. Epidemiological studies show AMD incidence and prevalence are consistently higher in women than men across all age groups, possibly due to hormonal differences, genetic susceptibility, or longer female life expectancy. Besides age and sex, several non-genetic and environmental factors are implicated in AMD development and progression. Smoking has been recognized as the most significant modifiable risk factor, with a well-established association with both early- and late-stage AMD (Lambert et al., 2016). Studies have demonstrated that smoking doubles the risk of developing late-stage AMD, likely because of its role in increasing oxidative stress, reducing macular pigment density, and impairing choroidal circulation. In addition to smoking, an unhealthy diet lacking essential nutrients such as antioxidants, omega-3 fatty acids, and carotenoids has been linked to an elevated risk of AMD. Moreover, prolonged exposure to ultraviolet (UV) light and high-energy visible (HEV) blue light has been proposed as a contributing factor to AMD development, although the strength of the evidence remains inconclusive (Smith et al., 2001). Similarly, iris color has been suggested to play a role, with lighter-colored irises potentially allowing greater light penetration into the retina, thus exacerbating oxidative stress. Another controversial risk factor is alcohol consumption, which some studies have correlated with AMD risk, though the precise relationship remains to be fully elucidated (Adams et al., 2012). In addition to lifestyle and environmental influences, systemic diseases, particularly cardiovascular conditions, have been implicated in AMD progression. Hypertension, hyperlipidemia, and atherosclerosis contribute to vascular dysfunction, inflammation, and compromised choroidal circulation, all of which exacerbate retinal degeneration and AMD progression (Cheung and Wong, 2014). Given that the retina is a highly metabolically active tissue with a rich vascular network, any systemic impairment of blood flow or endothelial function can negatively affect retinal health. Beyond its physiological consequences, AMD significantly af-

fects an individual's quality of life, leading to emotional distress, reduced independence, and a greater tendency toward anxiety, depression, and decreased life satisfaction (Brody et al., 2001). Vision impairment due to AMD can limit daily activities, such as reading, driving, and recognizing faces, further exacerbating psychosocial and emotional challenges. Furthermore, recent research suggests that AMD, particularly the atrophic (dry) form, may be associated with an increased risk of cognitive decline and neurodegenerative disorders including Alzheimer's disease (Woo et al., 2012). This correlation has led to the hypothesis that AMD and neurodegenerative conditions share common pathological mechanisms, such as mitochondrial dysfunction, chronic inflammation, and oxidative stress. Given the increasing prevalence of AMD due to global aging trends, understanding its risk factors is crucial for developing preventive and therapeutic strategies. Lifestyle modifications, including smoking cessation, dietary improvements, and cardiovascular health management, may reduce the incidence and progression of AMD. Further research is needed to clarify the interplay between genetic predisposition, environmental exposure, and systemic health in AMD pathogenesis, to advance targeted interventions and improve patient outcomes.

2.1. AMD Related Experimental Studies

Traumatic events affecting organisms and their genetic composition can lead to functional impairments in tissues and organs from various mechanical, chemical, thermal, or biological factors. While minor injuries are often addressed by the body's repair mechanisms, severe trauma can surpass the regenerative capabilities of affected tissues, causing irreversible functional deficits. When damage exceeds a critical threshold, endogenous healing processes become insufficient, necessitating external interventions to restore tissue integrity and function. In this context, stem cell-based therapies have emerged as a promising avenue for regenerative medicine, aiming to compensate for the body's limited self-repair ability (Jin et al., 2023). Stem cells possess self-renewal and multilineage differentiation capacities, enabling them to generate specialized cell types required for tissue restoration. Furthermore, they exert paracrine effects by secreting bioactive molecules that modulate the inflammatory response, enhance angiogenesis, and promote cellular proliferation and survival (Miceli et al., 2021). These properties have led to extensive research on stem cell transplantation, gene-edited stem cells, and exosome-based therapies, all of which aim to optimize tissue repair and functional recovery. Despite these advances, challenges such as low engraftment efficiency, immune rejection, and potential tumorigenicity remain critical hurdles that need to be addressed through further research and technological innovations.

Tissue engineering is an interdisciplinary field integrating medicine, biotechnology, materials science, and computational modeling. It plays a key role in regenerative and reparative medicine by combining cells, bioactive signaling molecules, and biomaterial scaffolds to restore or mimic biological tissues' structure and function. Initially a subdiscipline of biomaterials science, tissue engineering has evolved into an independent, expanding domain with advancements in 3D bioprinting, organoid technology, and decellularization techniques. These innovations have paved the way for engineered tissues and complex organ structures, addressing donor organ shortages and limitations of conventional transplantation methods. The goal is to replicate the body's physiological microenvironment, creating conditions supporting cell growth, differentiation, and functional integration within host tissue. By designing biomimetic scaffolds of biodegradable polymers, hydrogels, and nanocomposites, researchers have developed platforms facilitating cell adhesion, proliferation, and vascularization, improving tissue regeneration strategies. Tissue engineering has become intertwined with regenerative medicine, with stem cell-based approaches serving as the cornerstone of surgical interventions and organ reconstruction strategies.

As regenerative medicine advances, technologies like CRISPR gene editing, bioactive hydrogels, and AI-driven tissue modeling are refining regenerative therapies. CRISPR allows correction of genetic defects in stem cells, increasing therapeutic potential and reducing immune rejection risk. Bioactive hydrogels provide improved support for cell delivery and enhance tissue integration. AI and machine learning analyze datasets from regenerative medicine studies, accelerating discovery of optimal tissue growth conditions and predicting patient-specific responses. These innovations are bringing functional tissue and organ replacement closer to clinical reality, bridging experimental research and real-world applications. The integration of stem cell therapy, tissue engineering, and personalized medicine is poised to revolutionize healthcare, offer novel solutions for untreatable conditions, and improve patient outcomes in regenerative medicine.

3. Conclusion

In conclusion, AMD is a complex disorder involving gradual macula deterioration, leading to visual impairment in older individuals. AMD etiology includes oxidative stress, mitochondrial dysfunction, inflammation, and genetic susceptibility, contributing to RPE cell and photoreceptor degradation. Oxidative stress drives disease progression, causing lipid peroxidation, DNA damage, and protein alterations, resulting in cellular malfunctions and death. Mitochondrial deficiencies disrupt ATP pro-

duction and foster ROS accumulation. Environmental and lifestyle factors increase AMD risk. Current AMD treatments are stage-dependent, with anti-VEGF interventions effective for neovascular AMD. Dry AMD remains largely untreatable, limited to dietary changes and antioxidant supplements. Despite improved diagnostics, a cure for AMD remains elusive, emphasizing the need for new therapeutic strategies. Emerging regenerative medicine approaches show promise for AMD treatment. Stem cells capable of differentiating into RPE cells offer potential for arresting or reversing vision loss. Gene-editing technologies like CRISPR provide opportunities to address genetic mutations in AMD. Mitochondrial-targeted antioxidants and exosome-based therapies show promise for mitigating oxidative stress and restoring cellular function. Bioengineered scaffolds and 3D bioprinting may facilitate functional retinal construct creation. Integrating these innovative approaches into clinical practice will be crucial in transforming AMD management from symptomatic relief to curative interventions. A multidisciplinary strategy will be key to addressing AMD challenges and improving patient outcomes.

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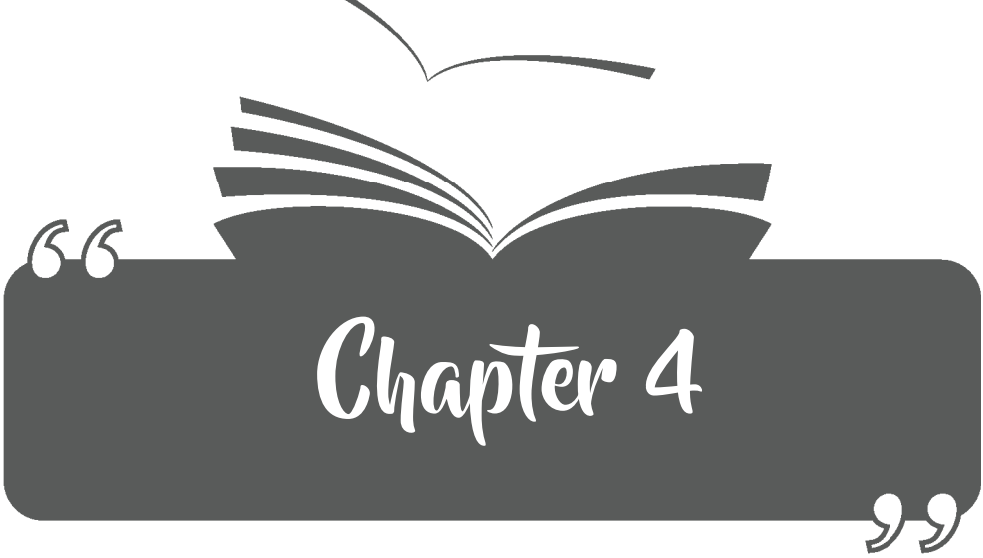

SURGICAL AND ENDOVASCULAR APPROACHES IN CRITICAL CAROTID ARTERY STENOSIS

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Introduction

Carotid artery stenosis (CAS) is a significant cause of cerebrovascular diseases and is one of the primary factors that increase the risk of ischemic stroke. CAS typically develops as a result of atherosclerotic processes and may present with transient ischemic attack (TIA) or ischemic stroke in affected patients. The natural course and management of the disease vary depending on the degree of stenosis, the presence of symptoms, and accompanying comorbidities.

Carotid artery stenosis is a major public health concern worldwide, particularly in the elderly population. Studies indicate that severe carotid stenosis (>70%) is detected in approximately 4-7% of individuals aged 65 years and older. Moreover, classical atherosclerotic risk factors such as hypertension, hyperlipidemia, smoking, diabetes mellitus, and obesity play a critical role in the development of CAS. It has been demonstrated that smokers have a 2.5-fold increased risk of developing carotid stenosis, while this risk is tripled in diabetic patients.

The atherosclerotic process begins with endothelial dysfunction, inflammation, and lipid accumulation, eventually leading to plaque formation in the arterial wall. The carotid bifurcation is a region with high hemodynamic stress and is one of the most common sites for atherosclerotic plaque deposition. As plaques progress, luminal narrowing occurs, potentially compromising cerebral perfusion. Unstable plaques, in particular, can lead to ischemic stroke through thrombus formation and embolization to the cerebral arteries.

Carotid artery stenosis is classified as asymptomatic or symptomatic. Asymptomatic stenosis is usually detected incidentally, whereas symptomatic stenosis presents with transient ischemic attack (TIA), amaurosis fugax, or stroke. In symptomatic patients, carotid stenosis greater than 50% raises the discussion of surgical or endovascular intervention. On the other hand, treatment decisions for asymptomatic patients should be individualized based on stroke risk assessment. With advancements in aggressive medical management, the annual stroke risk in asymptomatic patients has been reduced to below 1%.

Etiology

Carotid artery stenosis is a serious vascular pathology that primarily develops due to atherosclerotic processes and significantly contributes to cerebrovascular events. However, non-atherosclerotic causes can also lead to carotid artery stenosis. This section provides a detailed overview of

the mechanisms and risk factors involved in the development of carotid artery stenosis.

1. Atherosclerosis and Its Pathogenesis

Atherosclerosis is a progressive vascular disease characterized by systemic inflammation and lipid metabolism disorders. It is the most common etiology of carotid artery stenosis, involving endothelial dysfunction, lipoprotein infiltration, inflammation, thrombosis, and vascular remodeling.

1.1. Development of the Atherosclerotic Process

The atherosclerotic process progresses through the following stages:

- **Endothelial Dysfunction:** Chronic hypertension, hyperlipidemia, and cigarette smoke cause endothelial cell damage, increasing vascular permeability.

- **Lipid Accumulation:** Low-density lipoprotein (LDL) particles infiltrate the arterial wall and undergo oxidation. This oxidative process leads to macrophage phagocytosis of oxidized LDL, forming foam cells.

- **Inflammatory Response:** Inflammatory cells (monocytes, macrophages, and T cells) accumulate within the atherosclerotic plaque, releasing pro-inflammatory cytokines, which contribute to vascular thickening and fibrosis.

- **Plaque Stability and Complications:** Over time, mature atherosclerotic plaques may calcify or their fibrous caps may weaken, leading to rupture. Plaque rupture can result in thrombus formation, which may cause acute ischemic stroke.

1.2. Major Risk Factors for the Atherosclerotic Process

Several risk factors contribute to the development of atherosclerosis:

1.2.1. Modifiable Risk Factors

- **Hypertension:** High blood pressure accelerates atherosclerosis by causing endothelial damage.

- **Diabetes Mellitus:** Insulin resistance and hyperglycemia promote vascular inflammation, expediting the atherosclerotic process.

- **Smoking:** Nicotine and carbon monoxide impair vascular tone and contribute to atherosclerotic plaque formation.

- **Hyperlipidemia:** High LDL and low HDL levels are key factors that increase the risk of atherosclerosis.

- **Obesity and Sedentary Lifestyle:** Increased body mass index (BMI) and reduced physical activity accelerate the atherosclerotic process.

1.2.2. Non-Modifiable Risk Factors

- **Age:** Atherosclerosis progression accelerates with age. The prevalence of carotid stenosis ranges from 5-10% in individuals over 70 years old.

- **Gender:** Males have a higher risk of atherosclerosis compared to females.

- **Genetic Predisposition:** Individuals with a family history of early-onset atherosclerotic disease have an increased risk.

2. Non-Atherosclerotic Causes

Although atherosclerosis is the most common cause of carotid stenosis, some patients may develop stenosis due to non-atherosclerotic conditions.

2.1. Fibromuscular Dysplasia (FMD)

Fibromuscular dysplasia is a rare vascular disease that causes segmental stenosis in the carotid arteries, influenced by genetic and environmental factors. It is more common in young women and is often asymptomatic.

2.2. Vasculitides

Inflammatory diseases such as **Takayasu arteritis** and **giant cell arteritis** can affect large arteries, leading to carotid artery narrowing. These conditions are typically associated with systemic inflammation and autoimmune processes.

2.3. Carotid Artery Dissection

Carotid artery dissection occurs due to a tear in the inner layer of the arterial wall, leading to intramural hematoma formation and luminal narrowing. Trauma, connective tissue disorders (e.g., Marfan syndrome), and spontaneous causes contribute to dissection.

2.4. Radiation-Induced Stenosis

Patients who have undergone radiotherapy for head and neck malignancies may develop carotid artery stenosis due to radiation-induced fibrotic changes in the arterial wall. These stenoses often appear years after radiation exposure and increase the risk of stroke.

3. Conclusion

Although atherosclerosis is the most common cause of carotid artery stenosis, non-atherosclerotic causes should also be considered. Proper management of risk factors can slow the progression of atherosclerosis and reduce the risk of cerebrovascular events in affected patients. Treatment strategies should be tailored based on the patient's age, gender, comorbidities, and underlying pathological mechanisms (Table 1).

Table 1: Etiological Factors of Carotid Artery Stenosis

Etiological Factors	Description
Atherosclerosis	The most common cause, characterized by lipid accumulation and inflammation leading to plaque formation.
Hypertension	High blood pressure accelerates endothelial damage and the atherosclerotic process.
Diabetes Mellitus	Hyperglycemia induces vascular inflammation and increases atherosclerosis progression.
Hyperlipidemia	Elevated LDL and reduced HDL levels contribute to atherosclerosis development.
Smoking	Promotes oxidative stress and inflammation, leading to vascular damage.
Genetic Predisposition	More common in individuals with a family history of early-onset atherosclerosis.
Fibromuscular Dysplasia	A rare vascular disease that causes segmental arterial narrowing, particularly in young women.
Vasculitides	Inflammatory conditions such as Takayasu arteritis and giant cell arteritis affecting large vessels.
Radiation-Induced Stenosis	Fibrotic changes in the arterial wall due to previous head and neck radiotherapy.
Carotid Artery Dissection	Arterial wall tear leading to luminal narrowing due to trauma or spontaneous causes.

Pathophysiology

Carotid artery stenosis has complex pathophysiological mechanisms that affect cerebral perfusion and can lead to ischemic cerebrovascular events. Factors such as hemodynamic changes, plaque rupture, thrombus

formation, and embolization play a crucial role in this process. The pathophysiological mechanisms may progress differently in symptomatic and asymptomatic patients.

1. Pathophysiology of the Atherosclerotic Process

Atherosclerosis is a progressive vascular disease characterized by endothelial dysfunction, inflammation, and thrombotic activation. In the carotid arteries, the atherosclerotic process typically begins at the bifurcation due to the turbulent blood flow in this region.

1.1. Endothelial Dysfunction and Lipid Accumulation

Endothelial cells maintain vascular integrity by regulating vasodilation and antithrombotic mechanisms. However, factors such as hypertension, hyperlipidemia, and smoking impair endothelial function, leading to vascular damage. In this process, low-density lipoprotein (LDL) particles penetrate beneath the endothelium, undergo oxidation, and trigger an inflammatory response. Oxidized LDL is phagocytosed by macrophages, which then transform into foam cells, initiating atherosclerotic plaque formation. The accumulation of foam cells and the progression of inflammatory processes contribute to plaque instability and vascular narrowing, forming the basis of atherosclerosis.

1.2. Plaque Stability and Rupture

Atherosclerotic plaques are surrounded by a fibrous cap. Plaque stability depends on the thickness and composition of this cap:

- **Stable plaques** typically have a thick fibrous cap and low lipid content, posing a lower risk for thrombus formation.

- **Unstable plaques** have a thin fibrous cap and high inflammatory activity. The rupture of these plaques may lead to thrombus formation, increasing the risk of ischemic stroke.

2. Hemodynamic Changes

Carotid artery stenosis can significantly reduce cerebral blood flow. The severity of hemodynamic changes depends on the degree of stenosis:

- **Stenosis <50%:** Usually asymptomatic as cerebral autoregulation mechanisms maintain perfusion.

- **Stenosis between 50-70%:** Minimal hemodynamic effects, but a decrease in distal cerebral perfusion may begin.

- **Stenosis between 70-90%:** Critical reduction in cerebral perfusion with a higher risk of ischemic events.

- **Stenosis >90%:** High risk of cerebral hypoperfusion and ischemic stroke in patients with inadequate collateral circulation.

Cerebral autoregulation mechanisms attempt to compensate for the effects of carotid artery stenosis. However, beyond a certain degree of stenosis, collateral circulation becomes insufficient, resulting in cerebral hypoperfusion.

3. Thromboembolic Mechanisms and Embolization

Atherosclerotic plaques are the primary source of thromboembolic events. Plaque rupture can lead to thrombus formation, which may cause arterial embolization, increasing the risk of distal cerebral artery occlusion and ischemic stroke.

3.1. Thrombus Formation

Following plaque rupture, exposure of the subendothelial tissue activates the coagulation system, leading to thrombus formation. Initially, platelets adhere to the damaged area, aggregate, and initiate the clotting process. Subsequently, the coagulation cascade is activated, increasing fibrin formation and leading to thrombus enlargement, which narrows the arterial lumen and restricts blood flow, facilitating ischemic events.

3.2. Effects of Embolization

Embolization due to carotid artery stenosis occurs through the following mechanisms:

- **Dislodgement of plaque material into distal cerebral arteries**

- **Thrombus formation and embolization**

- **Microemboli obstructing small cerebral arteries (silent stroke)**

These embolization processes can result in **transient ischemic attack (TIA)** or **full-blown ischemic stroke**.

4. Clinical Consequences of Carotid Stenosis

The pathophysiological processes of carotid artery stenosis can lead to various clinical outcomes:

- **Asymptomatic Carotid Stenosis:** Patients may remain asymptomatic for an extended period if collateral circulation is sufficient and the plaque is stable.

- **Transient Ischemic Attack (TIA):** Microembolization and transient hypoperfusion can cause temporary neurological deficits.

- **Ischemic Stroke:** Occurs when thrombi or emboli completely occlude cerebral arteries, leading to permanent neurological damage.

5. Conclusion

The pathophysiology of carotid artery stenosis is complex, involving a combination of atherosclerosis, hemodynamic disturbances, and thromboembolic processes. Plaque rupture and thromboembolic events play a critical role in determining clinical outcomes.

Risk Faktörleri

Multiple factors contribute to the development of carotid artery stenosis. These risk factors can be categorized into **modifiable** and **non-modifiable** risk factors (Table 2). Managing modifiable risk factors can slow the progression of atherosclerosis and reduce the risk of ischemic stroke.

Table 2: Risk Factors for Carotid Artery Stenosis

Risk Factor	Description
Non-Modifiable Factors	Age, Gender, Genetic Predisposition
Modifiable Factors	Hypertension, Diabetes Mellitus, Hyperlipidemia, Smoking, Obesity and Sedentary Lifestyle, Dietary Habits, Alcohol Consumption
Other Factors	Chronic Kidney Disease, Elevated Homocysteine Levels

1. Non-Modifiable Risk Factors

Some individuals have an increased risk of developing carotid artery stenosis due to congenital or age-related factors. Although these factors cannot be altered, they significantly impact an individual's overall risk profile.

1.1. Age

The incidence of carotid artery stenosis increases with age. In individuals over 65 years, the prevalence of moderate to severe carotid stenosis

ranges between **5-10%**. Although atherosclerotic plaques tend to become more stable with aging, the risk of complications also rises. This is associated with vascular changes and structural deterioration of the arterial wall due to aging.

1.2. Gender

Carotid artery stenosis exhibits gender-related differences. It is more common in men than in women. In females, the disease tends to manifest later in life, potentially due to the protective effects of **estrogen** and other hormones on atherosclerosis.

1.3. Genetic Predisposition

Genetic factors play a significant role in the development of carotid artery stenosis. Individuals with a family history of **early-onset cardiovascular disease** or **carotid stenosis** have a higher risk of developing the disease. Genetic predisposition influences both the onset and progression of atherosclerosis, increasing an individual's susceptibility.

2. Modifiable Risk Factors

Many factors that accelerate or slow the progression of atherosclerosis are **modifiable**. Controlling these risk factors can slow disease progression and significantly reduce the risk of cerebrovascular events.

2.1. Hypertension

Chronic high blood pressure exerts mechanical stress on the arterial wall, leading to **endothelial damage** and the development of **atherosclerotic plaques**. In patients with carotid artery stenosis, hypertension increases the risk of **plaque rupture** and **thromboembolic events**, resulting in serious cerebrovascular complications. Blood pressure control is **crucial** for slowing the progression of atherosclerosis.

2.2. Diabetes Mellitus (DM)

Patients with type 2 diabetes have a **2 to 4 times** higher risk of developing atherosclerosis compared to healthy individuals. **Hyperglycemia** increases **oxidative stress**, leading to endothelial dysfunction and atherosclerotic plaque formation. Proper diabetes management is essential for slowing atherosclerosis progression and preventing cardiovascular events.

2.3. Hyperlipidemia

High levels of **LDL (low-density lipoprotein)** and low levels of **HDL (high-density lipoprotein)** accelerate the atherosclerotic process. Elevat-

ed **LDL** levels undergo oxidation, triggering **inflammation** and contributing to plaque accumulation in the arterial wall. **Statin therapy** and dietary modifications help stabilize atherosclerotic plaques and reduce the risk of stroke.

2.4. Smoking

Cigarette smoking promotes **vascular inflammation**, accelerating the formation of atherosclerotic plaques. **Smokers have a 2.5 times higher risk** of developing carotid artery stenosis compared to non-smokers. Toxic components in cigarette smoke **impair endothelial function**, increase **oxidative stress**, and activate **pro-coagulant mechanisms**.

2.5. Sedentary Lifestyle and Obesity

Physical inactivity and obesity are significant contributors to **metabolic syndrome**, which accelerates atherosclerosis progression. A sedentary lifestyle leads to **insulin resistance** and **dyslipidemia**, increasing **systemic inflammation** and negatively affecting vascular health. **Regular exercise** improves cardiovascular health by reducing inflammation and regulating lipid metabolism.

2.6. Dietary Habits

Diet plays a crucial role in atherosclerosis development. **Excessive consumption of saturated fats, trans fats, and refined carbohydrates** increases the risk of atherosclerosis. In contrast, a **Mediterranean diet**, rich in **fiber, antioxidants, and healthy fats**, has cardioprotective effects. Consuming **high-fiber foods, antioxidant-rich meals, and unsaturated fats** can preserve vascular health and slow the progression of atherosclerosis.

2.7. Chronic Kidney Disease

Impaired renal function increases **vascular inflammation**, elevating the risk of **atherosclerosis**. **Endothelial dysfunction** and **vascular calcification** are common in patients with chronic kidney disease (CKD), and these conditions are directly linked to the development of carotid artery stenosis. Regular monitoring of kidney function in patients with carotid stenosis is recommended to **reduce cardiovascular event risk**.

2.8. Alcohol Consumption

Excessive alcohol intake contributes to **hypertension** and **dyslipidemia**, promoting the development of atherosclerosis. **Chronic alcohol use** increases systemic inflammation and negatively affects vascular health.

However, some studies suggest that **moderate alcohol intake**, particularly **red wine**, which contains **polyphenols**, may have **cardioprotective effects**. Nevertheless, alcohol consumption should be evaluated **on an individual basis**, and excessive drinking should be avoided.

3. Management and Prevention of Risk Factors

The progression of carotid artery stenosis can be halted or slowed by implementing the following strategies:

1. **Blood Pressure Control:** Regular monitoring and appropriate antihypertensive therapy are recommended.

2. **Diabetes Management:** Maintaining **HbA1c levels below 6.5%** can reduce vascular complications.

3. **Lipid Profile Optimization:** LDL levels should be reduced through **statins** and **lifestyle modifications**.

4. **Smoking Cessation Programs: Nicotine replacement therapies** and **behavioral interventions** can help individuals quit smoking.

5. **Regular Exercise:** At least **150 minutes of moderate-intensity aerobic exercise per week** is recommended.

6. **Healthy Diet: Mediterranean-style diets**, rich in **anti-inflammatory** and **antioxidant** foods, should be encouraged.

7. **Limiting Alcohol Intake:** Excessive alcohol consumption should be avoided.

Effective management of these risk factors not only slows the progression of carotid artery disease but also significantly reduces **stroke risk**.

Diagnosis and Clinical Findings

The diagnosis of carotid artery stenosis is established through **clinical history assessment, physical examination findings, and advanced imaging techniques**. Distinguishing between asymptomatic and symptomatic patients is crucial in the diagnostic process. While symptomatic patients present with a history of **transient ischemic attack (TIA) or ischemic stroke**, asymptomatic cases are usually **incidentally detected** during routine screenings.

1. Clinical Findings

The clinical manifestations of carotid artery stenosis vary depending on the **degree of stenosis** and **embolization mechanism**. While symp-

tomatic patients primarily exhibit **neurological symptoms**, asymptomatic cases are often diagnosed through **physical examination or imaging studies**.

1.1. Asymptomatic Carotid Artery Stenosis

Asymptomatic patients are **frequently diagnosed incidentally** during **carotid auscultation** or **duplex ultrasonography** performed for unrelated reasons. These patients do not exhibit **neurological deficits**; however, **progression of stenosis** or **instability of atherosclerotic plaque** may lead to **sudden embolization**, significantly increasing the risk of **ischemic stroke**. Therefore, regular **follow-up** and **individualized treatment strategies** are essential for asymptomatic patients.

1.2. Symptomatic Carotid Artery Stenosis

Carotid artery stenosis is a major cause of **cerebrovascular diseases**, and **neurological deficits** may develop in symptomatic cases. Symptomatic carotid artery stenosis manifests as:

- **Transient ischemic attack (TIA)**
- **Reversible ischemic neurological deficit (RIND)**
- **Ischemic stroke**

These conditions result from **arterial embolization, thrombotic occlusion, or cerebral hypoperfusion**.

Transient Ischemic Attack (TIA)

TIA is a cerebrovascular event in which **neurological symptoms resolve completely within 24 hours**. It is caused by **temporary impairment of cerebral circulation** without **permanent infarction**. TIA is a **strong predictor of ischemic stroke**, requiring **early diagnosis and intervention**.

- **Amaurosis fugax:** Transient monocular vision loss due to **retinal artery embolization**.
- **Contralateral hemiparesis or hemianesthesia:** Temporary **motor or sensory deficits** in the extremities opposite to the affected carotid artery.
- **Dysarthria and aphasia:** If the **dominant hemisphere** is affected, speech disturbances may occur.

- **TIA-related stroke risk:** The **90-day stroke risk after TIA is 10-20%**, with the **highest risk occurring within the first 48 hours**.

Reversible Ischemic Neurological Deficit (RIND)

RIND is characterized by **neurological symptoms lasting longer than 24 hours but resolving within two weeks**.

- Unlike TIA, **RIND results in prolonged neurological deficits** but eventually leads to **complete recovery**.

- **Brain imaging may reveal small infarcted areas**, but patients **functionally recover**.

- RIND is associated with **high stroke risk**, necessitating **aggressive treatment**.

Some **recent guidelines** classify **RIND as an extended form of TIA**, rather than a distinct entity, considering it an **intermediate stage between TIA and ischemic stroke**.

Ischemic Stroke

Ischemic stroke is characterized by **neurological deficits lasting more than two weeks**, with radiological evidence of **cerebral infarction**. Carotid artery stenosis-associated strokes commonly involve infarction in the **middle cerebral artery (MCA) territory**.

- **Permanent motor and sensory deficits** may develop, including **hemiparesis, hemianesthesia, and visual loss**.

- **Cognitive impairment and speech disturbances** are common. **Aphasia** occurs with dominant hemisphere involvement, while **spatial neglect** is seen in non-dominant hemisphere involvement.

- **Stroke-related mortality and morbidity are high.** The **one-year mortality rate** after stroke in symptomatic carotid stenosis patients **can reach 20-30%**.

Early **thrombolytic therapy** (e.g., **tissue plasminogen activator [tPA]**) and **mechanical thrombectomy** may reduce ischemic stroke damage. However, in carotid artery stenosis-related strokes, the **timing of revascularization and selection of appropriate treatment** are crucial for long-term prognosis.

2. Physical Examination Findings

Physical examination is **a supportive tool** for detecting carotid artery stenosis but is **not diagnostic**.

- **Carotid bruit:** A **murmur** heard over the carotid artery due to **turbulent blood flow**. However, it can be present in **stenosis <50%** and may be **absent in completely occluded arteries**.

- **Blood pressure discrepancies:** Differences in blood pressure between arms may indicate **bilateral carotid stenosis** or **subclavian steal syndrome**.

- **Neurological deficits: Facial asymmetry, hemiparesis, sensory loss, or speech impairment** may be observed in acute stroke patients.

3. Diagnostic Methods

Non-invasive and invasive imaging techniques are used for the diagnosis of carotid artery stenosis. **Non-invasive methods are preferred** in most cases.

3.1. Non-Invasive Imaging Techniques

3.1.1. Carotid Duplex Ultrasonography (DUS)

Carotid **DUS** is the **first-line** imaging modality for carotid artery stenosis. It measures **blood flow velocities** and estimates stenosis severity using **peak systolic velocity (PSV) and end-diastolic velocity (EDV)**. Due to its **high accuracy, radiation-free nature, and ease of use**, DUS is the **preferred initial diagnostic tool**.

3.1.2. Magnetic Resonance Angiography (MRA)

MRA provides **detailed vascular imaging** using **magnetic resonance imaging (MRI)**. Contrast-enhanced MRA is particularly useful for assessing **stenosis severity**. It is **radiation-free** and offers **high soft tissue contrast**, making it a preferred **advanced imaging technique**.

3.1.3. Computed Tomography Angiography (CTA)

CTA provides **high-resolution cross-sectional imaging** of vascular structures. It is **advantageous** for evaluating the **anatomy of the carotid artery** and **stenosis severity**, as well as for **assessing plaque composition** (e.g., calcified plaques).

3.2. Invasive Imaging Techniques

3.2.1. Digital Subtraction Angiography (DSA)

DSA is the **gold standard** for assessing carotid artery stenosis. This **invasive** technique uses **contrast agents** to visualize hemodynamically significant stenoses and plays a critical role in planning **endovascular treatments**. However, due to the **high accuracy of non-invasive techniques (DUS, MRA, CTA)**, most patients can be diagnosed without DSA.

4. Risk Assessment and Clinical Approach

The management of carotid artery stenosis depends on **symptom status, stenosis severity, and additional risk factors**.

- **Asymptomatic Patients:**

  - **Stenosis <50%: Lifestyle modifications** and **medical therapy** are usually sufficient.

  - **Stenosis 60-80%:** Consider **carotid endarterectomy (CEA) or stenting** based on **individual stroke risk**.

  - **Aggressive medical therapy** (statins, antihypertensives, antiplatelet agents) is recommended.

- **Symptomatic Patients:**

  - **Stenosis >50%: CEA or endovascular intervention** should be considered.

  - **Urgent intervention is required for:**

    - **TIA or minor stroke within the past two weeks**

    - **Stenosis >70%**

  - If surgery is not feasible, **carotid artery stenting (CAS)** may be an alternative.

5. Conclusion

The diagnosis of carotid artery stenosis should be based on a combination of **clinical assessment and imaging studies** (Table 3). **Physical examination alone is insufficient**, but the presence of a **carotid bruit** may indicate **high-risk** patients. **Duplex ultrasonography is the most commonly used diagnostic tool**, with **MRA, CTA, or DSA** utilized in advanced cases. **Treatment decisions** should be individualized based on **stenosis severity and symptomatology**.

Table 3: Diagnostic Methods for Carotid Artery Stenosis

Diagnostic Method	Description
Clinical Assessment	Evaluating the patient's symptoms, including a history of transient ischemic attack (TIA) or stroke.
Physical Examination	Detection of carotid bruits and neurological deficits. However, bruits alone are not sufficient for determining stenosis severity.
Carotid Duplex Ultrasonography (DUS)	First-line imaging modality. Estimates stenosis severity by measuring blood flow velocities.
Magnetic Resonance Angiography (MRA)	Can be performed with or without contrast. Provides detailed visualization of the carotid artery anatomy and stenosis severity.
Computed Tomography Angiography (CTA)	Provides high-resolution imaging of vascular structures, especially useful for assessing plaque composition and surrounding bony structures.
Digital Subtraction Angiography (DSA)	The gold standard for diagnosing carotid artery stenosis. Preferred for endovascular interventions, but due to its invasive nature, it is mainly used in advanced cases.
Biomarkers and Blood Tests	High-sensitivity C-reactive protein (hs-CRP), homocysteine, and other inflammatory markers can help assess disease progression.

Treatment Approaches

The management of carotid artery stenosis is determined based on whether the patient is symptomatic, the degree of stenosis, overall health status, and associated risk factors. Treatment approaches are generally divided into three main categories: medical therapy, surgical treatment (carotid endarterectomy – CEA), and endovascular treatment (carotid artery stenting – CAS). In modern practice, a personalized approach is adopted, and treatment decisions are made by considering patient-specific risk factors.

1. Medical Therapy

Medical therapy is of critical importance for both asymptomatic and symptomatic patients with carotid artery stenosis. It aims to slow the progression of atherosclerosis, prevent thromboembolic events, and control cerebrovascular risk factors.

1.1. Antiplatelet and Anticoagulant Therapy

Antiplatelet therapy is one of the primary treatment strategies for preventing thromboembolic events associated with carotid artery stenosis. Aspirin (75–325 mg/day) or Clopidogrel (75 mg/day) inhibits platelet aggregation, thereby reducing the risk of thrombo-

embolic events. The combination of aspirin and clopidogrel is recommended for short-term use in high-risk patients, but long-term use should be approached cautiously due to an increased risk of bleeding. Oral anticoagulants (e.g., warfarin, direct oral anticoagulants – DOACs) should only be considered in patients at risk of stroke due to atrial fibrillation or cardioembolic sources. Given the potential for increased thrombotic risk in carotid artery stenosis, the suitability of anticoagulation therapy should be carefully assessed on an individual basis.

1.2. Lipid-Lowering Therapy

Statin therapy is widely used to stabilize atherosclerotic plaques and reduce inflammation. Statins such as atorvastatin, rosuvastatin, and simvastatin lower LDL levels and contribute to plaque stabilization. The target LDL level should be maintained below 70 mg/dL, and more aggressive lipid control should be implemented in high-risk patients. In cases where statin intolerance or insufficient LDL reduction occurs despite statin therapy, ezetimibe or PCSK9 inhibitors may be used as adjunctive therapies. Since lipid-lowering therapy has been shown not only to reduce LDL levels but also to decrease inflammatory processes, it is regarded as a crucial preventive treatment strategy in atherosclerotic disease.

1.3. Blood Pressure and Diabetes Control

Hypertension is one of the most significant factors accelerating the progression of carotid artery stenosis and increasing the risk of stroke. Blood pressure control plays a vital role in slowing the atherosclerotic process. ACE inhibitors (ramipril, perindopril) or ARBs (losartan, valsartan) are preferred due to their antihypertensive and anti-inflammatory effects. Generally, the target blood pressure should be maintained below 140/90 mmHg, but this target may vary depending on individual patient risk factors. Diabetes control is also essential in preventing atherosclerosis. HbA1c levels should be maintained below 6.5%, and the endothelial dysfunction and inflammatory processes caused by chronic hyperglycemia should be effectively managed. Proper diabetes management is considered one of the key factors in reducing the risk of stroke in patients with carotid artery stenosis.

1.4. Lifestyle Modifications

Lifestyle modifications play a fundamental role in managing the atherosclerotic process and preventing the progression of carotid artery stenosis.

- Smoking cessation is one of the most critical factors in halting the progression of atherosclerosis. Smoking increases vascular inflammation and contributes to plaque instability.

- A healthy diet, particularly the Mediterranean diet, is recommended. The Mediterranean diet is rich in antioxidants and has been shown to be effective in the prevention of atherosclerotic diseases.

- Regular physical activity, with at least 150 minutes of moderate-intensity exercise per week, is advised. Increasing physical activity helps prevent metabolic syndrome, maintains vascular health, and reduces overall cardiovascular risk.

Medical therapy plays a crucial role in both reducing the risk of stroke and slowing disease progression in patients with carotid artery stenosis. Therefore, an appropriate combination of medical treatment and lifestyle modifications should be planned based on individual patient characteristics.

2. Surgical Treatment: Carotid Endarterectomy (CEA)

Carotid endarterectomy (CEA) is considered the **gold standard** surgical intervention for reducing the risk of **stroke** in symptomatic **carotid artery stenosis**. The procedure aims to restore blood flow by surgically removing the **atherosclerotic plaque** from the stenotic segment of the **carotid artery**.

When performed with optimal indications, **CEA** significantly reduces stroke incidence and provides long-term neurological protection. **Endarterectomy** should be considered in symptomatic patients with **stenosis >50%** and in asymptomatic patients with **stenosis 60–80%**. The patient's overall health status, comorbidities, and surgical suitability play a critical role in decision-making.

2.1. Indications for Carotid Endarterectomy

Patient selection for **CEA** is based on the degree of **carotid artery stenosis**, the presence of symptoms, and overall health condition.

2.1.1. Symptomatic Patients

Symptomatic **carotid artery stenosis** includes patients with a history of **transient ischemic attack (TIA), minor stroke,** or **major ischemic stroke**.

- **50–69% stenosis:**

  - **CEA** is recommended as the first-line treatment.

  - In patients with a high risk of thromboembolic events, **early surgical intervention** reduces the risk of **recurrent stroke**.

- **≥70% stenosis:**

  - **Carotid endarterectomy** is the preferred surgical approach.

  - In high surgical risk patients, **carotid artery stenting (CAS)** may be considered as an alternative.

  - Performing surgery within **two weeks** significantly minimizes stroke risk.

2.1.2. Asymptomatic Patients

The necessity of **surgery** in **asymptomatic carotid stenosis** is still debated. However, in selected patients, **CEA** may provide long-term benefits compared to **medical therapy**.

- **60–80% stenosis:**

  - **CEA** is recommended for patients with a **long life expectancy**.

  - Surgery can be considered in patients **not adequately controlled** with medical therapy.

  - In patients with additional risk factors (**diabetes, smoking, hypertension**), surgery should be considered.

- **<50% stenosis:**

  - **Medical therapy** is recommended.

  - Surgery is **not indicated**.

2.2. Carotid Endarterectomy Technique

CEA is typically performed under **general anesthesia**, though **local anesthesia** may be used in certain cases. The key surgical steps include:

1. **Incision and exposure** – The **skin** and **subcutaneous tissues** are incised to access the surgical site.

2. **Isolation of the carotid arteries** – The **carotid arteries** are carefully dissected, and **clamps** are applied to temporarily interrupt blood flow.

3. **Plaque removal** – The **atherosclerotic plaque** is meticulously extracted.

4. **Closure of the artery** – The artery is closed using either **primary suturing** or **patch angioplasty** (Dacron or a vein graft).

5. **Restoration of blood flow** – Blood flow is re-established, and **cerebral perfusion** is evaluated.

In some patients, **intraoperative shunting** may be required to maintain **cerebral perfusion** during the procedure.

2.3. Advantages and Risks of Carotid Endarterectomy

Advantages

- **Significantly reduces stroke risk**.
- High **long-term patency rates**.
- Lower **restenosis rates** compared to **CAS**.
- Lower risk of **hemorrhagic complications** than **CAS**.

Risks and Complications

- **Perioperative stroke risk: ~2–3%**.
- **Cranial nerve injuries (5–7%)** – May affect the **hypoglossal, vagus,** or **facial nerve**.
- **Airway compression due to hematoma** – Risk is higher in **hypertensive patients**.
- **Restenosis development** – May occur in the **long term**.

Following **CEA**, **regular ultrasound follow-up** and **continuation of antiplatelet therapy** are recommended to ensure long-term **vascular patency** and stroke prevention.

3. Endovascular Treatment: Carotid Artery Stenting (CAS)

Carotid artery stenting (CAS) has been developed as a **minimally invasive alternative** for patients who are **not suitable for surgery** or are at **high surgical risk**.

In this procedure, a **stent is placed via catheterization** into the stenotic segment to **widen the vessel lumen** and **improve cerebral blood flow**.

CAS has become an important treatment option, particularly for patients at **high surgical risk**.

3.1. Indications for Carotid Artery Stenting

CAS may be considered as an alternative to surgery in the following patient groups:

- **High-risk surgical patients:**
  - Patients with **advanced age** or a history of **severe cardiovascular disease**.
- **Patients with bilateral carotid artery stenosis:**
  - **CAS** may be preferred in cases of **bilateral involvement** to avoid surgery.
- **Patients with recurrent stenosis after previous CEA:**
  - **CAS** can be a suitable option in cases of **restenosis** following **CEA**.
- **Patients with a history of head and neck radiation therapy:**
  - In patients with **fibrosis and extensive scar tissue**, **CAS** may be a **safer alternative**.

3.2. Advantages and Disadvantages of CAS

Advantages

- **Less invasive** than **CEA**.
- **Does not require general anesthesia**.
- **Shorter hospital stay**.

Disadvantages

- **Higher perioperative stroke risk** compared to **CEA (4–5%)**.
- **Lower long-term efficacy** than **surgery**.
- **Risk of embolization** during stent placement, requiring the use of **embolic protection devices**.

While **CAS** is a **valuable option** for **high-risk surgical patients**, it has a **higher perioperative stroke risk** than **CEA**, necessitating **careful patient selection**.

4. Decision-Making in Treatment Selection

A **personalized approach** is essential in the treatment of **carotid artery stenosis** (Table 4). The **choice of treatment** is determined based on whether the patient is **symptomatic** and the **degree of stenosis**.

Table 4: Treatment Approach Based on Patient Groups in Carotid Artery Stenosis

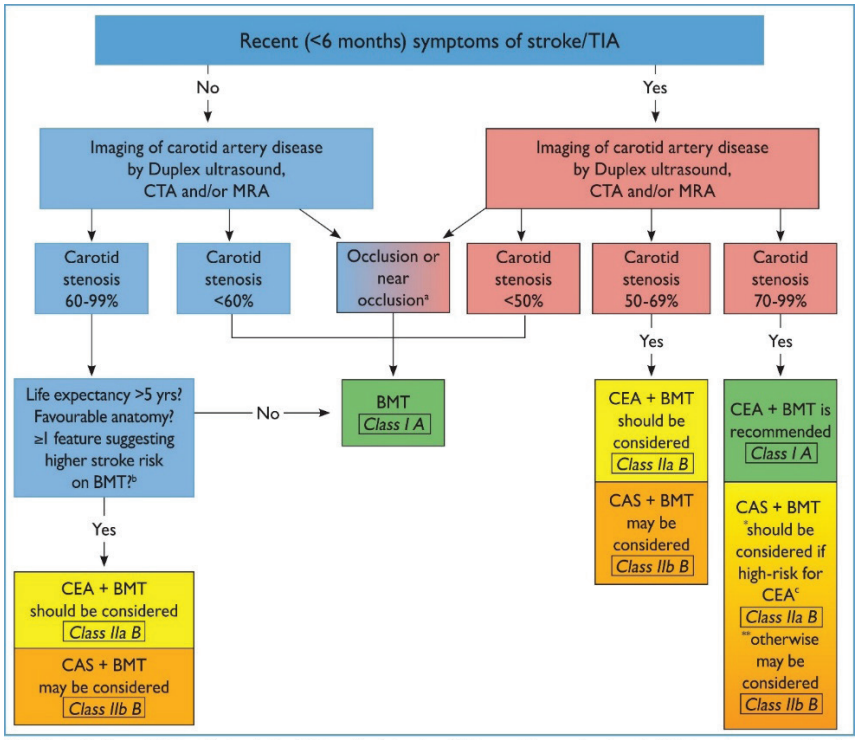
Patient Group	Degree of Stenosis	Treatment Option
Asymptomatic Patient	<50%	Medical therapy
Asymptomatic Patient	60–80%	Medical therapy ± CEA
Symptomatic Patient	50–69%	CEA (first-line option)
Symptomatic Patient	>70%	CEA (first-line option) or CAS (for high-risk patients)

The **most effective approach** in **carotid artery stenosis treatment** is a **combination of medical therapy** along with **surgical or endovascular intervention**, tailored to **patient-specific factors**.

5. Conclusion

The **management of carotid artery stenosis** should be determined based on the **patient's symptoms, degree of stenosis,** and **associated risk factors** (Figure 1). **Medical therapy** should be the **standard treatment** for all patients. **Carotid endarterectomy (CEA)** remains the **preferred surgical option** for symptomatic patients, while **carotid artery stenting (CAS)** should be considered in patients who are **not suitable for surgery** or are at **high surgical risk**.

Figure 1: Treatment Algorithm for Carotid Artery Stenosis



BMT = best medical therapy; CAS = carotid artery stenting; CEA = carotid endarterectomy; CTA = computed tomography angiography; MRA = magnetic resonance angiography; TIA = transient ischaemic attack.
[a]With post-stenotic internal carotid artery narrowed to the point of near occlusion.
[b]See Table 4.
[c]Age > 80 years, clinically significant cardiac disease, severe pulmonary disease, contralateral internal carotid artery occlusion, contralateral recurrent laryngeal nerve palsy, previous radical neck surgery or radiotherapy and recurrent stenosis after CEA.

Kaynak: *Editor's Choice – 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS), Aboyans, VictorDocument Reviewers, et al., European Journal of Vascular and Endovascular Surgery, Volume 55, Issue 3, 305 - 368*

Monitoring and Management of Treatment

The **long-term management** of patients with **carotid artery stenosis** involves both **monitoring the effectiveness** of the applied treatment and developing strategies to **prevent new cerebrovascular events**. **Monitoring and management** include **clinical follow-up, imaging techniques,** and **assessment of adherence to medical therapy**.

1. Follow-up of Patients on Medical Therapy

Patients with **asymptomatic or symptomatic carotid stenosis** who are managed solely with **medical therapy** should be **regularly evaluated**.

Their follow-up includes **clinical assessment, imaging studies,** and monitoring **compliance with medical treatment**.

For patients on **medical therapy**, an **initial routine examination** should be conducted **every six months**, followed by **annual check-ups** to monitor **blood pressure, lipid profile, diabetes control,** and **smoking cessation**. Patients who develop **TIA** or **minor stroke symptoms** should undergo **urgent evaluation**.

The **most commonly used method** for monitoring the **progression of carotid stenosis** is **carotid duplex ultrasonography**. Follow-up intervals are determined based on the **degree of stenosis**:

- **<50% stenosis: Ultrasound every 1–2 years**
- **50–70% stenosis: Ultrasound every 6–12 months**
- **>70% stenosis: Ultrasound every 3–6 months**

If **plaque progression** is suspected, **magnetic resonance angiography (MRA)** or **computed tomography angiography (CTA)** may be required.

Patients on **medical therapy** should maintain **regular antiplatelet therapy**, adhere to **lipid-lowering treatment**, and keep **LDL levels below 70 mg/dL**. **Hypertension and diabetes** should be **well controlled**, and **smoking cessation** and **lifestyle modifications** should be regularly evaluated.

2. Post-Carotid Endarterectomy Follow-up

Patients undergoing **carotid endarterectomy (CEA)** require **regular follow-up** to prevent **early and late complications**.

In the **early postoperative period (first 30 days)**, patients should undergo **neurological evaluation**. **Blood pressure must be carefully controlled**, avoiding both **hypotension and hypertension**. The **surgical site** should be **monitored** for **hematoma** or **infection**.

Due to the risk of **restenosis**, patients should undergo **regular ultrasonographic evaluation**.

- **First 6 months: Ultrasound every 6 months**
- **After 6 months: Annual ultrasound follow-ups**

The incidence of **restenosis** after **CEA** is approximately **10–15%**, particularly in patients with **uncontrolled hypertension and hyperlipidemia**.

In the **long-term follow-up**, **antiplatelet therapy** and **statin use** should be **continued**, as these treatments are **essential** for **preventing recurrent stenosis** and **reducing cerebrovascular event risk**.

3. Post-Carotid Artery Stenting Follow-up

Patients undergoing **carotid artery stenting (CAS)** require **closer follow-up** due to the risk of **restenosis** and **stent thrombosis**.

During the **first 30 days**, patients should be carefully monitored for **cerebral ischemia** and **embolic events**. **Hypertension and hypotension** should be prevented, and **blood pressure regulation** must be ensured. To **reduce perioperative stroke** and **thrombus formation**, **dual antiplatelet therapy** (**Aspirin + Clopidogrel**) should be administered for **at least 1 month**, preferably **3–6 months**.

In **long-term follow-up**, **carotid ultrasound** should be performed **every 3–6 months** during the **first year** to assess **stent patency**. After one year, patients should be followed **annually**. **Restenosis after CAS** occurs **more frequently than after surgery**, with an incidence ranging from **5–20%**. Patients developing **stent thrombosis** or **late restenosis** may require **additional interventions** such as **balloon angioplasty** or **revascularization**.

Medical therapy adherence should be **closely monitored** after **CAS**, with **regular blood pressure and lipid level assessments**. **Antiplatelet therapy should not be discontinued**, and patients should be advised to **avoid smoking**.

4. Key Complications in Treatment Follow-up and Management

Early detection and appropriate management of post-treatment complications are among the most critical factors determining **patient prognosis** (Table 5).

Table 5: Complications Associated with Carotid Artery Stenosis Treatment and Management

Complication	Incidence (%)	Recommended Management
Perioperative stroke (after CEA)	2–3%	Intensive neurological monitoring, blood pressure control
Perioperative stroke (after CAS)	4–5%	Early ultrasound assessment for stent thrombosis
Restenosis (after CEA)	10–15%	Frequent ultrasound follow-up in high-risk patients
Restenosis (after CAS)	5–20%	Repeat balloon angioplasty if necessary
Hypertension (may occur with both procedures)	20–30%	Control with ACE inhibitors or calcium channel blockers

5. Long-Term Management of Patients

The primary goal of **long-term follow-up** in patients with **carotid artery disease** is to **prevent recurrent stenosis** and **minimize the risk of cerebrovascular events**. This process should be supported by **regular clinical check-ups, risk factor management,** and **lifestyle modifications**.

To prevent **stroke and cardiovascular events**, patients should be **regularly evaluated**, and **risk factors such as blood pressure, lipid profile, and diabetes** should be continuously monitored and maintained at **optimal levels**. **Hypertension should be controlled**, **LDL levels should be maintained below 70 mg/dL**, and **HbA1c levels** should be adjusted to **target values in diabetic patients**.

Patients should **continue lifelong antiplatelet therapy**. **Aspirin or Clopidogrel** plays a **critical role in preventing thromboembolic events**. In patients who have undergone **CEA or CAS**, the duration of **dual antiplatelet therapy** should be determined **individually**.

Long-term **lipid-lowering therapy** should be ensured. **Statins** are essential for **atherosclerotic plaque stabilization and inflammation reduction**, making them the **first-line therapy** for achieving **target LDL levels**. In patients with **statin intolerance**, alternative agents such as **Ezetimibe or PCSK9 inhibitors** should be considered.

Smoking cessation programs and **lifestyle changes** should be encouraged. **Smoking is a major risk factor** for the **progression of atherosclerosis**, and **patients should receive support to quit smoking**.

Regular **physical activity** and **healthy eating habits** are fundamental to **improving cardiovascular health**. Patients should engage in at least

150 minutes of moderate exercise per week. In terms of nutrition, **Mediterranean diet**, known for its **anti-inflammatory properties**, should be recommended. Personalized **exercise and dietary counseling** should be provided to help slow disease progression.

6. Conclusion

Long-term follow-up of patients treated for **carotid artery disease** is **crucial** for preventing **recurrent stenosis, stroke,** and **cardiovascular events**. Patients on **medical therapy** should be monitored with **regular ultrasound** and **risk factor management**.

Patients who have undergone **carotid endarterectomy (CEA)** should undergo **annual ultrasound screening** to detect restenosis and should continue **antiplatelet therapy**. In patients who have undergone **carotid artery stenting (CAS)**, the risk of **restenosis is higher**, and **more frequent follow-up** is recommended within the **first year**.

Long-term **treatment success** depends on **multidisciplinary follow-up** and **patient adherence to therapy**.

General Conclusion

Carotid artery stenosis is a major cause of **cerebrovascular disease**, leading to severe clinical outcomes such as **transient ischemic attack (TIA)** and **ischemic stroke**. The **natural course of the disease** varies depending on the **degree of stenosis, presence of symptoms,** and **associated risk factors**. **A patient-specific, individualized approach** is essential in treatment management, with **medical therapy, surgical interventions,** and **endovascular techniques** forming the core components of this process.

The **primary etiological factor** in **carotid artery disease** is **atherosclerosis**, which is influenced by **modifiable risk factors** such as **hypertension, diabetes mellitus, hyperlipidemia, smoking,** and a **sedentary lifestyle**. Effective **management of these factors** is critical in **slowing disease progression** and **reducing stroke risk**.

The **diagnosis** of **carotid artery stenosis** is based on a combination of **clinical evaluation** and **imaging modalities**. **Carotid duplex ultrasonography** is the **first-line diagnostic tool**, while more advanced cases may require **magnetic resonance angiography (MRA), computed tomography angiography (CTA),** or **digital subtraction angiography (DSA)**.

There are **three main approaches** to the **treatment of carotid artery stenosis**. **Medical therapy** is the **first-line treatment** for all patients and

includes **antiplatelet therapy, statins, antihypertensive medications,** and **diabetes management**. Additionally, **lifestyle modifications, smoking cessation, dietary adjustments,** and **regular exercise** should be incorporated.

Carotid endarterectomy (CEA) is the **preferred surgical option** for **symptomatic patients with >50% stenosis**. It may also be considered in **selected asymptomatic high-risk patients** with **60–80% stenosis**. **CEA** has **high long-term patency rates** and a **low recurrence rate of restenosis.**

Carotid artery stenting (CAS) is recommended for **patients at high surgical risk** or those with **anatomical constraints unsuitable for surgery**. During the **stenting procedure**, the use of **embolic protection devices** is essential. However, **CAS has a higher long-term restenosis risk** compared to **CEA**.

The **success of treatment** is directly related to **effective long-term follow-up**. Patients who undergo **CEA** or **CAS** should be monitored with **regular ultrasound examinations** to **detect restenosis at an early stage**. **High-risk patients** require **lifelong antiplatelet therapy** and **aggressive risk factor management**.

Individualized management of **carotid artery stenosis** is crucial for **stroke prevention**. Advances in **both surgical and endovascular treatment options** have significantly **reduced stroke risk** in affected patients. However, the **optimal treatment strategy** should be determined based on **each patient's clinical and anatomical characteristics**. **Long-term follow-up** remains **one of the most important factors in stroke prevention**, and strategies should be developed to **enhance patient adherence** to treatment.

Key Points

1. What Did We Know?

- Carotid artery stenosis develops as a result of atherosclerosis and is one of the leading causes of ischemic stroke.

- Hypertension, hyperlipidemia, smoking, and diabetes mellitus are among the most significant modifiable risk factors for the disease.

- Symptomatic carotid artery stenosis, particularly with ≥50% narrowing, is a critical factor that increases stroke risk.

- In asymptomatic patients, treatment decisions should be made based on an individualized stroke risk assessment.

- Carotid duplex ultrasonography (DUS) is the first-line diagnostic tool with high sensitivity in determining the degree of stenosis.

- Carotid endarterectomy (CEA) is considered the gold standard surgical treatment for ≥50% symptomatic stenosis.

- Carotid artery stenting (CAS) is an alternative treatment for high-risk surgical patients or those anatomically unsuitable for surgery.

- Medical therapy (antiplatelet agents, statins, antihypertensives, and diabetes control) is mandatory for all patients and plays a critical role in preventing disease progression.

- Treatment success is directly linked to regular ultrasound monitoring and long-term risk factor management.

2. What Is New?

- Personalized treatment approaches have become more prominent in carotid artery disease. The choice between surgery and endovascular therapy is now based on individual risk factors.

- Recent studies suggest that aggressive medical therapy in asymptomatic patients results in low stroke risk, leading to questions about the necessity of surgery, particularly in patients with 60–80% asymptomatic stenosis.

- The use of embolic protection devices (EPCs) in CAS procedures has increased, and safer stenting protocols have been developed. New-generation stents and distal filtration techniques have improved the safety and efficacy of CAS.

- Recent meta-analyses comparing CEA and CAS indicate that CEA has a lower long-term restenosis rate, whereas CAS may be associated with fewer perioperative complications in select patient groups.

- New biomarkers and genetic studies are being used for early diagnosis and risk assessment in carotid artery disease. High-sensitivity CRP (hs-CRP) and inflammatory markers are being explored for their role in predicting disease progression.

- Artificial intelligence-assisted imaging techniques have led to significant advancements in the detection of carotid artery stenosis and

stroke risk prediction. Automated plaque analysis software now allows for a better assessment of plaque stability and thromboembolic risk.

- Medical therapy strategies have set more stringent LDL targets. Recent guidelines recommend lowering LDL levels to <55 mg/dL in high-risk patients with carotid stenosis.

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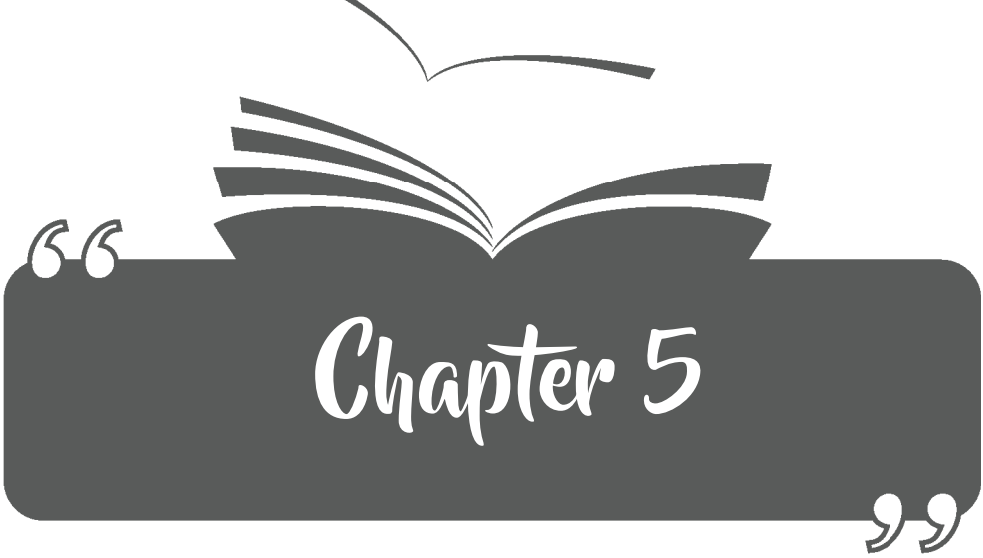

NATURAL DISASTER AND WOMEN'S HEALTH

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Introduction

Natural disaster without direct humanitarian response and long periods of epidemics, drought and famine. as well as volcanoes, earthquakes,negative environmental impacts that threaten human life, such as events (Habtezion, 2016)

Globally, women have been identified as the population most in need of assistance in times of disasters, and women and children are many times more likely to die compared to men (Habtezion, 2016). On February 6, 2023 in Turkey earthquakes are recognized to cause damage all over the country that is difficult to repair for a long time. Disaster-induced in order to heal the damage, since the day of the earthquake, both the country and foreign aid teams, health professionals and people from all sectors of society are working as one to heal the scars caused by this devastating earthquake (World Health Organization, 2013).

Post Traumatic Stress Disorder in Disasters

Disasters affect a wide range of mental and physical health traumatic events. Posttraumatic stress disorder, probably the most researched post-disaster psychiatric disorder (Neria et al., 2008). Post-traumatic stress disorder is when a person threatens psychological and/or physical integrity possible exposure to a traumatic event that recognized as a psychopathological consequence (Yehuda et al., 2015).

People with post-traumatic stress disorder, traumatic memories, feelings and emotions of an event through sensory flashbacks or nightmares may experience repeatedly or avoidance, irritability, hypervigilance, difficulty sleeping, weakness concentration or emotional withdrawal experience (Neumayer et al., 2007). Posttraumatic stress disorder is more with high suicide risk and suicidal ideation and post-traumatic persistent headaches such as other adverse clinical outcomes (Mckinney et al., 2017)

Reproductive Health and Importance in Disasters

In other words, a situation that is beyond the control of individuals by expression not directly realized by human beings, physical for the individual/society as a whole or for a particular group, economic and social losses, and the stops or interrupts the flow of the affected individual/ the negative effects that society has insufficient capacity to cope with natural/environmental events are defined as disasters (Ilgın ve Karagöl, 2022; Fatema et.al., 2019). All individuals are at risk from the effects of a disaster. They are under. But children, women, pregnant women, puerperas

and the elderly because they are vulnerable and more exposed to a great risk because they need care (Maher , 2019).

Losses in the aftermath of disasters, and the impact of losses. The depth and persistence of health problems are striking. More affected by natural disasters than men. Women are also vulnerable to the impacts of natural disasters. Therefore, women's injury and even death rates is higher compared to men (Ilgın ve Karagöl, 2022; Fatema et.al., 2019).

- Maternal and newborn morbidity and mortality;

- Risk of premature birth

- Fetal growth and development restriction, low birth weight newborn

- Inadequate prenatal (pregnancy) care and follow-up

- Breastfeeding problems

- Risk of spontaneous abortion, safety of unwanted pregnancies termination of pregnancy (miscarriage)

- Infections

- Unintended pregnancies as a result of sexual violence

- In contracting sexually transmitted infections (STIs) increase

- Increase in HIV transmission

- The psyche, including sequelae of trauma and depression health problems arise (Özmen and Sayın, 2021; Bilge and Hotun Şahin, 2018; Sendai Framework for Disaster Risk Reduction, 2015).

Society as well as mental and physical abilities fundamental rights to health and well-being that also focus on development are often violated against women in disasters. Whereas women's health is not only about the well-being of the family, but also It also closely affects the whole society (Sohrabizadeh et.al., 2016). The earthquake process itself is the most important and the aftershocks that may occur will be a source of stress. destruction, poor sanitation, lack of access to health services and not receiving the necessary care, resulting in inappropriate increased health behaviors, injuries and shelter The presence of problems is a cause of intense psychosocial stress. Early mental problems are characterized by shock, anxiety and sleep disturbances, and evolve into mood disorders in the ongoing process (Fatema et.al., 2019; Ren etl., 2014; Zhang and Ho, 2011).

In a study, postpartum after an earthquake puerperium during this period, the puerperium also has a similar psychological and physical problems. But in addition malnutrition, need for support in infant care baby, such as hearing, breastfeeding and changing diapers lack of a suitable environment for the care of infants and have additional problems, such as worrying about their other children (Suzuki et.al. 2022). In addition, early postpatum maternal and for the baby. Maternal mortality 60% of deaths after childbirth and 60% of deaths in the puerperium 50% occur within the first 24 hours. Similar in a way, the newborn period is also an important part of the baby's life is a critical period. Because two-thirds of infant deaths 4 weeks after birth and 60% of newborn deaths occurs 7 days after birth. Living the physical and emotional burden of the disaster, labor and delivery burden of the puerperal period and the puerperal recovery and adversely affects or disrupts the process of uterine involution can cause health problems. During this period the risk of bleeding and infection for the mother can be fatal (Yusefni et.al., 2022).

Women's Reproductıve Health After The Earthquake

Menstrual Period: Menstruation is a physiological process that begins in adolescence and ends with menopause. Symptoms during menstruation are good When unmanaged, it causes gynecological problems, urinary infections, anemia and psychosocial problems. Fear of bad odor, embarrassment and fear of menstrual bleeding can lead to isolation. In the general chaos after an earthquake, the focus is primarily on basic needs. Nutrition, shelter, clean water, heating or emergency health care needs are met to meet these needs among them. Women's menstrual needs not spoken about, and there are no pads and other hygienic requirements are often ignored (Budhathoki et al., 2018; Ünür, 2021).

Pregnancy: Pregnant women are more vulnerable in natural disaster situations. An earthquake can cause severe stress on pregnant women. factor and adversely affects intrauterine development. Preterm labor in pregnant women exposed to natural disasters threat, low birth weight baby, low apgar score, and perinatal factors such as smaller than normal head circumference complications can occur. After natural disasters babies are born with long-term physical and mental development, obesity and metabolic diseases increase has been reported (Palmeiro-Silva et al., 2018)

Birth: Another problem faced by pregnant women after the earthquake the major challenge is the possibility of complications. Unsafe and unhealthy childbirths carried out in conditions where both the mother and can also lead to infections and infections that can threaten the baby's

life the risk of developing other complications increases. Many pregnant women do not have access to trained health giving birth at home without the help of staff to do it at home without a specialist. At home without an expert births have life-threatening consequences for mother and baby (Amarpoor Mesrkanlou et al. 2023; Ahmed et al., 2023).

Breastfeeding: Breast milk is the most reliable food for the baby in times of disaster source. Breastfeeding is particularly encouraged in this process and should be supported. With formula food infections and diarrhea are more common in formula-fed babies because they do not benefit from the protective Deprem and Women's Reproductive Health antibodies in breast milk. In addition, formula can be contaminated if clean water, bottles, kettles, etc. are not available to prepare the formula. At the same time, it can be difficult to obtain a continuous supply of formula. As a result, malnutrition in formula-fed infants may be associated with problems and dehydration may develop (United States Breastfeeding Committee (USBC), 2018; American Academy of Pediatrics, 2020).

Family planning: One's choice and use of contraceptive methods general health, age, frequency of sexual intercourse, number of partners and future childbearing varies depending on the request (WHO, 2023). In a study conducted after the Haiti earthquake, modern decreased use of contraception, unintended pregnancies and unmet family planning services have reportedly increased (Behrman and Weitzman, 2016). In another study, earthquake is generally considered to be a sign of contraceptive methods does not change the use of the service and reported decreased access to methods (Strid et al., 2022).

Urogenital Infections: Symptoms of urogenital infection in women and lack of knowledge on the subject the occurrence of these infections after disasters and facilitates its spread. Natural phenomena such as earthquakes women's urogenital health after disasters infections and sexually transmitted infections (STI) risks, symptoms, early treatment and protection from infections in existing conditions about the issues (Rajabi et al. al., 2022).

Intervention in Emergencies and Disasters and Nurses

Disaster Management; in the event of a disaster emergency, rapid and appropriate decisions need to be taken, the most important thing to remember issue is the ongoing/continuity of disaster management, is a complex process (Yusefni et.al., 2022; Thobaity et.al., 2016). An effective disaster management does not happen spontaneously/automatically, mostly to assess uncertainty in the natural world and to manage people, strategy, processes, technology and is a structured, disciplined approach

that aligns knowledge. The aim of the disaster management process is to minimize risks and maximizing the benefit of the preparations made (Thobaity et.al., 2016).

Naturally, disasters are short and human health, while causing long-term health problems and is a major threat to public health poses a threat. Health interventions are therefore the most valid should be planned based on research evidence. However to people during a disaster, especially health services there is little research on how to present it (Kohan et.al., 2016).

Disaster Nursing

Nurses respond to life-threatening disasters during disaster response to eliminate conditions that affect and impair health or with evidence-based practices in order to minimize other approaches to health management, helping and caring in collaboration with professionals (45). Health team is strong in numbers and has close ties with the community nurses have important roles at every stage of disasters (Al-Maaitah et.al., 2016; Taşkıran and Baykal, 2017; Tel, 2016).

Disaster nursing, "nursing knowledge and skills in disasters the systematic use of the disaster and the health impacts of the disaster reduce harm and eliminate life-threatening hazards the development of applications designed to remove the " (Taşkıran and Baykal, 2017).

Nurses employed in different health care fields, with a wide range of public health or health professionals on a daily basis collaborates and significantly improves population health outcomes to improve and develop a community-wide community response to disasters. health system leaders, individuals to build resilience and are well positioned to partner with families and important human resources in the fight against unpredictable disasters resources. Therefore, they should be prepared for disasters in terms of socio-cultural and economic development of societies is very important (Taşkıran and Baykal, 2017; Veenema et.al., 2016). ICN (International Council of Nurses (International Council of Nurses) clinician, educator, every nurse, whether a researcher or manager, is a disaster. to plan and implement its care and preparation. emphasizes the need to have appropriate skills (Taşkıran and Baykal, 2017; Al-Maaitah et.al., 2016 ; Stewart , 2012)

Also, to improve nurses' disaster protocols, pre-disaster planning, post-disaster assessment, safety in decision-making during an emergency/disaster explanation and infection control practices participation is also emphasized. In addition to emergencies and ways to intervene, re-

porting of situation events, rapid health assessment of physical and mental victims, applying basic first aid, isolating the patient the need for identification and isolation of the patient placement are also core disaster competencies for nurses (Songwathana P, Timalsina, 2021; Moradi et.al., 2020).

Conclussion

Nurses should take an active role in the disaster management process. Thus, nurses are able to improve health and prevent health threats. The elimination of hazards and the improvement of health protection processes in an effective manner and they would make an important contribution. As a result, women's post-disaster health social by professionals, economically, physically and emotionally should be supported. In the light of this information, midwives, obstetric nurses and psychiatrists health nurses are aware of the impact of disasters on women's mental negative effects on health for training and consultancy they must.

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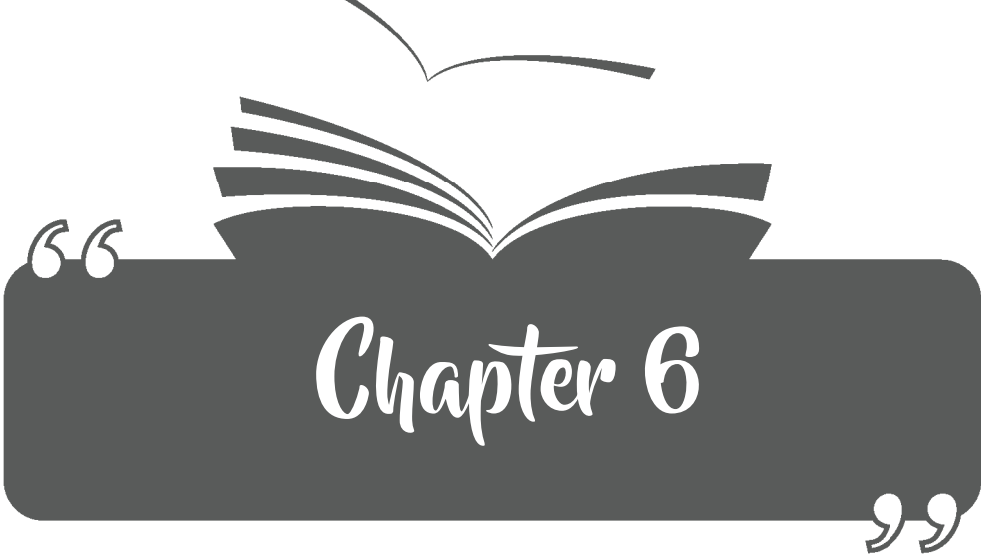

ALZHEIMER'S DISEASE: PATHOGENESIS, STEM CELL MODELS, AND MICROENVIRONMENT

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1. Introduction

Dementia is a neurological disorder characterized by a progressive decline in cognitive and behavioral functions, including thinking, memory, and reasoning, to a degree that significantly disrupts an individual's daily activities and quality of life. The condition manifests across a spectrum of severity, ranging from mild impairment, where daily functioning begins to be affected, to advanced stages in which individuals become entirely reliant on caregivers for fundamental tasks, ultimately leading to mortality. Although dementia predominantly occurs in older adults, it is not an unavoidable consequence of aging. Among its various forms, Alzheimer's disease (AD) is the most prevalent, accounting for approximately 60–70% of all cases.

The pathogenesis of Alzheimer's disease is complex and involves multiple mechanisms, including the accumulation of amyloid-beta plaques, synaptic dysfunction, hyperphosphorylation of tau protein, oxidative stress, and inflammatory responses. In recent years, the critical role of the extracellular matrix (ECM) in the neurodegenerative process has gained increasing attention. The ECM plays a fundamental role in maintaining the structural and functional stability of the nervous system by regulating various neurophysiological processes, ranging from inter-neuronal signal transmission to synaptic plasticity. Alterations in ECM components in Alzheimer's disease are considered a significant factor contributing to disease progression.

This section will discuss the molecular mechanisms and pathogenesis of Alzheimer's disease, examine its relationship with the ECM in detail, and explore current approaches to experimental models of the disease. A deeper understanding of Alzheimer's disease will facilitate the development of novel therapeutic strategies, representing a critical step in managing the disease and slowing its progression.

2. Pathogenesis of Alzheimer's Disease

Alzheimer's disease (AD) was initially identified in 1907 by the German neuropsychiatrist Alois Alzheimer, who documented a case characterized by progressive memory impairment, language deficits, and behavioral disturbances (Alzheimer et al., 1995). The postmortem evaluation of this subject indicated the presence of numerous abnormal accumulations, now recognized as amyloid plaques, in addition to disorganized fiber bundles (neurofibrillary tangles and tau fibrils). These tangles and plaques in the brain remain the primary diagnostic criteria for Alzheimer's disease today.

In most Alzheimer's patients, symptoms first appear in the mid-60s. Short-term memory loss and forgetfulness are hallmark symptoms of the disease. As the disease progresses, cognitive impairments such as speech difficulties, attention deficits, problems with executive functions (e.g., planning and decision-making), apraxia, and recognition difficulties emerge. Additionally, psychiatric symptoms such as depression and hallucinations, as well as behavioral changes like aggression, may develop during disease progression.

Age is the most significant risk factor influencing the prevalence of Alzheimer's disease. If the disease manifests before the age of 65, it is classified as early-onset familial Alzheimer's disease (EOFAD), which is typically inherited in an autosomal dominant manner. When the disease develops after the age of 65, it is referred to as late-onset Alzheimer's disease (LOAD) or senile Alzheimer's disease (Bekris et al., 2010). However, sporadic LOAD generally exhibits a complex inheritance pattern involving multiple genes with small contributions, in conjunction with environmental factors.

Regardless of whether Alzheimer's disease is early or late onset, or whether it arises from genetic or environmental factors, its fundamental pathological hallmarks include the extracellular accumulation of abnormal amyloid-beta (Aβ) plaques and the intracellular hyperphosphorylation of tau proteins, leading to the formation of neurofibrillary tangles. These pathologies contribute to synaptic disconnection, neuronal death, and ultimately, brain atrophy (Tackenberg et al., 2020).

Four genetic loci have been identified as key contributors to the etiology of Alzheimer's disease (AD): the amyloid precursor protein (*APP*) gene on chromosome 21, *presenilin 1* (*PSEN1*) on chromosome 14, *presenilin 2* (*PSEN2*) on chromosome 1, and *apolipoprotein E* (*APOE*) on chromosome 19. To date, over 300 mutations have been documented in these genes. The *APP* gene encodes an integral membrane protein that is widely expressed in various tissues, with a notable concentration at neuronal synapses. Alternative splicing of *APP* results in at least ten different isoforms. The proteolytic processing of *APP* generates amyloid-beta (*Aβ*) peptides ranging from 36 to 43 amino acids in length, with *Aβ40* and *Aβ42* being particularly implicated in AD pathology (Bekris et al., 2010). Among the most well-characterized mutations in the *APP* gene are KM670/671NL ("Swedish APP") and V717I ("London APP").

The proteolytic cleavage of *APP* is mediated by secretases, a group of enzymes responsible for degrading membrane-bound proteins. *PSEN1* and *PSEN2* encode the catalytic subunits of γ-secretase,

a key enzyme involved in *APP*processing. In early-onset familial Alzheimer's disease (EOFAD), *PSEN1* mutations are the most frequently observed. The cleavage pathway of *APP* determines whether neurotoxic peptides are generated. When *APP* is initially cleaved by α-secretase followed by γ-secretase, a non-toxic P3 fragment is produced. However, when β-secretase initiates cleavage, followed by γ-secretase processing, neurotoxic *Aβ* peptides are formed, which accumulate extracellularly to form amyloid plaques. Mutations in *PSEN1* and *PSEN2* have been shown to increase the *Aβ42/Aβ40* ratio, a key factor contributing to AD pathogenesis (Kwak et al., 2020). Notably, *Aβ42* is a highly hydrophobic peptide with a strong propensity to aggregate into fibrillar structures, further promoting plaque formation and neurodegeneration.

Another key pathological feature of Alzheimer's disease is the accumulation of neurofibrillary tangles. Tau, a microtubule-associated protein, is synthesized through alternative mRNA splicing and plays a crucial role in stabilizing neuronal microtubules under normal physiological conditions. Tau was first discovered approximately 35 years ago as a heat-stable protein involved in microtubule assembly (Weingarten et al., 1975). It gained significant attention when it was identified as the main component of paired helical filaments, which form the neurofibrillary tangles observed in the brains of Alzheimer's patients (Grundke-Iqbal et al., 1986). In these structures, tau undergoes abnormal hyperphosphorylation, leading to the formation of neurofibrillary tangles (Grundke-Iqbal et al., 1986). Furthermore, hippocampal atrophy, one of the earliest and most affected regions in Alzheimer's disease, has been linked to the presence of hyperphosphorylated tau protein.

A histopathological feature observed in Alzheimer's disease is the presence of Hirano bodies, which are bright eosinophilic rod-shaped structures. These inclusions are detected in the hippocampus during the later stages of the disease and are thought to be associated with RNA accumulation (Mitake et al., 1997).

3. Experimental Stem Cell Models in Alzheimer's Disease

One of the primary challenges in developing experimental models for Alzheimer's disease (AD) using human tissue is the limited availability of high-quality postmortem samples. Additionally, the scarcity of primary human neural stem cells and neurons presents a significant obstacle for researchers in the field. The advent of induced pluripotent stem cells (iPSCs) has partially addressed these limitations (Takahashi et al., 2007). Currently, iPSCs can be derived from various human donor cell types, enabling the development of patient-specific cell models. iPSC lines gen-

erated from individuals with Alzheimer's disease have been extensively characterized, revealing pathological hallmarks such as increased amyloid-beta (*Aβ*) production, particularly *Aβ42*, and tau hyperphosphorylation (Yagi et al., 2011). Some of these iPSC-derived models also exhibit additional AD-related abnormalities, including elevated GSK3β activation, an increased number of large endosomes (Israel et al., 2012), and intracellular accumulation of *Aβ* oligomers (Kondo et al., 2013).

Despite their advantages, iPSC-based experimental models of Alzheimer's disease present several challenges, akin to other in vitro models. Notable limitations include the lack of standardized protocols for generating and maintaining these cell lines, the potential persistence of pre-existing epigenetic modifications from donor cells following reprogramming, and phenotypic variability among different iPSC lines (Drummond et al., 2017). Another significant challenge is the need to induce an aging phenotype in differentiated neurons to recapitulate AD pathology, which remains technically demanding.

Among the various cell types utilized in Alzheimer's research, neural progenitor stem cells (NPSCs) represent a valuable resource. Since their initial identification, neural stem cells have garnered considerable interest due to their therapeutic potential in neurodegenerative disorders. During embryonic development, these cells differentiate into radial glial progenitors, which give rise to both neurons and glial cells in the nervous system. While most neural progenitor stem cells are depleted after development, a subset persists in specific regions of the adult vertebrate brain, contributing to neurogenesis throughout life. In mammals, neural stem cells have been identified in distinct brain regions, including the subgranular zone of the dentate gyrus in the hippocampus, the subventricular zone surrounding the lateral ventricles, and the dorsal α1 and α2 regions of the hypothalamus, along with the adjacent median eminence (Andreotti et al., 2019).

Due to difficulties in sourcing primary human neural progenitor cells, researchers frequently use established cell lines. One of the most widely utilized human neural progenitor cell lines is ReNcell VM (Sordini et al., 2021; Choi et al., 2014). ReNcell VM is derived from the ventral mesencephalon region of the human fetal brain. Karyotypic analyses have demonstrated that ReNcell VM maintains a normal diploid karyotype even after prolonged passage (>45 passages) in culture (Kim et al., 2015). ReNeuron Group, the developer of ReNcell VM, reports that these cells can be efficiently differentiated into dopaminergic neurons in vitro and that neurons differentiated from ReNcell VM exhibit electrophysiological activity. ReNcell has been widely used in research on neurotoxicity, neu-

rogenesis, electrophysiology, neurotransmitter and receptor functions, and neurodegenerative disorders such as Alzheimer's disease (Song et al., 2019).

Alzheimer's disease remains a major scientific challenge due to its complex pathology, which is not yet fully understood. To address these gaps, researchers have developed a variety of disease models. Traditional cell culture models provide only a limited representation of the in vivo environment. To better mimic cell-cell and cell-matrix interactions that normally occur within the extracellular matrix (ECM), three-dimensional (3D) Alzheimer's models have been developed. These models are based on tissue scaffolds composed of hydrogels or Matrigel, which allow for enhanced physiological interactions between neurons and glial cells, or scaffold-free environments that facilitate the development of three-dimensional organoids (Choi et al., 2014; Raja et al., 2016).

A study published in *Nature* in 2015 marked a significant milestone in the development of *in vitro* Alzheimer's disease models, addressing several challenges present in previous models. In this study, researchers created a 3D environment using Matrigel and introduced genetic modifications associated with Alzheimer's disease into neural stem cells via vector-mediated gene transfer, followed by neuronal differentiation. Over time, extracellular accumulation of amyloid-beta plaques, intracellular tau accumulation and phosphorylation, and the formation of neurofibrillary tangles were observed (Choi et al., 2014). This model has since been widely adopted by researchers studying Alzheimer's disease mechanisms and testing potential drug candidates, receiving nearly 1,000 citations.

Scientists emphasize the growing need for research on Alzheimer's disease, as it is influenced not only by genetic factors but also by environmental and epigenetic mechanisms (Drummond et al., 2017). Researchers also highlight the necessity of employing diverse approaches to better delineate the causal relationships, differences, and similarities among various forms of the disease. They stress the importance of leveraging existing knowledge and techniques to generate novel insights that could ultimately unravel the intricate processes underlying Alzheimer's disease (Zetterberg et al., 2014; Riemens et al., 2020).

4. Microenvironment in Alzheimer's Disease

To date, approximately 250,000 studies related to Alzheimer's disease have been indexed in PubMed, with 60% of them conducted in the last decade. While these studies encompass a wide range of approaches, definitions, and experimental models, the number of studies examining the extracellular matrix (ECM) structure in this aging-related disease re-

mains remarkably low. Nonetheless, the existing studies provide valuable insights.

The first data on the relationship between ECM status and Alzheimer's disease emerged in 1990, revealing that collagen IV, laminin, and fibronectin in brain tissue were localized with amyloid plaques in the brains of Alzheimer's patients (Howard et al., 1990). In 2017, increased expression of ECM proteins such as collagen IV and fibronectin was detected in the brains of individuals with Alzheimer's disease (Lepelletier et al., 2017). Some studies suggest that proteoglycans possess neuroprotective properties in Alzheimer's pathogenesis (Suttkus et al., 2016), whereas others highlight that proteoglycans support amyloid beta and tau fibrillization and protect beta-amyloids from proteolytic degradation (Van Horssen et al., 2003; Karamanos et al., 2018).

A recent proteomic study conducted on a murine AD model demonstrated significant increases in levels of various ECM protein components, such as hyaluronan, tenascin, and neurocan, parallel to disease pathology (Sethi et al., 2017). These and similar findings indicate structural changes in the ECM associated with Alzheimer's disease. Brain ECM components have been reported to influence amyloid β degradation mechanisms due to age-related alterations (Bondareff, 2013). The results of several studies reinforce the notion that ECM proteins are closely associated with Alzheimer's pathology (Reed et al., 2019). In a 2021 publication, Ma et al. emphasized the need for further research into ECM functions in Alzheimer's disease (Ma et al., 2020). As is well known, organ aging occurs not only at the cellular level but also in the ECM, which provides a niche for cells, regulates their interactions, and plays active roles in division, secretion, cellular migration, and numerous physiological processes. ECM composition also undergoes significant changes with aging.

Two important studies published in 2021 demonstrated that ECM aging leads to severe functional losses and disorganization in cells (Ozcebe et al., 2021; Acun et al., 2021). In their myocardial infarction model, Ozcebe and colleagues reported that as ECM age increased, the cellular response to ischemia weakened, cells became unresponsive to therapeutic drugs, and the aging phenotype accelerated. Conversely, when ECM age was rejuvenated, cells re-entered the proliferation phase, protective effects against stress were observed in aged cells, and young ECM cells responded to infarction-related drugs (Acun et al., 2021). A similar study conducted at Harvard showed that increased ECM age in the liver led to impaired primary hepatocyte function and reduced growth factors (Acun et al., 2021). These findings suggest that ECM aging may be a fundamental

factor underlying systemic dysfunctions in multiple organs, particularly in age-related conditions such as Alzheimer's disease.

4.1. Decellularized Organ Matrices in Alzheimer's Disease

Tissues and organs generally consist of two components: cells and structural scaffolds composed of various proteins. These structural scaffolds, which host cells, are known as the extracellular matrix (ECM). The process of isolating these two components by removing cells while preserving the ECM is called decellularization (Uygun et al., 2010). With advancements in technology, complete organ decellularization has become feasible, allowing for the full extraction of an organ's ECM (Scarritt et al., 2015).

Three key criteria have been established for successful decellularization (Crapo et al., 2011): (I) the presence of less than 50 ng of double-stranded DNA per milligram of dry extracellular matrix (ECM) weight, (II) DNA fragment lengths shorter than 200 base pairs, and (III) the absence of detectable nuclear staining in 4',6-diamidino-2-phenylindole (DAPI) or hematoxylin-eosin (HE) staining. ECM plays a crucial role in regulating various cellular processes, including mitogenesis, chemotaxis, and differentiation.

Before these advancements, three-dimensional (3D) cell culture studies primarily relied on synthetic biomaterials such as polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA), and chitosan. However, these materials require additional modifications to incorporate integrin-binding peptide motifs, such as arginine-glycine-aspartic acid (RGD) and tyrosine-isoleucine-glycine-serine-arginine (YIGSR), to enhance cell adhesion and signaling (Boateng et al., 2005). Furthermore, as synthetic polymers, they lack natural biological relevance to human tissue.

Matrigel, a non-synthetic alternative, has been widely utilized in cell culture as a basement membrane matrix due to its composition resembling ECM. However, it is important to note that Matrigel is a gelatinous protein mixture derived from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells, which may pose limitations for certain biomedical applications.

Following DeQuach et al.'s 2011 study on porcine brain decellularization (DeQuach et al., 2011), protocols for brain decellularization have been developed (Baiguera et al., 2014), leading to the widespread use of decellularized brain matrices in various research studies. These matrices have been employed to create 3D neuronal cultures and brain organoids (Simsa et al., 2021). Unlike products derived from sarcoma cells or syn-

thetic sources, decellularized brain matrices are naturally formed structures developed by bodily cells. Multiple studies have highlighted that brain ECM significantly contributes to the functional maturation, differentiation, and long-term viability of human neural stem cells, making it a crucial resource for in vitro brain model development (Sood et al., 2019; Simsa et al., 2021). Additionally, brain ECM hydrogels are frequently proposed as superior alternatives for advanced brain organoid studies due to their natural composition (Simsa et al., 2021).

Medberry et al. conducted a study comparing hydrogel forms derived from brain and spinal cord ECM with a non-central nervous system ECM hydrogel (urinary bladder matrix). Their findings demonstrated that brain ECM promotes neurite elongation and axonal repair (Medberry et al., 2012).

To emphasize, decellularized matrices are the most familiar structures to cells. Recent studies have clearly demonstrated that ECM undergoes significant alterations during aging (Ozcebe et al., 2021; Acun et al., 2021). Given that Alzheimer's disease is predominantly age-related and its etiology remains largely unknown, researchers should shift their focus toward ECM modifications. We believe that Alzheimer's studies utilizing decellularized brain ECM obtained from both young and aged individuals would provide the most accurate in vivo-like representation among current in vitro experimental models. Existing in vitro Alzheimer's models have largely been either two-dimensional (2D) (Kondo et al., 2013) or reliant on biomaterials like Matrigel (Choi et al., 2014), which is derived from mouse sarcoma cells and not naturally present in the human body. Furthermore, Matrigel is a non-specific hydrogel derived from murine tumors and does not capture the complexity of the brain's protein environment (Simsa et al., 2021).

In summary, decellularized brain ECM represents a highly valuable resource for modeling neurodegenerative diseases such as Alzheimer's. The integration of this natural microenvironmental component into Alzheimer's research is crucial for developing more accurate disease models.

5. Conclusion

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by complex pathogenesis and multifaceted molecular mechanisms. The disease is primarily associated with the accumulation of amyloid-beta (Aβ) plaques, hyperphosphorylation of tau protein leading to neurofibrillary tangles, synaptic dysfunction, oxidative stress, and neuroinflammatory responses. These pathological features contribute to neuronal loss and cognitive decline, ultimately resulting in severe impair-

ments in memory, reasoning, and daily functioning. Despite decades of research, the exact mechanisms underlying Alzheimer's disease remain incompletely understood, and no curative treatment has been developed.

In recent years, increasing attention has been directed toward the role of the extracellular matrix (ECM) in the pathogenesis of Alzheimer's disease. The ECM provides structural and biochemical support to neural cells and plays a critical role in regulating cellular communication, migration, and homeostasis. Alterations in ECM composition and function have been implicated in neurodegenerative processes, including those observed in Alzheimer's disease. Specifically, changes in ECM components have been shown to influence the aggregation of amyloid-beta and the hyperphosphorylation of tau, two hallmark pathological events in AD progression. Additionally, ECM remodeling has been linked to impaired neuroplasticity and synaptic dysfunction, further exacerbating disease pathology. However, despite these findings, the precise mechanisms by which ECM modifications contribute to Alzheimer's disease remain largely unexplored, necessitating further investigation (Ma et al., 2020).

Experimental models have provided valuable insights into Alzheimer's disease pathology and have facilitated the development of potential therapeutic strategies. Induced pluripotent stem cell (iPSC) and neural progenitor cell models allow for the study of molecular and genetic factors contributing to AD, while three-dimensional (3D) models enable a more physiologically relevant exploration of cell-ECM interactions. These advanced models have helped to bridge the gap between in vitro and in vivo research, offering a more comprehensive understanding of disease mechanisms. Additionally, the utilization of decellularized brain ECM has emerged as a promising approach for studying Alzheimer's disease in a microenvironment that closely mimics in vivo conditions. Such models can provide crucial insights into how ECM alterations affect disease progression and may lead to the identification of novel therapeutic targets.

Given the complexity of Alzheimer's disease, a multidisciplinary approach is essential for advancing our understanding and developing effective interventions. Research integrating genetics, epigenetics, bioinformatics, and neuroimmunology has the potential to reveal novel molecular pathways involved in AD pathology. Furthermore, environmental factors, including lifestyle, diet, and exposure to toxins, may significantly influence disease onset and progression. Therefore, comprehensive studies that combine cellular, molecular, and systemic approaches will be crucial in uncovering the intricate interactions between genetic predisposition and environmental influences in Alzheimer's disease.

In light of these considerations, future research should prioritize investigating the role of the ECM in Alzheimer's disease, particularly in relation to age-related changes in brain microenvironments. Understanding how ECM remodeling affects neuronal health and function may provide new avenues for therapeutic development. Strategies targeting ECM components, such as matrix metalloproteinases (MMPs), proteoglycans, and integrins, could offer innovative treatment options aimed at restoring a healthy neural microenvironment and mitigating neurodegeneration.

In conclusion, Alzheimer's disease remains a major public health challenge with significant societal and economic burdens. While considerable progress has been made in identifying key pathological features of the disease, many aspects of its etiology and progression remain elusive. Advancing our knowledge of the interactions between neurons, glial cells, and the ECM may lead to groundbreaking discoveries that pave the way for novel therapeutic interventions. Ultimately, a deeper understanding of ECM dynamics in Alzheimer's disease could be instrumental in developing effective strategies for disease prevention, early diagnosis, and targeted treatments.

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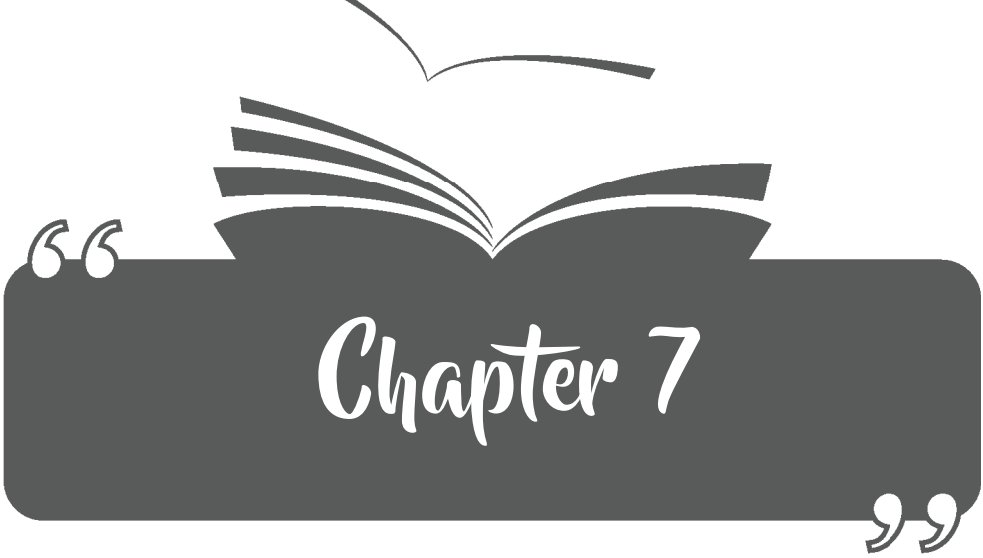

SPERM CRYOPRESERVATION IN ALL ASPECTS

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1.Introduction

Sperm cryopreservation (freezing for storage) is a technique that allows sperm cells to be frozen and stored for long periods while maintaining their vitality and functionality for the purpose of preserving male fertility (1). This study aims to provide an overview of scientific research on sperm cryopreservation and report recent advancements in the field.

History of Cryopreservation

The first scientific observations on sperm freezing began in 1776 with Spallanzani's studies examining the effects of cold temperatures on semen. Spallanzani determined that frozen sperm could retain a certain level of motility once thawed. In 1886, Montegazza became the first scientist to propose the idea of preserving human sperm by freezing. During the 1940s, glycerol was accidentally discovered to protect sperm cells from damage during the freezing process. This discovery allowed human sperm stored at -79°C on dry ice to become usable. In 1949, Polge and colleagues successfully froze and thawed human sperm using glycerol. In 1953, Bunge and Sherman published the first report of fertilization and embryo development achieved with frozen sperm. The 1960s and 1970s were notable for research into cryoprotectants (protective agents) and the effects of freezing on biological material, leading to the establishment of cryobiology principles.

In 1964, the first successful live birth was achieved through the use of human sperm samples that had been cryopreserved with glycerol and stored in liquid nitrogen. This breakthrough demonstrated the potential for preserving male fertility through sperm cryopreservation, marking a pivotal moment in the field of reproductive medicine and cryobiology. The ability to store sperm at ultra-low temperatures while maintaining its viability for future use opened new possibilities for assisted reproductive technologies (ART), paving the way for subsequent advancements in fertility treatments.

A significant milestone in the field of sperm cryopreservation came in 1972 with the establishment of the first live cell freezing bank (2,3). Authorities such as the American Society for Reproductive Medicine (ASRM) and the U.S. Food and Drug Administration (FDA) have issued various guidelines and regulations to ensure the safety and effectiveness of this process.

Technological advancements have increased the efficiency of sperm freezing and thawing methods, leading to significant improvements in reducing cellular damage and enhancing viability rates (4,5) (**Figure1.**)

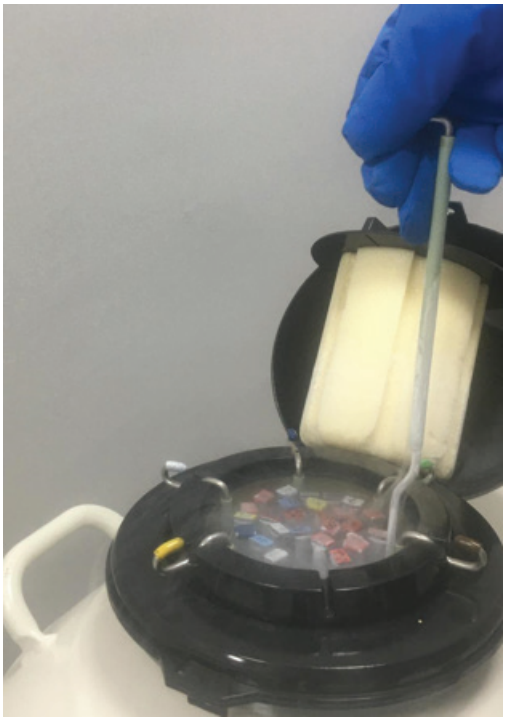

Figure 1. *Sperm cryopreservation conditions in a nitrogen tank. The figure illustrates the freezing process, including temperature stages, storage levels, and the positioning of cryovials or straws within the liquid nitrogen tank.*

2.Indications for Sperm Cryopreservation

Semen can be collected, frozen, and stored before procedures that hinder or impair fertility, as a guarantee against potential infertility in the future. For this purpose, sperm cryopreservation is recommended in the following cases:

- Male factor infertility such as azoospermia or severe oligozoospermia.
- Preservation of fertility prior to treatments like chemotherapy, radiotherapy, or surgical interventions for testicular tumors, leukemia, or lymphoma.
- Patients at risk of deteriorating sperm parameters over time.
- Individuals working in military or other high-risk professions planning to have children in the future.

- Storage of sperm samples prior to assisted reproductive techniques (ART) (6,7).

3. Principles of Cryopreservation

The five key stages of the cryopreservation procedure are as follows according to Porcu et al., (8) (**Figure 2.).**

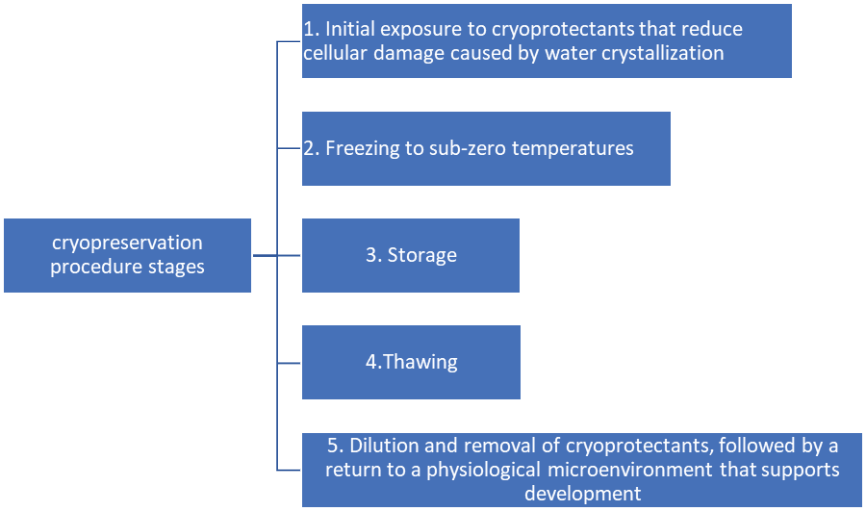

Figure 2. *Cryopreservation procedure stages*

One of the primary challenges in sperm cryopreservation is the decline in essential parameters, such as motility and viability. To ensure the success of this process, it is crucial to preserve the structural integrity and functional properties of the cells while minimizing cellular damage (9).

4. Cryoprotectants

Cryoprotective substances are used in cryopreservation and are believed to protect cells from freezing-induced damage during the process. However, they are toxic to cells. These substances possess a high H2-binding capacity.

Cryoprotectants do not always need to enter the cell to demonstrate their protective effects, as the cell membrane is where damage is most intense and rapid. Cryoprotective substances demonstrate their effectiveness in two ways:

- **By entering the cell:** Glycerol, DMSO (Dimethyl sulfoxide), and ProH (Propylene glycol) are cryoprotectants capable of penetrating the cell. They lower the formation of intracellular ice crystals to -40°C and protect the cell from the toxic effects of the solution.

- **By remaining outside the cell:** Cryoprotectants that remain outside the cell include monosaccharides (glucose, hexose), disaccharides (sucrose), and trisaccharides (raffinose). These protect the cell membrane against osmotic pressure changes and prevent excessive swelling of the cell during the thawing process (10).

In sperm freezing solutions, 5–15% glycerol is preferred as the intracellular agent, but the most effective results are obtained with ethylene glycol. Sucrose is chosen as the extracellular agent. Sucrose is a large molecule that cannot pass through the cell membrane. Thus, it initiates osmotic dehydration during the cooling process and prevents cell lysis during thawing. It also facilitates the removal of intracellular cryoprotectants from the cell through concentration gradients.

To enhance the post-thaw viability rates of frozen sperm, more complex solutions have been developed by adding auxiliary cryoprotectants like glycine, egg yolk, and citrate. The most commonly used among these is glycerol egg yolk citrate (GEYC) (11).

Spermatozoa in different stages of maturation exhibit distinct cryobiological properties and require different freezing-thawing techniques. Stage-specific cryopreservation of sperm is possible. After leaving the testis, spermatozoa undergo physical and chemical changes in the lipid and protein composition of the plasma membrane. Ejaculated sperm show higher freezing-thawing sensitivity compared to epididymal or testicular sperm (12).

5. Damage Caused by Cryopreservation in Sperm Cells

There are various opinions regarding the storage durations for frozen sperm. According to Gao (2000) and Gilmore et al. (2000), the primary cause of damage in cells preserved in liquid nitrogen at -196°C is the gradual fragmentation of DNA due to low-level radiation exposure over time. This radiation amounts to 0.1 rad/year, and the estimated time required for 63% of typical mammalian cells to die is approximately 2,000 years. However, regulations set by ethical committees and assisted reproduction centers in each country govern the storage durations of gamete cells (13,14).

The unique structure of spermatozoa and their plasma membrane, their high mitochondrial content, low cytoplasmic volume, and limited antioxidant levels make them vulnerable to damage caused by reactive oxygen species (ROS) (15).

As a result, the freezing-thawing process can lead to alterations in sperm motility, morphology, and DNA integrity. As is well known, antioxidants are fundamental defense factors against oxidative stress caused by free radicals (16). Therefore, the recently developed antioxidant-enriched environments and optimal freezing techniques aim to preserve sperm quality.

During the freezing process, cells are exposed to two primary physical stresses:

- Direct effects of low temperature.
- Structural changes caused by ice formation.

In the freezing process, the biological form of water changes—it crystallizes and transforms in structure. During freezing, the extracellular solution spontaneously or via seeding crystallizes at -5°C to -10°C. While the extracellular environment crystallizes, the intracellular environment remains unfrozen due to the influence of the cell membrane. According to a consensus, intracellular freezing is dangerous, whereas extracellular ice is harmless. The crystallization of extracellular water increases the concentration of substances in the environment. This creates a difference in the concentration of certain chemicals inside and outside the cell. This altered structure causes some fluid to leave the cell, resulting in an increased concentration of dissolved substances and intracellular crystallization. This effect occurs during both the freezing and thawing processes and is influenced by the cooling rate, potentially to varying degrees (17).

6. Freezing Rate in the Cryopreservation Process

The freezing rate during the process must match the cell's specific characteristics. Sudden temperature drops can cause structural disruptions in the cell, altering membrane permeability and cytoskeleton structure. Damage caused by freezing can lead to reduced sperm motility after thawing, decreased sperm-oocyte fusion ability, and negatively impact fertilization capacity due to plasma membrane lipid peroxidation. This has been associated with the increase in free oxygen radicals, such as hydrogen peroxide and superoxide anions. To minimize the adverse effects of sperm freezing, it is recommended to add antioxidant substances such as ascorbic acid, vitamin E, and catalase to the culture medium.

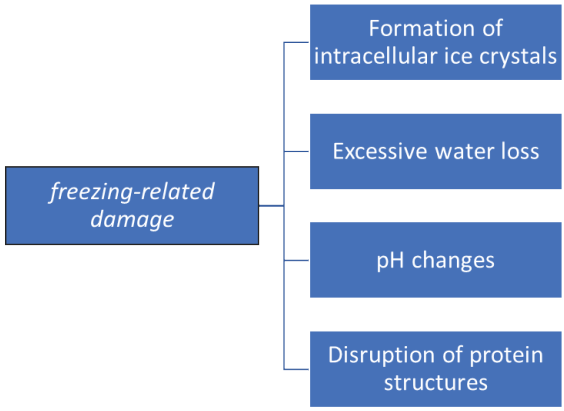

Figure 3. *The primary causes of freezing-related damage*

In addition to the factors summerized above (**Figure 3**) factors like environmental temperature, membrane permeability, concentration changes, the surface area-to-volume ratio of cells, properties of cryoprotectants, and membrane structure also play determining roles in the extent of cellular damage during freezing.

Apart from the direct toxic effects of cryoprotectants, cellular damage resulting from the release and activation of lysosomal enzymes must also be considered. Electron microscopy analyses reveal that the cell membrane is the earliest structure to be damaged during the freezing process. Structural alterations in the cell membrane include protrusions, undulations, vesicle formation, leakage of cytoplasmic contents, and ruptures resulting from the loss of membrane integrity. These changes directly affect the survival rate and functionality of sperm cells, making them critical factors in determining the success of freezing and thawing processes (18-20).

7. Cryopreservation Methods

The primary objective of the cryopreservation process is to enable the long-term storage of living cells or tissues at extremely low temperatures, while minimizing cellular damage and preserving their functional integrity. The ideal freezing rate is determined by the specific characteristics of the material being preserved, as different biological entities may respond differently to cooling and thawing procedures.

There are two major techniques for cryopreservation:

- freeze-thaw processes
- fast freezing, namely vitrification.

The key difference between them is the total avoidance of ice formation in vitrification The physical definition of vitrification is the solidification of a solution at low temperature, not through ice crystallization, but via an extreme increase in viscosity during cooling to reach the optimal temperature of -196°C within a few minutes. In conventional slow freezing, a significant amount of ice crystals is formed. Slow freezing techniques are widely used in tissue cryopreservation and single sperm freezing processes. On the other hand, the vitrification method is preferred for freezing fragmented sperm tissues, semen, and aspirations. Vitrification has been recently introduced into Assisted Reproduction Technique programs as well.

The most significant distinction between vitrification and slow freezing protocols lies in the elevated concentrations of cryoprotectants employed during vitrification. Studies have demonstrated that vitrification of human spermatozoa with non-permeable cryoprotectants, such as human serum albumin (HSA) and sucrose, can successfully preserve sperm function without substantial deterioration in key physiological parameters. This preservation of cell integrity is achieved with minimal damage to mitochondrial activity and acrosomal status, crucial components for successful fertilization (21). Additionally, the use of sucrose and dextran-based cryoprotectants during the vitrification of human spermatozoa has been shown to enhance sperm motility and progressive motility, while significantly improving DNA integrity and reducing DNA fragmentation when compared to traditional slow freezing methods. These improvements in sperm quality are particularly critical for maintaining fertility potential during long-term cryopreservation (22).

8. Conclusion

Sperm cryopreservation is recognized as a fundamental method for preserving male fertility. This technique plays a crucial role in reproductive medicine, particularly for individuals undergoing cancer treatments, dealing with genetic diseases, undergoing surgeries, or affected by advanced age that could negatively impact fertility.

Today's cryopreservation techniques allow for the long-term preservation of sperm cells at low temperatures while continuously being improved to minimize cellular damage during freezing and thawing processes.

Conventional cryopreservation methods involve protecting sperm cells using permeable or non-permeable cryoprotectants and subjecting them to low temperatures. However, intracellular ice crystal formation during the freezing process can disrupt membrane integrity, adversely

affecting sperm motility, DNA integrity, and acrosomal reaction ability. During the thawing process, osmotic stress, sudden temperature changes, and increased reactive oxygen species (ROS) can cause cellular damage. Thus, efforts to enhance the efficiency of sperm cryopreservation continue through advancements in new-generation technologies.

In recent years, biotechnology and artificial intelligence (AI) -based approaches have offered significant opportunities for improving cryopreservation processes. AI-supported sperm selection is a promising innovation aimed at improving sperm quality post-cryopreservation. This method focuses on selecting the healthiest sperm cells based on their morphological and physiological characteristics. This ensures that high-quality sperm are isolated prior to freezing, enhancing the success of the cryopreservation process.

In addition, nanotechnology-based cryoprotectants stand out as noteworthy innovations in the field of cryopreservation. Nano-bioengineering cryoprotectants, designed to minimize the toxic effects of traditional cryoprotectants and protect cell membranes more effectively, are believed to play a significant role in increasing sperm survival rates. Particularly, lipid nanoparticles and hydrogel-based cryoprotectants show potential to regulate intracellular water loss and minimize ice crystal formation.

Research on bioengineering-assisted freezing protocols provides new strategies to enhance cryopreservation efficiency. Microfluidic systems aim to reduce cellular stress through controlled cooling and warming processes. Furthermore, antioxidant-based solutions integrated into the cryopreservation process help counteract oxidative stress caused by ROS, preserving the functional properties of sperm cells.

In the future, cryopreservation technologies are expected to reach more advanced levels through artificial intelligence, nanotechnology, and bioengineering. By improving the success rates of sperm freezing-thawing processes, more effective and individualized solutions can be offered in reproductive medicine. AI-supported algorithms can optimize sperm selection, while nanotechnological cryoprotectants have the potential to minimize cellular damage. (23-27). These advancements in cryopreservation could pave the way for revolutionary developments in reproductive biology, particularly by achieving higher pregnancy rates in infertility treatments.

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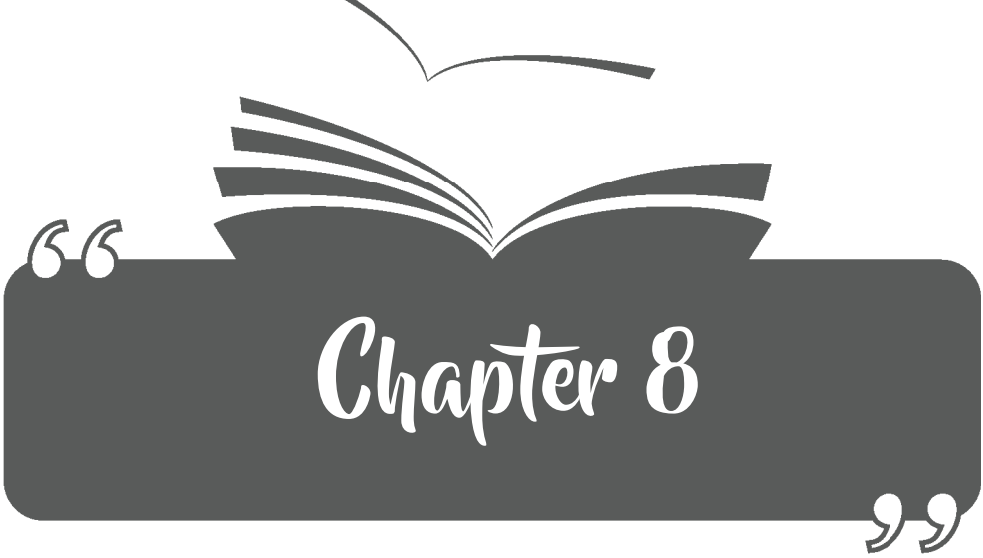

IN VIVO ANIMAL MODELS IN GLUCOSE METABOLISM RESEARCH

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Introduction

There are various types of models used in scientific research. These models are classified based on their execution methods and the materials used, including in vivo, in vitro, in silico, in virtuo, in petto, and in abstrakto (Genç & Salman, 2018). Among these methods, the use of live animals (in vivo) has been gaining increasing importance due to the advantages it offers (Genç, 2017). One of the primary advantages of using live animal models in research is their ability to accurately represent the relationships between cells, tissues, organs, and systems (Genç et al., 2020). The small body size of laboratory animals, their low dietary consumption, rapid generation cycles, ease of intervention, high offspring numbers, and the possibility of creating models specific to the study are among the favorable features that facilitate their breeding and use in research. Moreover, experiments conducted with animals obtained and raised under standardized conditions, in compliance with research standards, provide highly reliable and internationally accepted results, independent of time constraints, ensuring the most realistic and accurate findings (Genç, 2018). The use of animal models with known genetic characteristics in metabolic research is considered the most advantageous method for establishing a well-structured research hypothesis, preventing unexpected developments during the research process, and better controlling metabolic dynamics (Genç, 2019). Obtaining the most accurate results in scientific studies depends not only on selecting an appropriate and reliable model but also on controlling environmental factors such as light, diet, sterilization, noise, humidity, temperature, and personnel movement. The ability to regulate these factors becomes even more critical in complex studies, particularly in metabolic research. To ensure the health of laboratory animals, innovative approaches such as the use of novel biotics (Genç & Özocak, 2021), various dietary supplements, physical enrichment devices, and other advancements have been increasingly implemented. Since animal models are commonly used to study complex phenomena, it is essential to standardize the methods employed. Due to the complexity factor, it is widely acknowledged that selecting the most appropriate models is crucial, especially for research in metabolism, endocrine disruption, reproduction, and developmental metabolism (Genç & Salman, 2018). However, thanks to legal regulations that establish standardized guidelines for laboratory animal breeding and usage in many countries worldwide, the use of animals in research has become more reliable. This increased reliability is a crucial factor that significantly enhances the quality of research.

Live Animal Models

In modern scientific research, the most commonly used live animal species due to their physiological and genetic characteristics include mice, rats, rabbits, guinea pigs, gerbils, hamsters, zebrafish, and axolotls. In addition to these species, advancements in technology and scientific progress have led to the inclusion of other animal species with increasingly well-understood physiological and genetic traits in research. Various organisms that thrive in different environments, such as fruit fly larvae (Drosophila), nematodes (*Caenorhabditis elegans*), Trichoplax adhaerens, and *Ephydatia muelleri*, are also utilized in scientific studies due to their unique characteristics (Neff, 2018). Laboratory animals are further classified microbiologically and genetically based on their use in research.

Microbiological Classification of Laboratory Animals

- **Conventional Animals:** These animals are maintained under standard care and feeding conditions without any specific microbiological barrier. Their microbiological population is not entirely known on an individual basis.

- **Colonization-Resistant Flora (CFR) Animals:** These animals possess not only a unique bacterial flora but also allow the presence of other microorganisms. They serve as the initial group for establishing a Specific Pathogen-Free (SPF) colony.

- **Specific Pathogen-Free (SPF) Animals:** These animals do not carry any pathogenic microorganisms. They live in a sterile environment where contamination by any pathogenic microorganism is strictly prevented.

- **Gnotobiotic Animals:** These animals contain only known microorganisms and do not carry any biological material from other organisms except for specified microbes.

- **Germ-Free (GF) or Axenic Animals:** These animals do not carry any microorganisms. To maintain genetic and microbiological standardization, they undergo routine quarterly and annual microbiological screenings during breeding and maintenance.

Genetic Classification of Laboratory Animals

- **Genetically Undefined Animals:** These include outbred animals, genetically heterogeneous species, segregation hybrids, and selectively outbred animals.

- **Partially Genetically Defined Animals:** This category includes outbred mutant animals, outbred transgenic animals, and animals in developmental stages.

- **Genetically Defined (Isogenic) Animals**: These include inbred strains, F1 hybrids, coisogenic, congenic, consomic, collaborative, conplastic, recombinant inbred, recombinant congenic, inbred transgenic, segregation inbred, and mutant inbred strains.

These animals can be selected based on their specific characteristics to align with research hypotheses and serve as models. There are various methods available for developing animal models (İrer et al., 2004). Depending on their characteristics, animal models can either arise spontaneously or be induced. Spontaneous models are either normal animals that share phenotypic similarities with humans or animals that exhibit abnormal traits due to spontaneous mutations within a species (Maurer & Quimby, 2015).

1- Spontaneous or Transgenic Models

These models utilize animals that either have a natural predisposition to a disease or develop the disease spontaneously. They are obtained by controlling their genetic structures through gene technology methods. Examples of such models include: Gunn rat (*hereditary hyperbilirubinemia*), BB Wistar rat (*Type 1 diabetes mellitus*), Brattleboro rat (*neurogenic diabetes insipidus*), Watanabe rabbit (*hypercholesterolemia*) (Andrews et al., 1979; Maurer & Quimby, 2015).

2- Diet-Induced Models

These models are developed by feeding animals a specifically formulated diet based on the research hypothesis. The composition of the diet plays a crucial role in inducing the desired physiological or pathological condition. Diet-induced models are commonly used to study conditions such as obesity, hypercholesterolemia, and diabetes.

3- Drug- and Chemical-Induced Models

This method involves administering pharmaceutical agents or chemical substances to animals in various ways to create a model based on the research hypothesis. These substances act as active agents that induce specific physiological or pathological conditions. Example: Diabetes model induction using streptozotocin (STZ) and alloxan – These compounds are known as diabetogenic agents that selectively damage pancreatic β-cells, leading to diabetes (Kim et al., 2002).

4- Virus-Induced Models

This method involves using virological agents to induce disease in animals based on the research hypothesis. Various viruses have been identified for their ability to induce diabetes models. Examples of viruses used in diabetes models: Encephalomyocarditis virus (EMC), Coxsackie B virüs, Kilham rat virus (KRV), Rubella virüs, Cytomegalovirus (CMV), Venezuelan equine encephalitis virüs (Jun & Yoon, 2001; Pickup & Williams, 2002).

5- Surgically-Induced Models

These models are created through surgical interventions based on the research hypothesis. Examples: Partial pancreatectomy and lesion induction in the ventromedial hypothalamus used for developing diabetes models (Pickup & Williams, 2002). Hyperthermic intraperitoneal chemotherapy (HIPEC) applied for certain cancer research models (Ocak et al., 2019; Bük et al., 2020). Nephrectomy can be used to create chronic kidney disease models (Askari et al., 2016).

Animal Models in Glucose Metabolism Research

Diabetes Models

The advantages of using live animal models in the study of human diseases are well recognized. However, despite these advantages, diseases induced in laboratory and experimental animals—while often closely resembling human conditions—do not perfectly replicate the pathophysiological characteristics observed in humans (Pickup & Williams, 2002). For diabetes modeling, non-human primates (NHPs) offer significant advantages due to their metabolic physiology and genetic similarity to humans (99% similarity), as well as their anatomical resemblance. Their suitability for translational methods, the feasibility of interventional procedures such as blood sampling, endoscopy, and serial procedures, and the ability to perform laparoscopic biopsies make them valuable models for studying diabetes. However, their use is limited by several factors, including high maintenance costs, the need for specialized facilities, long life cycles, and ethical considerations. These constraints make non-human primates less commonly used compared to other animal models. Large-bodied mammals such as pigs and dogs offer several advantages as diabetes models due to their physiological similarities to humans, their comparable size and shape, and similar pharmacokinetics. Additional advantages include their ability to produce multiple offspring, their suitability for long-term cannulation, and the structural resemblance of their pancreas and Langerhans islets to those of humans. However, their use is

limited by certain disadvantages, including the high costs associated with breeding and housing, the need for specialized facilities, and their long life cycles. These factors make their application in diabetes research more challenging compared to smaller animal models. Non-Mammalian Animal Models (Drosophila, *Caenorhabditis elegans, Trichoplax adhaerens, Ephydatia muelleri*) offer several advantages in diabetes research. Their short life cycles (except for zebrafish), the availability of complete genome RNAi libraries, and their ability to model obesity-like and Type 2 Diabetes Mellitus (T2DM)-like conditions make them valuable tools. Additionally, their low maintenance costs further enhance their appeal as research models. However, a significant drawback is their physiological and anatomical differences from mammals, which may limit the direct translational relevance of findings to human diabetes. The advantages of rodents, which are the most preferred in modeling diabetes and other metabolic disorders today, are the ability to create a large number of obesity and T2DM models, being amenable to genetic manipulation, reaching sexual maturity in a short time (4-8 weeks), short gestation period (average 21 days), the capacity to give multiple offspring (6-12 offspring/birth), being compatible with the applicability of metabolic phenotyping technology, and the opportunity to produce and house at lower costs. However, their disadvantages are that they have different pancreatic Langerhans island structures than humans and that monogenic models do not represent most human diseases (Kleinert et al., 2018).

Table I: Some rodents are suitable as models for diabetes-related conditions.

Strain /method	Species / Diet	Model
Polygenic		
C57BL/6J	M/ HFD	Obesity, IR, Dyslipidemia
SWR/J	M/HFD	Dyslipidemia
A/J	M/HFD	Obesity, Dyslipidemia
C3H/HeJ	M/HFD	Obesity, IR, T2DM, Dyslipidemia
DBA/2J	M/HFD	Obesity, IR
NZO	M/CHO	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
TALLYHO/Jng	M/SD, HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
DIO-sensitive Sprague Dawley	R/HFD	Obesity, IR, Dyslipidemia
UCD-T2DM	R/SD, HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
Sand rat	G/SD, HFD	Obesity, IR, T2DM, Dyslipidemia, Pathological islet
Goto–Kakizaki	R/SD, HFD	Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
Monogenic		
C57BL/6J–ob/ob	M/SD, HFD	Obesity, IR, Dyslipidemia
C57BLKS/J–db/db	M/SD, HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
Otsuka Long–Evans Tokushima obese	R/SD, HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
fa/fa	R/SD, HFD	Obesity, Hyperglycemia, IR, Dyslipidemia
Zucker Diabetic obese	R/SD, HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
Koletsky	R/SD, HFD	Obesity, IR, Dyslipidemia
Experimental		
Low dose streptozotosin	M,R/HFD	Obesity, Hyperglycemia, IR, T2DM, Dyslipidemia, Pathological islet
VMH lesion	M , R / S D , HFD	Obesity, IR, Dyslipidemia

CHO, carbohydrate-rich diet, DIO, diet-induced obesity, R: rat, M: mouse, G: gerbil DR, diet resistance, HFD: high-fat diet, IR, insulin resistance, SD, standard diet; T2DM, type 2 diabetes mellitus; VMH, ventromedial hypothalamus.

The table was compiled from Kleinert et al., (2018).

Model Development Using Chemical Agents

In this model development method, a significant portion of the beta cells is targeted for damage, aiming to reduce insulin production. As insulin levels decrease, the animal develops hyperglycemia. The induction of diabetes should be carried out 5 to 7 days before conducting the animal experiment (Dufrane et al., 2006). The use of Streptozotocin and Alloxan is quite common for inducing diabetes through chemical methods. These two chemical compounds compete with glucose in metabolism (Bansal et al., 1980). Since both solutions are chemically unstable, they must be pre-

pared immediately before injection to ensure their full effectiveness (King et al., 2017). Alloxan and Streptozotocin are toxic glucose analogues. Glucose Transporter Protein 2 (GLUT2) plays an important role in their accumulation in beta cells.Therefore, dose adjustments should be made according to the animal species and application method (Lee et al., 2010). Although both agents are used to create T1DM and T2DM conditions, they are mostly used for the T1DM model because they do not directly trigger insulin resistance (Radenkovic et al., 2016). Streptozotocin is an antibiotic and antineoplastic nitrosourea derivative isolated from Streptomyces achromogenes and has a toxic effect on beta cells. With this feature, it is suitable for use in creating a diabetes model in experimental animals (Bolzan & Bianchi, 2002). The routes of administration of streptozotocin can be intraperitoneal (IP) or intravenous (IV). After administration (Szkudelski, 2001), it is retained by GLUT2 in the beta cell membrane and leads to cell death by being reduced to glucose and methyl nitrite. In addition, it causes damage to mitochondrial DNA and disrupts energy metabolism. It prevents glucose-induced insulin secretion (Lenzen, 2008). Poly (ADP-ribose) polymerase (PARP) is activated, NAD+ is depleted, cellular ATP decreases, and insulin production is inhibited (Sandler & Swenne, 1983). For diabetes model induction using streptozotocin, both high-dose administration and repeated low-dose administration can be employed. High-dose application leads to a faster and more effective T1DM model induction, but it is important to note that it also results in a higher mortality rate (Zhang et al., 2023). Examples of low doses include 100-200 mg kg-1 in mice (Dekel et al., 2009) and 35-65 mg kg-1 in rats (Srinivasan and Ramarao, 2007). This dosage level leads to rapid destruction of beta cells and the development of hyperglycemia. However, it should not be overlooked that pancreatic islet regeneration may occur after STZ treatment. This shows that it should be ensured that the improvement in symptoms of glycemia is not due to the regeneration of endogenous beta cells (Grossman et al., 2010). Alloxan is another chemical substance used to create a diabetes model. Alloxan, which is retained by beta cells, causes free radical formation in the cell. Since the defense mechanisms of beta cells are not developed, the risk of damage is quite high. With this mechanism, alloxan is effective in creating a model by damaging the cell (Nerup et al., 1994). The radical formation mechanism of alloxan is formed by reducing it to dialuric acid, oxidizing it again to alloxan, causing a cycle that causes superoxide formation, and the formation of hydrogen peroxide from the radicals formed. Beta cell DNAs are damaged by this mechanism. A dose of 50 to 200 mg/kg is appropriate for creating a model in mice and 40 to 200 mg/kg in rats. For subcutaneous (SC) and intraperitoneal (IP) administration, three times the intravenous (IV) dose may be required (Szkudelski, 2001). A high döşe (one singal dose) of 200 mg/kg body weight

can be administered as intraperitoneal injection. However, Federiuk et al. (2004) reported a 10% mortality rate with this protocol. Sathya et al. (2013) administered 150 mg/kg of alloxan hydrate to Sprague-Dawley rats weighing 150–200 g via IP injection after an overnight fast. After the injection, the animals were given access to food and water and after 72 h, blood glucose levels reached 200 mg/dL, indicating successful induction of Type 2 Diabetes Mellitus (T2DM). A dose of 100 mg/kg has been reported to be long-lasting and sufficient for a successful diabetes model in rabbits (Wang et al., 2010).

High-Fat Diet-Induced Diabetes Model

In this method, subjects are given high-calorie diets prepared using fat sources and/or sugar and sodium-enriched rations to induce obesity ad libitum and the animals are monitored. The Diet-Induced Obesity (DIO) model successfully reflects the human obesity model because slow weight gain and secondary insulin resistance develop in this model (Kleinert et al., 2018). An example of this model is the study conducted by Li et al. (2020); in this study, a high-fat diet (cooked lard 10%, sucrose 20%, cholesterol 2.5%, cholesterol 1.0% bile salts and normal diet 66.5%) was given to 8-week-old healthy male Sprague-Dawley (SD) rats for four weeks to create a T1DM model. The models continued this diet for 12 weeks and low-dose STZ (25 mg/kg, once a day, for two days) was administered to induce diabetes.

Genetically Predisposed Strains for Diabetes

The genetic characteristics of animals obtained from these strains by inbred technique may be the main reason for the negative effects in creating DIO. Due to these genetic characteristics, they offer significant advantages for diabetes research. The C57BL6/J strain is frequently used in developing DIO models. Its main advantages include the occurrence of glucose intolerance, severe obesity, excessive fat accumulation and moderate insulin resistance (Surwit et al., 1988; Winzell and Ahren, 2004). On the other hand, some strains such as SWR/J and A/J mice are particularly interesting models for studying human obesity resistance due to their lower susceptibility to DIO and its complications (West et al., 1992; Leibowitz et al., 2005).

Spontaneous Autoimmune Animal Models for Type 1 Diabetes

Genetic and environmental factors are of great importance for the development of T1DM. In the development of the disease, the destruction of beta cells by T cells, the disruption of insulin production, and the autoimmune disorder are the notable stages of the mechanism (Ramos et al.,

2021). Since this form of diabetes is more commonly observed in children and adolescents, it is also referred to as juvenile diabetes (Kitada et al., 2016). To create these tables, NOD mice, BB rats and LEW.1AR1/Ztm-iddm rats are mostly preferred (Lenzen et al., 2001; Yang and Santamaria, 2006). These animals are spontaneous autoimmune models and the Akita mouse can be given as an example of a non-autoimmune model (Zhang et al., 2023). Insulitis develops in NOD mice within 3–4 weeks, and during this period, called the pre-diabetic phase, immune system cell infiltration (B, NK cells and CD4+ and CD8+ lymphocytes) into the pancreatic islets is observed (Yoon & Jun, 2001). When approximately 90% of pancreatic insulin levels are eliminated, a clear diabetic phenotype typically occurs within 10–30 weeks. For model development, females are preferred because they have a higher incidence of diabetes (60–90%), while in males this rate varies between 10% and 30% (Pozzilli et al., 1993; Hanafusa et al., 1994). A rapid model can be achieved by cyclophosphamide application in the NOD lineage (Caquard et al., 2010). T cell transfer from NOD mice to non-diabetic recipient mice, also known as adoptive transfer, is also possible (Christianson et al., 1993). When using NOD mice as a diabetes model, it is essential to maintain strict microbiological standards to prevent microbial contamination, ensuring they are housed in specific pathogen-free (SPF) environments. Additionally, factors such as sex differences influencing disease development and the variable onset of the diabetic phenotype must be considered. Compared to chemically-induced diabetes models, the use of NOD mice as a Type 1 Diabetes model is more expensive due to these factors (King, 2012). BB strain rats, which were derived from Wistar rats, are also used in diabetes research. In this strain, a diabetic phenotype can be induced with a 90% success rate at 8 to 16 weeks of age, regardless of sex. When diabetes symptoms are severe, insulin treatment is essential for life. The insulitis picture in these animals is marked by the presence of T cells, pancreatic β cells, macrophages and NK cells, as well as lymphopenia due to the decrease in CD4+ T cells and the near depletion of CD8+ T cells (Mordes et al., 2004).However, since this immune profile does not resemble human diabetes, it is considered a limitation of BB rats in diabetes research. Additionally, unlike NOD mice, BB rats do not have a peri-insulitis phase before the onset of insulitis. Despite these differences, BB rats are highly valuable for studying genetic factors involved in Type 1 Diabetes (Wallis et al., 2009). They are also suitable models for studying islet transplantation tolerance induction and diabetic neuropathy (Hartoft-Nielsen et al., 2009; Holmberg et al., 2011; Zhang et al., 2007). LEW.1AR1/-iddm rats are a spontaneously arising line from the LEW.1AR1 congenic line with a defined MHC haplotype used as a model of T1DM. The diabetic phenotype becomes evident within approximately 8–9 weeks (King, 2012). The diabetes induction rate is

around 60% in both sexes. One week before the onset of hyperglycemia, the animals enter a pre-diabetic phase characterized by islet infiltration (Jorns et al., 2005). Unlike NOD and BB rats, LEW.1AR1/-iddm rats do not develop other autoimmune disorders. Additionally, since these animals exhibit high survival rates even after developing overt diabetes, they are suitable for studying diabetic complications (Mathews, 2005). Komeda Diabetes-Prone (KDP) rats were derived from the Long-Evans Tokushima Lean (LETL) strain through a selective breeding program of approximately 60 days. Bu sıçanlarda görülen poliüri ve hiperglisemi tablosu ile ağırlık kaybı özellikleri T1DM özellikleridir (Komeda ve ark., 1998). Similar to other autoimmune diabetes models, beta-cell destruction in KDP rats occurs via insulitis (King & Austin, 2017). KDP rats exhibit pancreatic infiltration of immune cells along with proinflammatory cytokine expression (IL-1 and TNF-α) in both macrophages and T cells. The primary proinflammatory cytokines expressed in the pancreas of these rats are IFN-γ and TNF-α (Jörns et al., 2014). Genetic studies on KDP rats revealed that the majority of genetic susceptibility to spontaneous T1DM is linked to the MHC region on chromosome 20 and the IDDM/KDP locus on chromosome 11 (Yokoi et al., 2002). These data suggest that KDP rats are the most suitable animal model for studies of autoimmunity in T1DM (Yokoi et al., 2002).

Generation of an Insulin-Dependent Type 1 Diabetes Model via Genetic Manipulation

The Akita mouse model is derived from the C57BL/6NSlc mouse. It carries a mutation in the insulin 2 gene that impairs proinsulin metabolism and is a non-autoimmune model.This leads to misfolded protein accumulation, resulting in endoplasmic reticulum (ER) stress and subsequent beta-cell dysfunction. From one month of age onwards, hyperglycemia, hypoinsulinemia, polyuria and polydipsia develop, and eventually severe insulin-dependent diabetes mellitus develops. Homozygous animals can survive for more than 12 weeks. Beta cell depletion streptococin-treated mice offer the potential to be a suitable model for transplantation studies (Mathews et al., 2002). They are also known to be a suitable model for the production of Type 1 diabetic macrovascular disorders (Zhou et al., 2011) and neuropathy (Drel et al., 2011). It can also be used as a model for endoplasmic reticulum stress studies in islets and some pathological conditions of type 2 diabetes (Chen et al., 2011). Since the INS gene is responsible for the development of permanent neonatal diabetes mellitus (PNDM) in humans, a genetically modified pig model with a mutation in the INSC94Y gene (similar to the INSC96Y mutation in humans) has been developed for diabetes research. In this transgenic model, severe hyperglycemia occurs 24 hours after birth and persists in the following

stages (Renner et al., 2013). Immunological and cellular characterization studies using various techniques have demonstrated that these transgenic pig strains serve as an excellent model for Type 1 Diabetes Mellitus (T1DM) (Zhang et al., 2023).

Creating a Type 1 Diabetes Model with Viruses

Knowing the role of viruses in the pathogenesis of T1DM (van der Werf et al., 2007), their use in models that induce beta cell destruction is being used.With this method, beta cell destruction is achieved through viruses to create a type 1 diabetes model. Beta cells can be directly destroyed or they can develop as a result of an autoimmune reaction (Jun and Yoon, 2003). Coxsackie B virus (Jaidane et al., 2009), encephalomyocarditis virus (Shimada and Maruyama, 2004) and Kilham rat virus (Ellerman et al., 1996) are examples of viruses used as triggers in the development of diabetes.In pre-diabetic Non-Obese Diabetic (NOD) mice, Coxsackievirus infection can induce diabetes. Similarly, diabetes induction can be achieved in transgenic mice expressing rat viral neo-antigens through Lymphocytic Choriomeningitis Virus (LCMV) infection (Christoffersson et al., 2020). Viral agents are known to play a role in diabetes development. It is suggested that the Coxsackievirus B (CVB) group in particular may be the cause of virus-induced Type 1 Diabetes (T1D). In a study by Stone et al. (2018), a CVB1 vaccine was shown to sustain CVB1-induced diabetes in SOCS1-transgenic NOD mice through cytokine signaling regulation. EMC-D virus can also be used to induce diabetes, but obtaining a reliable model requires a host genetic background that is susceptible to the virus. Additionally, some strains exhibit moderate susceptibility to EMC-D, suggesting that low-susceptibility genes may also contribute to disease etiology (Keiichiro et al., 2018). When modeling human diseases in animals, it is essential not to overlook complex biological mechanisms. The importance of this factor has been emphasized in numerous studies. Tyrosine Kinase 2 gene plays a role in the DM model created using EMC-D virus in mice. Polymorphisms in the 5'UTR of this gene are known to be the TYK2 promoter variant, which has a role in increasing the risk of diabetes. This variant is thought to be a susceptible gene in the formation of virus-induced DM in humans (Nagafuchi et al., 2015; Keichiiro et al., 2017). Virus-induced diabetes depends on both the diabetogenicity of the virus and the susceptibility of the host. Therefore, to accurately establish a model of human virus-induced diabetes in living animals, it is essential to incorporate TYK2 and other promoter variants into animal models (Keichiiro et al., 2018). There is also a transgenic virus model that reacts with antigens that cause immune responses in beta cells and causes beta cell damage, due to the effect of Lymphocytic Choriomeningitis Virus (LCMV), which does not cause spontaneous beta cell destruction

(von Herrath et al., 1997). Although positive results have been obtained in studies on model development, and some Type 1 Diabetes (T1D) cases in humans have been associated with viruses (van der Werf et al., 2007; Richardson et al., 2009), the extent to which viruses contribute to the pathogenesis of T1D remains uncertain. Insulin resistance (IR) affects glucose and lipid absorption and retention in muscle cells, causing an increase in blood sugar and triglyceride levels (Misra et al., 2008). With the development of chronic hyperglycemia, some neuropathic, nephropathic and retinopathic phenomena and a tendency to cardiovascular diseases may be observed. Insulin resistance and functional impairment in β-cells are observed in Type 2 Diabetes Mellitus (T2DM), therefore insulin resistance and beta cell failure can be studied with T2DM animal models. Most of these animal models are obese, which is valuable in mirroring the close relationship between obesity and T2DM development in humans (Fang et al., 2019; King, 2012). Type 2 DM animal models are basically divided into two according to whether they are obese or not. Leprdb/db, Lepob/ob, KK mice, Zucker fatty and Zucker diabetic fatty rats are examples of spontaneous T2DM obesity models. The Goto-Kakizaki (GK) rat is known as a non-obese spontaneous T2DM model. With these models, it is possible to study the pathophysiology, obesity, and leptin signaling pathways in T2DM disease (Zhang et al., 2023).

Creating a Type 2 Diabetes Model with Chemicals

It is possible to create T2DM models using streptozotocin and alloxan. Barragán et al. (2019) developed a hyperglycemia model with a single STZ injection (90 mg/kg body weight) at birth and 10% sucrose (SSB; 10% or 30% sucrose) sweetened liquid beverage for 7 weeks after weaning in 39 male and 37 female Wistar rats. It was observed that animals developed symptoms seen in T2DM, such as hyperglycemia, moderate insulin resistance, and hyperactive neuralgia during the postpubertal adult period. Wang et al (2020) demonstrated that IP injection of STZ (60 mg/kg body weight) into SD rats could be a model of diabetic peripheral neuropathy. For model formation, different concentrations of DPDs (Diphenyl diselenide) were administered once a day for 12 weeks by gavage. This demonstrated that DPDs could be used as a new treatment for diabetic peripheral neuropathy. In low-dose STZ applications, the number of repetitions varies between 2-7, and the exact number varies depending on the hypothesis and variables of the study. In the study by Wang et al. (2019), a T2DM model was created by administering 50 mg/kg STZ daily in 0.1 M sodium citrate (pH4.5) to LyzM-Cre-Ubc9fl/fl, KO mice at 8 weeks of age.

Monogenic Type 2 Diabetes Models

Monogenic obesity is defined as obesity caused by a mutation or deletion in a single gene. Monogenic mutations are rarely encountered in human obesity causes, but these models are still frequently used in animal studies. A key characteristic of these models is leptin signaling defects, which lead to hyperphagia (excessive eating) due to impaired leptin function (Ranadive et al., 2008; King, 2012). Lepob/ob mice are a strain that has developed a mutation in chromosome 6 (mutation in the leptin protein) in C57BL/6 mice and is characterized by extreme obesity (Zhang et al., 2007). Obesity (3 times normal body weight) and hyperinsulinemia begin to be seen in mice starting from the age of 2 weeks. Hyperglycemia is observed at the age of 4 weeks, blood glucose levels peak at the age of 4-5 months and begin to decline as they age (Lindström, 2007; Park et al., 2011). These mice, whose pancreatic islet volumes are significantly reduced due to excessively high insulin secretion, are infertile, have irregular body temperatures, are weak, and exhibit hyperlipidemia (Chehab et al., 1996; Bock et al., 2003, Lindström, 2007). Despite the above-mentioned characteristics, although they do not fully meet the human type 2 diabetes picture, these extreme obesity, persistent hyperinsulinemia and insulin resistance are suitable animal models for studies of insulin sensitizers, anti-obesity drugs and drugs used in the treatment of hyperglycemia, which increase peripheral insulin sensitivity and reduce body weight (Chakrabati et al., 2003; Cheung et al., 2005; Olivares et al., 2017). Obesity becomes evident at 3–4 weeks of age, hyperinsulinemia develops as early as 2 weeks, and hyperglycemia manifests between 4–8 weeks of age (King, 2012). Since hyperinsulinemia, hyperglycemia, and renal dysfunction occur at different stages and pancreatic islet cells are highly sensitive to glucotoxicity, insulin dependence develops in these animals for hyperglycemia control (Kitada et al., 2016). Mortality typically occurs at approximately 10 months of age, coinciding with persistent hyperglycemia, severe pancreatic islet destruction, and myocardial damage (Olivares et al., 2017). Due to the presence of advanced reactive gliosis and vascular leakage, this model is particularly valuable for studying late-stage diabetes progression (Cheung et al., 2005). Zucker obese (ZF) and Zucker diabetic fatty (ZDF) rats are animals with a leptin mutation that develop obesity within four weeks along with hyperphagia. They also exhibit hyperinsulinemia, hyperlipidemia, hypertension, and impaired glucose tolerance (Srinivasan & Ramarao, 2007). Zucker obese rats were derived from a crossbreeding of the M line with Sherman rats and carry mutations in the leptin receptor. In these animals, which may also exhibit reduced appetite (Yokoi et al., 2002), significant fat accumulation begins around four weeks of age, and by the fifth week, advanced adiposity is observed (Wang et al.,

2014). When modeling with these strains, it is important to note certain differences from the human condition. In genetically weak heterozygous rats, plasma insulin levels return to normal, and glucagon levels decrease by the 30th week (Janssen et al., 1999), whereas such a phenomenon is not observed in humans (Augstein & Salzsieder, 2009). However, mild hyperglycemia, which significantly impacts diabetic nephropathy in humans (D'Angelo et al., 1979), and hypertension (Seaquist & Ibrahim, 2010) serve as facilitating factors in modeling diabetes-related complications. Unlike Lepob/ob mice, ZF models do not develop a pronounced diabetic phenotype but exhibit hyperinsulinemia and impaired glucose tolerance beginning after one month (Olivares et al., 2017). Due to these factors, ZF rats may be more suitable for **pre-diabetes studies** rather than fully replicating human diabetes (Zhang et al., 2023). Obesity is less in ZDF rats, whereas beta cell apoptosis is high and therefore insulin resistance is severe (Pick et al., 1998). Hyperinsulinemia emerges at 8 weeks of age, but insulin levels progressively decline over time (Shibata et al., 2000). While male ZDF rats develop diabetes between 8–10 weeks of age, this condition is not observed in females (Srinivasan & Ramarao, 2007). ZDF rats also exhibit hyperlipidemia, hypertension, and impaired glucose tolerance, along with larger pancreatic islets with irregular borders (Katsuda et al., 2014; Olivares et al., 2017). In addition, due to the relationship between the increase in DNA content in pancreatic islets and serum insulin levels, the hypothesis that hyperinsulinemia may develop in ZDF rats due to islet hyperplasia seems acceptable (Tokuyama et al., 1995).Excessive fat accumulation, elevated triglyceride and cholesterol levels, and lipotoxicity in skeletal muscle and pancreatic islets due to fatty acid metabolism dysfunction further contribute to disease progression in ZDF rats (Lee et al., 1997; Shimabukuro et al., 1998). With the mentioned features, it can be said that ZDF rats can be a good model in human T2DM research. Another laboratory animal used to study the obesity-diabetes relationship is the KK mouse. Symptoms observed in CC mice include obesity (moderate), excessive diet consumption, excessive urination, hyperinsulinemia, and insulin resistance, as well as pancreatic islet hypertrophy and muscle and fat tissue degranulations. In addition to these, other symptoms observed have been reported to include plasma hyperinsulinemia, insulin sensitivity differences and nephropathy (Ikeda, 1994). In these mice, the induction of T2DM through a high-fat diet, particularly in an age-dependent manner (Bleisch et al., 1952), promotes increased islet number and size, hyperinsulinemia, and insulin resistance compensation. This adaptation helps maintain stable blood glucose levels and preserve β-cell health (Ikeda, 1994; Oyadomari et al., 2002). A good example of a spontaneous non-obese T2DM model is Goto-Kakizaki (GK) rats selectively derived from hyperglycemic Wistar rats. Due to impaired islet cell development,

glucose intolerance emerges by two weeks of age, and with a high-glucose diet at four weeks, they develop a stable hyperglycemic profile (Ostenson et al., 1993). In young GK rats, pancreatic islets exhibit a starfish-like appearance, which is a structural indicator of glycolipid toxicity (Al-Awar et al., 2016). This morphological change is caused by fibrotic tissue formation between endocrine cell chains (Ostenson & Efendic, 2007).

Polygenic Type 2 Diabetes Models

Polygenic models are more suitable for human disease modeling, as they allow the study of various genotypes and provide advantages in replicating human Type 2 Diabetes Mellitus (T2DM). These models are particularly useful for better understanding obesity, glucose homeostasis, and diabetic complications. In these strains, male offspring tend to be more dominant at birth (Leiter, 2009). Considering the importance of sex differences in scientific research, this factor should be carefully taken into account. In KK mice, hyperinsulinemia and insulin resistance in various muscle and adipose tissues can be modeled. In this strain, pancreatic islets appear hypertrophic and degranulated, and nephropathy-related findings are also present (Ikeda, 1994). In OLETF rats, diabetes develops in a sex-linked manner, predominantly affecting males. The symptoms seen in the early period (6-20 weeks of age) are islet cell infiltration and degeneration, while hyperplasia may be observed at 20-40 weeks. In later stages, fibrotic changes and connective tissue formation occur (Kawano et al., 1994). The New Zealand Obese (NZO) mouse is a strain characterized by hyperphagia and leptin resistance. Between 9 and 12 weeks of age, these mice develop hyperleptinemia (Leiter & Reifsnyder, 2004). Because of their insensitivity to peripheral leptin administration and their responsiveness to central leptin administration, it is suspected that the mechanism of leptin transfer across the blood-brain barrier is defective (Halaas et al., 1997). Due to a disruption in hepatic fructose 1,6-bisphosphatase regulation, NZO mice develop hepatic insulin resistance and hyperinsulinemia (Andrikopoulos et al., 1993). As blood glucose levels rise, glucose intolerance emerges, and the condition worsens with age, affecting approximately 50% of males (Haskell et al., 2002). Hayvanlarda 3 ila 6 aylık yaşlarda adacık hiperplazisi ve hipertrofi görülebilir. Bu evrede beta hücre kaybı görülmez. Yetişkinlerde latent otoimmün diyabet tablosu şekillenir (Junger ve ark., 2002). Bir başka spontan obezite ve T2DM'nin tamamı ise TallyHo/Jng fareleridir (Kim ve ark., 2005). These mice display increased fat accumulation, elevated free fatty acids, cholesterol, and triglycerides in the blood. Hyperglycemia in males is moderate and appears between 10 and 14 weeks of age. However, further studies are needed to establish their reliability for diabetes research (Leiter et al., 2009; Buck et al., 2011). NoncNZO10/LtJ mice are well suited for diabetic wound studies (Fang et

al., 2010), as they develop hepatic and muscle insulin resistance at 8 weeks of age, chronic hyperglycemia at 12 weeks of age, and diabetic nephropathy at 12 months of age (Leiter, 2009).

Genetically Modified Type 2 Diabetes Models

These models aim to stably maintain specific characteristics over time. To achieve this, specific gene fragments are modified using techniques such as Cre/loxP, CRISPR/Cas9, Zinc-Finger Nuclease (ZFN), and TALEN technologies. There are important elements used in gene locus studies in T2DM tables. Examples of these are glucose transporter protein-4, insulin receptor, substrate, insulin-like growth factor I receptor, peroxisome proliferator activated receptor and PAX4 genes. Examples of strains obtained by this process are IRS-1. IRS-2, IRS-1–/– , IRS-3–/–, Syn4+/- mouse, INSC94Y pig and PAX4+/- rabbit (Zhang ve ark., 2023). IRS-1 and IRS-2 strains have different but similar physiological properties. The important roles of IRS-3 and IRS-4 are on the growth, development and glucose homeostasis of the organism, while the effects of insulin hormone are on the growth, development and glucose homeostasis of the organism. IRS-1/IRS-3 strain exhibits early fat atrophy, severe hyperglycemia, hyperinsulinemia, insulin resistance, glucose intolerance and islet hyperplasia (Laustsen et al., 2002). Therefore, it is suitable for being a DM model. The Syn4+/- mouse strain, generated by zygote-stage deletion of the Syntaxin-4 gene, exhibits a 50% reduction in systemic glucose absorption, leading to impaired glucose tolerance. These mice show significantly reduced insulin-stimulated GLUT4 translocation in skeletal muscle, while insulin-stimulated glucose uptake and metabolism in adipose tissue and the liver remain normal (Yang et al., 2001). Peroxisome Proliferator-Activated Receptor (PPAR) knock-out mice are also used in diabetes research, as the expression patterns of PPAR- in human and mouse tissues are highly similar (Su et al., 2004). Metabolic syndrome, which includes obesity, insulin resistance, hyperglycemia, hypertension, hypertriglyceridemia, and low serum HDL cholesterol levels, is regulated by PPAR- as a transcription factor (Grundy, 2016). Possible symptoms in these mice include decreased plasma leptin levels, lipodystrophy, decreased lipocalin levels, organomegaly, IR, increased free fatty acids and hypotension (Pap et al., 2016). Given these characteristics, PPAR knock-out models are valuable for studying diabetes within the broader scope of metabolic syndrome research (Grundy, 2016). Transcription Factor 4 (PAX4) is essential for the presence and maintenance of pancreatic beta cells during pancreatic development in mammals (Napolitano et al., 2015). It is a key factor associated with T1DM, T2DM and MODY9(Maturity-Onset Diabetes of the Young) in adults (Anık et al., 2015), as well as ketosis-prone diabetes and other diabetes subtypes (Mauvais et al., 2004). In PAX4+/- rabbit models,

generated through PAX4 gene knock-out, the phenotype includes reduced beta-cell numbers, increased alpha-cell numbers, persistent hyperglycemia, diabetic nephropathy, hepatopathy, myopathy, and cardiomyopathy (Xu et al., 2018). Because of these features, PAX4+/- rabbits have a very important place to investigate DM conditions and their complications (Zhang et al., 2023).

Type 2 Diabetes Models Induced by High-Fat Diet

High-fat diet models are suitable for creating a picture of obesity, hyperinsulinemia, and disrupted glucose homeostasis that is amenable to undercompensation in the islets (Winzell ve Ahren, 2004). This method replaces a normal diet (typically consisting of ~26% protein, ~63% carbohydrates, and ~11% fat by caloric content) with a high-fat diet where approximately 58% of energy is derived from fat. Careful monitoring of food intake is necessary to ensure proper consumption. Since environmental factors play a more significant role in obesity development than genetic predisposition, diet-induced obesity (DIO) models are considered more relevant than genetically engineered models. However, the animal's metabolism must be compatible with obesity induction. The heterogeneous response of C57BL/6 mice to HFD indicates that the observed metabolic effects are not solely dependent on genetic factors (Burcelin et al., 2002). Thus, even in diet-induced models, targeting beta-cell function highlights the importance of genetic susceptibility (Surwit et al., 1995; Bachmanov et al., 2001; King, 2012). In Jerusalem gerbils (*Psammomys obesus*), different metabolic stages can be classified as: normoglycemic and normoinsulinemic (Level A), normoglycemic and hyperinsulinemic (Level B), hyperglycemic and hyperinsulinemic (Level C), hyperglycemic and insulinopenic (Level D) (Shafrir et al., 2006). These animals do not exhibit hyperphagia, but develop hyperinsulinemia, obesity, and diabetes when exposed to a high-energy diet (Ziv et al., 1999). The Nile grass rat (*Arvicanthis niloticus*) naturally develops obesity, dyslipidemia, and hyperglycemia under normal dietary conditions, making it a suitable model for metabolic syndrome studies (Noda et al., 2010).

Non-Rodent Animal Models for Diabetes

Non-Rodent T1DM Models

Large mammals can be used as diabetes models. Dogs, pigs, and primates possess a comparable portal vein size and islet structure similar to humans, making them suitable for hyperglycemic diabetic animal models. These characteristics also provide clinical guidance for diabetes research (Sawaya et al., 2020). Unlike rodents, non-rodent animals do not naturally develop Type 1 Diabetes (T1DM). Therefore, T1DM mod-

els in these species are predominantly induced through pancreatectomy or streptozotocin (STZ) administration (King, 2012). Pancreatectomy is a method used to induce hyperglycemia in animals, and its effects have been demonstrated in dogs (McClaran et al., 2007), pigs (Kin et al., 2005), and primates (Zhu et al., 2014). However, due to its invasive surgical nature, hypoglycemia management must be carefully monitored (He et al., 2011). Type 1 diabetes models in non-rodent animals can also be induced using chemical agents, primarily STZ and alloxan. A dose of 50 mg/kg is sufficient for creating a permanent diabetes model in rats and Cynomolgus monkeys, while 150 mg/kg is required in pigs. However, partial recovery from diabetes symptoms may occur four weeks post-administration. Increasing the dose to 200 mg/kg may lead to kidney and liver toxicity (Dufrane et al., 2006). A combination of pancreatectomy and STZ administration is also an option, as it allows for lower STZ usage (He et al., 2011). Additionally, low-dose repeated STZ administration is another alternative method (Wei et al., 2011).

Non-Rodent T2DM Animal Models

In Type 2 Diabetes Mellitus (T2DM) research, non-rodent animals have also been used. Cats offer advantages in modeling various aspects of T2DM, including disease progression, clinical presentation, physiological mechanisms, and pathological changes. The development of T2DM in middle age is similar in cats and humans. In both species, an association between obesity and IR, damage to beta cells, and islet amyloid formation can be seen (O'Brien, 2002; Henson & O'Brien, 2006). Certain pig breeds also exhibit T2DM-like phenotypes, making them valuable for diabetes research. In pigs, injection of streptozotocin combined with feeding on a diet rich in fat and carbohydrates can produce a model of T2DM that closely resembles human anatomy and metabolism and is associated with atherosclerosis (Bellinger et al., 2006A T2DM model can be induced by low-dose STZ injection in dogs fed a high-fat diet. This method produces a model that accurately reflects the hyperbolic relationship between insulin sensitivity and insulin production observed in humans. Additionally, it demonstrates how multiple organs (pancreas, lien, and adipose tissue) coordinate their functions to maintain normal blood glucose levels (Ionut et al., 2010). In recent years, zebrafish (*Danio rerio*) and Drosophila have been increasingly used in T2DM research due to their favorable physiological and genetic characteristics, enabling quantitative and high-throughput analyses. The 87% genetic similarity between zebrafish and humans is strikingly evident in pancreatic structure and glucose metabolism. Zebrafish have some features that offer advantages over other laboratory animals. Early maturation, rapid generation, easy housing conditions, the ability to establish a large number of colonies with their small size, and

their low economic costs, having a transparent embryo and the course of internal organ differentiation are among these. These features make zebrafish a highly suitable model for studying the mechanisms and progression of diabetes. Similarly, Drosophila, whose genetic sequencing is well-established, has been widely used in research due to its biological similarities to humans. It is particularly useful in fundamental diabetes research, especially for studying gene regulation processes (Zhang et al., 2023).

Conclusion

Research findings emphasize the importance of considering animal physiology, human physiology, and metabolic characteristics when developing rodent and non-rodent animal models for diabetes studies. Additionally, the chemical properties and precise dosing of the agents used must be carefully determined. With advancements in genetic science, the development of model animals with specific genetic traits for diabetes research is becoming increasingly feasible. While rodents remain the most widely used animal models, the expanding understanding of new species and the promotion of animal welfare suggest that the use of alternative animal models will continue to grow. With greater species diversity, the ability to model human diabetes more accurately and specifically will significantly improve.

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