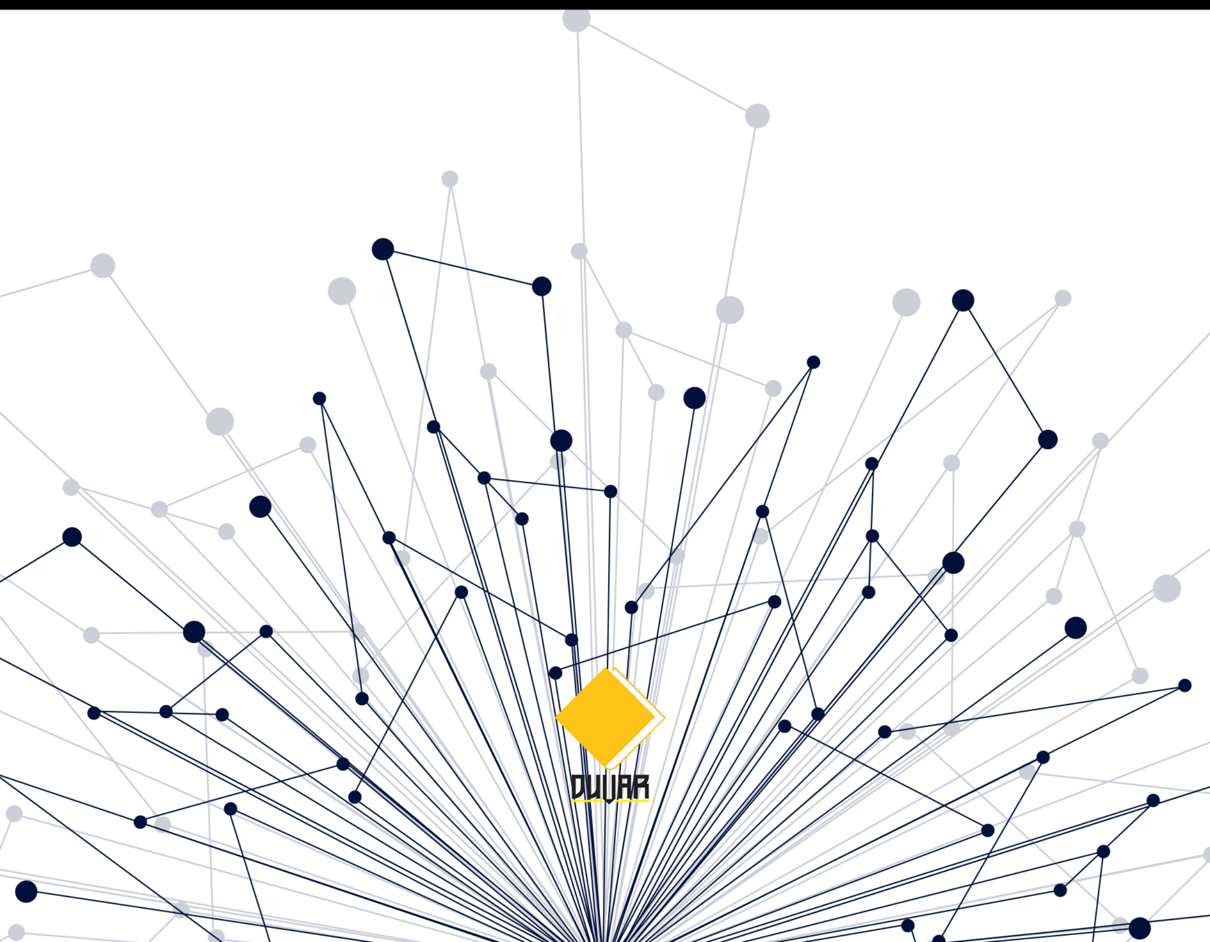
THEORETICAL AND APPLIED RESEARCH IN HEALTH SCIENCES

Editors
Assoc. Prof. Dr. Sadettin DEMİREL
Asst. Prof. Dr. Feyza DERELİ



THEORETICAL AND APPLIED RESEARCH IN HEALTH SCIENCES

Editors
Assoc. Prof. Dr. Sadettin DEMİREL
Asst. Prof. Dr. Feyza DERELİ

Theoretical And Applied Research In Health Sciences
Editors: Assoc. Prof. Dr. Sadettin DEMİREL
Asst. Prof. Dr. Feyza DERELİ

Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : October -2025
Publisher Certificate No: 49837
ISBN: 978-625-8734-07-2

853 Sokak No:13 P.10 Kemeraltı-Konak/İzmir
Tel: 0 232 484 88 68
www.duvaryayinlari.com
duvarkitabevi@gmail.com

TABLE OF CONTENTS

Chapter 1

Drugs Causing Anticholinergic Burden and Management of Anticholinergic Burden in the Geriatric Population

Melisa Sultan TEKŞAHİN[1], Ahmet ÇAKIR[2]

Although a universally accepted definition is lacking in the literature, individuals aged 65 years and above are conventionally regarded as belonging to the elderly population (1). In this age group, the aging process is characterized as a biological phenomenon marked by progressive physiological alterations occurring at both the tissue and organismal levels (2). The most prominent aspect of this process is the decrease in functional units in the organs over time (3). The aging process is accompanied by differences in the biochemical structure of tissues, a gradual decline in physiological capacity, and increased susceptibility to disease (2). These changes in the body cause multiple diseases to gradually emerge (4).

With aging, there is a decrease in total body water and lean body mass. In contrast, the increase in body fat leads to changes in the distribution profile of drugs. The distribution volume increases, especially in lipophilic drugs, leading to longer half-lives and accumulation in the body (5).

The kidneys are the elimination site for waste products and drug metabolites in the blood (6). It is known that there is a progressive decrease in glomerular filtration rate (GFR) of approximately 8 mL/minute every ten years (7). The age-related decline in GFR is associated with a decrease in arterial blood flow and functional nephron count, as well as with diseases such as diabetes and hypertension (6). Accurate estimation of kidney function is critically important in the care of geriatric patients (8). In geriatric patients, a significant decrease in GFR can lead to impaired drug excretion by the kidneys, toxicity due to increased blood concentrations, and the development of significant chronic kidney disease (CKD) (9). Regular GFR monitoring is recommended for medications used in long-term treatments prescribed for chronic conditions with renal impairment (10). Certain medications, such as vancomycin, gentamicin, tobramycin, some

[1] Research assistant, Inonu University, Faculty of Pharmacy, ORCID ID: 0009-0008-0514-9476
[2] Assistant Professor, Inonu University, Faculty of Pharmacy, Department of Clinical Pharmacy, ORCID ID: 0000-0002-9843-1604

antiepileptics (phenytoin, carbamazepine, oxcarbazepine, valproic acid), and methotrexate, may require dose reduction in the elderly (11).

With age, liver mass and hepatic blood flow decrease, and the bioavailability of drugs undergoing extensive first-pass metabolism increases. First-pass clearance decreases by approximately 1% per year after age 40, leading to increased plasma drug levels with oral doses in older adults and an increased risk of side effects and toxicity (12).

The prevalence of chronic diseases such as Alzheimer's disease, cardiovascular diseases, stroke, and diabetes increases significantly with aging. Additionally, it is common for older individuals to have multiple chronic conditions. This complicates treatment processes, leading to increased hospital admissions and multiple drug use (13). When evaluating medication use in the geriatric population in terms of associated risks, if these risks outweigh the potential benefits, the medication may be classified as potentially inappropriate. Potentially inappropriate medication (PIM) use is associated with adverse drug reactions, cognitive and physical decline, increased fall frequency, higher hospitalization rates, and increased mortality rates (14). In this population, the use of PIMs in individuals receiving polypharmacy requires serious evaluation and careful clinical approach in prescribing processes (15). Although there is no universally accepted definition, the simultaneous use of five or more medications is referred to as polypharmacy (13). In addition, hyperpolypharmacy is most commonly defined as the simultaneous use of ten or more medications (16, 17). Older individuals pose a challenge in this regard because physicians struggle to maintain the delicate balance between the need to limit the number of prescribed medications and the potential benefits of administering potentially beneficial treatments (18). Polypharmacy and inappropriate prescribing are important indicators of inappropriate medication use and can lead to increased healthcare expenditures due to adverse drug reaction (ADR), drug-drug and drug-disease interactions, treatment non-compliance, and increased morbidity and mortality (19). The physiological changes that occur with aging significantly affect the pharmacokinetic and pharmacodynamic properties of drugs. This situation leads to changes in the side effect profile, increased drug interactions, and adverse drug reactions in elderly individuals exposed to polypharmacy (5). It has been reported that the incidence of ADRs increases with age and that the rate of hospital admissions due to ADRs in individuals aged 65 and over is approximately twice as high as in young adults (20).

The susceptibility of older adults to adverse drug reactions and other undesirable effects of drug exposure is not solely related to advancing age. It is also associated with the gradual accumulation of patient-specific pathophysiological changes. Pharmacokinetics, pharmacodynamics, and clinical outcomes are influenced by patient-specific factors, including age, gender, ethnicity, genetics, disease processes, polypharmacy, drug dosage and frequency, social factors, and many others (2).

These changes associated with the aging process make geriatric individuals more susceptible to the side effects of medications. This situation has clinically significant consequences, particularly with regard to anticholinergic drugs that affect the central nervous system and the autonomic nervous system. It is crucial for clinicians to adhere to appropriate prescribing principles in order to prevent polypharmacy and related adverse drug reactions in elderly individuals. Some systematic assessment tools developed for this purpose facilitate the identification of PIMs use in geriatric patients (21). Determining appropriate criteria to prevent PIMs in elderly patients is of vital importance. The literature contains tools aimed at reducing the side effects of polypharmacy. These tools differ in scope and application. Some provide a general assessment by focusing on medications that should be avoided in all elderly individuals, while others adopt a more personalized approach by considering factors such as life expectancy, existing diseases, and individual clinical characteristics. Tools are categorized as explicit and implicit. Explicit tools have predefined criteria-based lists and are applied based on records without requiring clinical judgment. Implicit tools, on the other hand, involve individualized assessments based on the evaluator's clinical expertise (22).

The most commonly used criteria for assessing potentially inappropriate prescribing in individuals aged 65 and older are: Beers Criteria (23), TIME Criteria (24), PRISCUS List (25), START-STOPP Criteria (26), FORTA List (27), NORGEP (28) and EU(7)-PIM List (29). Beers Criteria are the most frequently used tool for assessing potentially inappropriate prescribing (22).

Medication management in geriatric patients requires special consideration in terms of both pharmacological efficacy and potential adverse effects. Therefore, certain drug classes are considered potentially risky in geriatric patients. Benzodiazepines, antipsychotics, nonsteroidal anti-inflammatory drugs (NSAIDs), anticholinergic drugs, and antidiabetics with a high risk of hypoglycemia are among the risky drug groups (30). To support the safe prescribing of high-risk medications, the tools mentioned in clinical practice are widely used.

When high-risk medications are evaluated among themselves, drugs with anticholinergic properties are of particular importance in the geriatric population. These drugs are used in the treatment of common clinical conditions such as depression, urinary incontinence, allergic diseases, and Parkinson's disease.

When evaluated in terms of their side effect profile in elderly individuals, they have been associated with cognitive decline, delirium, falls, and increased mortality (31).

Therefore, the assessment of anticholinergic burden and potentially inappropriate prescribing plays a critical role in geriatric medication management.

The purpose of this section is to examine the adverse effects associated with anticholinergic drug use in the geriatric population and their clinical outcomes, as well as to present evidence-based strategies for managing anticholinergic burden.

Anticholinergic Drug Burden

Various medications and drug classes are associated with cognitive impairment, ranging from acute confusion to chronic deficits. In this situation, anticholinergic drugs play an important role, because the cholinergic system plays a critical role in cognitive functions such as attention, awareness, and processing of environmental stimuli (32). Anticholinergic drugs are commonly included in the standard treatment of conditions such as urinary incontinence, asthma, and psychiatric disorders in older adults, with prescription rates ranging from 20% to 50% (33). Approximately 10% of individuals aged 65 and older are estimated to use anticholinergic drugs on a regular basis (34, 35). Many medications commonly prescribed to older adults exert anticholinergic effects by inhibiting acetylcholine, a neurotransmitter essential for learning and memory, through interactions with the central nervous system (36). Numerous studies have reported an association between anticholinergic drug use and an elevated risk of adverse outcomes, including dementia, cognitive impairment, and delirium (37, 38). In addition, constipation, dry skin, mouth, and eyes are among the side effects (39). Anticholinergic side effects stem from drugs' affinity for muscarinic receptors, which is the primary determinant of anticholinergic burden. Serum Anticholinergic Activity (SAA), measured via a radioreceptor assay, has long been regarded as the gold standard for quantifying this muscarinic receptor binding. However, its utility is constrained by several factors: it is expensive, requires specialized laboratory facilities, and presents limitations in clinical settings (40). Various anticholinergic scoring systems have been created to predict the likelihood of side effects associated with anticholinergic drugs. These scales include the Anticholinergic Drug Scale (41), Anticholinergic Burden Classification (42), Clinician-rated Anticholinergic Score (43), Anticholinergic Risk Scale (44), Anticholinergic Cognitive Burden Scale (45), Anticholinergic Activity Scale (46), and Anticholinergic Loading Scale (47). Among these, the most validated is the Anticholinergic Cognitive Burden (ACB) Scale (48). In this scale, drugs are graded based on their anticholinergic potential, ranging from 0 (no activity) to 3 (highest activity). Medications with high anticholinergic burden scores are listed in Table 1 (36, 45, 49).

Table 1. Medications with high anticholinergic burden

Medication	**Pharmacological group**	**Medication**	**Pharmacological group**
Chlorpromazine	Antipsychotics	Carbinoxamine	Antihistamines
Clozapine		Chlorpheniramine	
Levomepromazine		Clemastine	
Olanzapine		Cyproheptadine	
Perphenazine		Dexchlorpheniramine	
Quetiapine		Dicyclomine	
Thioridazine		Dimenhydrinate	
Trifluoperazine		Diphenhydramine	
Atropine	Anticholinergic	Doxylamine	
Biperiden		Hydroxyzine	
Benztropine		Meclizine	
Darifenacin		Promethazine	
Fesoterodine		Amitriptyline	Antidepressants
Hyoscyamine		Amoxapine	
Orphenadrine		Clomipramine	
Oxybutynin		Desipramine	
Procyclidine		Doxepin	
Propantheline		Imipramine	
Propiverine		Nortriptyline	
Scopolamine		Opipramol	
Solifenacin		Protriptyline	
Tolterodine		Trimipramine	
Trihexyphenidyl		Paroxetine	
Each medication yields 3 points according to the ACB Scale.			

As anticholinergic burden increases in the elderly population, outcomes associated with neurocognitive impairment have been found to increase (50). An overall score of three or above signifies a clinically meaningful anticholinergic burden (45). There is a significant association between increased anticholinergic drug burden and readmission to hospitals and emergency departments (51). Medications with low to moderate anticholinergic burden scores become risky for patients when used concomitantly. Medications with low to moderate anticholinergic burden scores are listed in Table 2 (36, 49).

Table 2. Medications with low to moderate anticholinergic burden

Medication	**Pharmacological group**	**Medication**	**Pharmacological group**
Amantadine*	Anti-Parkinson Agent	Cortisone	Corticosteroid
Bromocriptine		Dexamethasone	
Carbidopa-Levodopa		Hydrocortisone	
Entacapone		Methylprednisolone	
Pramipexol		Prednisolone	
Selegiline		Prednisone	
Bupropion	Antidepressants	Codeine	Opioids
Escitalopram		Fentanyl	
Fluoxetine		Hydromorphone	
Fluvoxamine		Morphine	
Maprotiline*		Meperidine*	
Mirtazapine		Methadone*	
Nortriptyline		Oxycodone	
Sertraline		Tramadol*	
Fluphenazine*	Antipsychotics	Atenolol	Cardiovascular
Haloperidol		Benazepril	
Loxapine*		Betaxolol	
Pimozide*		Captopril	
Ziprasidone		Digoxin	
Zuclopenthixol*		Diltiazem	
Alprazolam	Benzodiazepines	Furosemide	
Chlordiazepoxide		Hydralazine	

Clonazepam		Isosorbide mononitrate	
Clorazepate		Isosorbide dinitrate	
Diazepam		Metoprolol	
Flurazepam		Nifedipine	
Lorazepam		Quinidine	
Midazolam		Trandolapril	
Baclofen*	Myorelaxant	Triamterene	
Cyclobenzaprine *		Warfarin	
Each medication yield 2 points according to the ACB Scale.			

The ACB should be calculated for each geriatric patient to prevent possible PIM situations. Patients with multimorbidity or discharged from acute illness are often on multiple medications; they are at risk of using PIMs and medications with anticholinergic burden (52, 53). Studies have shown that indicators of drug use, including the presence of PIM, polypharmacy and anticholinergic burden, are associated with increased frequency of health care needs, cognitive impairment and mortality (54, 55). In light of these considerations, a detailed evaluation of patients' pharmacotherapy is warranted, especially regarding medications associated with elevated anticholinergic burden.

Anticholinergic Burden Management

ACB management is critically important in the geriatric population. The use of drugs with anticholinergic properties further increases clinical risks, particularly in elderly populations receiving polypharmacy. Therefore, regular monitoring and management of anticholinergic burden is essential.

Interventions usually begin with determining the appropriate method after calculating the anticholinergic burden. Various strategies have been developed to reduce the ACB in order to avoid adverse outcomes (56). There is no clear information in the literature regarding the implementation of the developed strategies. It is essential to make decisions based on patient-specific factors such as comorbidities, polypharmacy, and drug suitability.

ACB can be reduced through regular medication reviews. Discontinuing medication may be appropriate for drugs with limited effectiveness or those that have been used inappropriately for a long time (56, 57). It has been shown that deprescribing in older adults reduces side effects, improves medication adherence, and lowers healthcare costs (58). This situation emphasizes the individualization of treatment as a component of personalized care in high-risk populations (56).

Another approach to ACB management is the evaluation of alternative options. This refers to reassessing safe alternatives to re-establish the appropriate treatment for the patient. It is an important step in maintaining the same therapeutic efficacy while minimizing side effects. For example, in conditions such as depression and urinary incontinence, drugs with low anticholinergic effects may be preferred (59).

ACB can be reduced in patients through regular medication reviews. Treatment success can be achieved by evaluating comorbidities and concomitant medications in patients. At the same time, evaluating the patient's compliance profile and monitoring cognitive functions and other side effects can also be beneficial.

A multidisciplinary approach is increasingly important in ACB management. Collaboration between clinical pharmacists, physicians and nurses plays a critical role in ensuring proper medication reconciliation, reviewing patients' medication lists, evaluating appropriate medications and offering alternatives, assessing anticholinergic effects, and implementing medication discontinuation plans (60, 61). In addition to clinical pharmacists, community pharmacists, who are among the most accessible healthcare professionals in primary care, also make significant contributions to providing medication support in ACB management (62).

In conclusion, ACB management is not limited to discontinuing or changing specific medications. It is a comprehensive process that requires patient-centered individualized assessment, continuous monitoring, and multidisciplinary collaboration. In clinical practice, discontinuing inappropriate medications, exploring alternative treatment options, regularly reviewing medications, and using ACB calculation tools constitute the core components of this process. Managing anticholinergic burden directly contributes to improving cognitive and physical health outcomes in the geriatric population. It also offers positive contributions to the healthcare system in terms of costs when evaluated in terms of side effects and increased hospital admissions.

REFERENCES

1. Shreya D, Fish PN, Du D. Navigating the Future of Elderly Healthcare: A Comprehensive Analysis of Aging Populations and Mortality Trends Using National Inpatient Sample (NIS) Data (2010-2024). Cureus. 2025;17(3):e80442.
2. Rodrigues MC, Oliveira C. Drug-drug interactions and adverse drug reactions in polypharmacy among older adults: an integrative review. Rev Lat Am Enfermagem. 2016;24:e2800.
3. Ngcobo NN. Influence of Ageing on the Pharmacodynamics and Pharmacokinetics of Chronically Administered Medicines in Geriatric Patients: A Review. Clin Pharmacokinet. 2025;64(3):335-67.
4. Mangoni AA, Jarmuzewska EA. Incorporating pharmacokinetic data into personalised prescribing for older people: challenges and opportunities. Eur Geriatr Med. 2021;12(3):435-42.
5. Mangoni AA, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol. 2004;57(1):6-14.
6. Goyal K, Afzal M, Altamimi ASA, Babu MA, Ballal S, Kaur I, et al. Chronic kidney disease and aging: dissecting the p53/p21 pathway as a therapeutic target. Biogerontology. 2024;26(1):32.
7. Wazzan AAA, Tommelein E, Foubert K, Bonassi S, Onder G, Somers A, et al. Development and Application of the GheOP(3)S-Tool Addendum on Potentially Inappropriate Prescribing (PIP) of Renally Excreted Active Drugs (READs) in Older Adults with Polypharmacy. Drugs Aging. 2018;35(4):343-64.
8. Cartet-Farnier E, Goutelle-Audibert L, Maire P, De la Gastine B, Goutelle S. Implications of using the MDRD or CKD-EPI equation instead of the Cockcroft-Gault equation for estimating renal function and drug dosage adjustment in elderly patients. Fundam Clin Pharmacol. 2017;31(1):110-9.
9. Doogue MP, Polasek TM. Drug dosing in renal disease. Clin Biochem Rev. 2011;32(2):69-73.
10. Sesso R, Prado F, Vicioso B, Ramos LR. Prospective study of progression of kidney dysfunction in community-dwelling older adults. Nephrology (Carlton). 2008;13(2):99-103.
11. Ebert N. [Novel equations for estimating renal function: significance for drug dose adjustment]. Inn Med (Heidelb). 2024;65(3):280-5.
12. Ruiz A, DiCristina S. Absorption to Excretion: The Aging Body’s Take on Drugs – A Review of Pharmacokinetic Changes and their Impact on Medication Management. Current Pharmacology Reports. 2025;11(1):42.

13. Pazan F, Wehling M. Polypharmacy in older adults: a narrative review of definitions, epidemiology and consequences. Eur Geriatr Med. 2021;12(3):443-52.
14. Rantsi M, Hyttinen V, Jyrkka J, Vartiainen AK, Kankaanpaa E. Process evaluation of implementation strategies to reduce potentially inappropriate medication prescribing in older population: A scoping review. Res Social Adm Pharm. 2022;18(3):2367-91.
15. Endalifer BL, Kassa MT, Ejigu YW, Ambaye AS. Polypharmacy, drug-drug interactions, and potentially inappropriate medications among older adults: a cross-sectional study in Northeast Ethiopia. Front Public Health. 2025;13:1525079.
16. Toh JJY, Zhang H, Soh YY, Zhang Z, Wu XV. Prevalence and health outcomes of polypharmacy and hyperpolypharmacy in older adults with frailty: A systematic review and meta-analysis. Ageing Res Rev. 2023;83:101811.
17. Rana R, Choi J, Vordenberg SE. Older adults' willingness to consider deprescribing when experiencing hyperpolypharmacy. J Am Pharm Assoc (2003). 2022;62(4):1189-96.
18. Weng MC, Tsai CF, Sheu KL, Lee YT, Lee HC, Tzeng SL, et al. The impact of number of drugs prescribed on the risk of potentially inappropriate medication among outpatient older adults with chronic diseases. QJM. 2013;106(11):1009-15.
19. Wastesson JW, Morin L, Tan ECK, Johnell K. An update on the clinical consequences of polypharmacy in older adults: a narrative review. Expert Opin Drug Saf. 2018;17(12):1185-96.
20. Lavan AH, Gallagher P. Predicting risk of adverse drug reactions in older adults. Ther Adv Drug Saf. 2016;7(1):11-22.
21. De Las Salas R, Vaca-Gonzalez C, Eslava-Schmalbach J, Torres-Espinosa C, Figueras A. Tackling potentially inappropriate prescriptions in older adults: development of deprescribing criteria by consensus from experts in Colombia, Argentina, and Spain. BMC Geriatr. 2023;23(1):682.
22. Mose K, Lundby C, Rasmussen L, Pottegard A. Tools for Evaluating Potentially Inappropriate Medication Use in Older Adults: A Scoping Review Assessing Suitability for Register-Based Research. Basic Clin Pharmacol Toxicol. 2025;137(4):e70094.
23. American Geriatrics Society Beers Criteria Alternatives P, Steinman MA. Alternative Treatments to Selected Medications in the 2023 American Geriatrics Society Beers Criteria(R). J Am Geriatr Soc. 2025;73(9):2657-77.

24. Bahat G, Erdogan T, Can B, Ozkok S, Ilhan B, Tufan A, et al. Cross-Cultural Adaptation and Clinical Validation of TIME Criteria to Detect Potentially Inappropriate Medication Use in Older Adults: Methodological Report from the TIME International Study Group. Drugs Aging. 2025;42(1):57-67.
25. Mann NK, Mathes T, Sonnichsen A, Pieper D, Klager E, Moussa M, et al. Potentially Inadequate Medications in the Elderly: PRISCUS 2.0. Dtsch Arztebl Int. 2023;120(1-2):3-10.
26. Abegaz TM, Birru EM, Mekonnen GB. Potentially inappropriate prescribing in Ethiopian geriatric patients hospitalized with cardiovascular disorders using START/STOPP criteria. PLoS One. 2018;13(5):e0195949.
27. Schulze Westhoff M, Groh A, Schroder S, Proskynitopoulos PJ, Jahn K, Klietz M, et al. Potentially inappropriate medications according to PRISCUS list and FORTA (Fit fOR The Aged) classification in geriatric psychiatry: a cross-sectional study. J Neural Transm (Vienna). 2022;129(11):1367-75.
28. Nyborg G, Brekke M, Straand J, Gjelstad S, Romoren M. Potentially inappropriate medication use in nursing homes: an observational study using the NORGEP-NH criteria. BMC Geriatr. 2017;17(1):220.
29. Erszegi A, Csupor D, Bodo G, Engi Z, Bahar MA, Matuz M, et al. High rate of potentially inappropriate medication use in older people: a case-control study. Geroscience. 2024;46(5):5217-33.
30. Choo A. Potentially inappropriate medicines for older people: consensus-based lists. Aust Prescr. 2025;48(4):128-32.
31. Poonawalla IB, Xu Y, Gaddy R, James A, Ruble M, Burns S, et al. Anticholinergic exposure and its association with dementia/Alzheimer's disease and mortality in older adults. BMC Geriatr. 2023;23(1):401.
32. Lemstra AW, Eikelenboom P, van Gool WA. The cholinergic deficiency syndrome and its therapeutic implications. Gerontology. 2003;49(1):55-60.
33. Campbell N, Boustani M, Limbil T, Ott C, Fox C, Maidment I, et al. The cognitive impact of anticholinergics: a clinical review. Clin Interv Aging. 2009;4:225-33.
34. Chuang YF, Elango P, Gonzalez CE, Thambisetty M. Midlife anticholinergic drug use, risk of Alzheimer's disease, and brain atrophy in community-dwelling older adults. Alzheimers Dement (N Y). 2017;3(3):471-9.
35. Esin E, Ergen A, Cankurtaran M, Yavuz BB, Halil M, Ulger Z, et al. Influence of antimuscarinic therapy on cognitive functions and quality of

life in geriatric patients treated for overactive bladder. Aging Ment Health. 2015;19(3):217-23.
36. Lopez-Alvarez J, Sevilla-Llewellyn-Jones J, Aguera-Ortiz L. Anticholinergic Drugs in Geriatric Psychopharmacology. Front Neurosci. 2019;13:1309.
37. Taylor-Rowan M, Edwards S, Noel-Storr AH, McCleery J, Myint PK, Soiza R, et al. Anticholinergic burden (prognostic factor) for prediction of dementia or cognitive decline in older adults with no known cognitive syndrome. Cochrane Database Syst Rev. 2021;5(5):CD013540.
38. Pieper NT, Grossi CM, Chan WY, Loke YK, Savva GM, Haroulis C, et al. Anticholinergic drugs and incident dementia, mild cognitive impairment and cognitive decline: a meta-analysis. Age Ageing. 2020;49(6):939-47.
39. Kalisch Ellett LM, Pratt NL, Ramsay EN, Barratt JD, Roughead EE. Multiple anticholinergic medication use and risk of hospital admission for confusion or dementia. J Am Geriatr Soc. 2014;62(10):1916-22.
40. Lampela P, Taipale H, Lavikainen P, Hartikainen S. The effect of comprehensive geriatric assessment on anticholinergic exposure assessed by four ranked anticholinergic lists. Arch Gerontol Geriatr. 2017;68:195-201.
41. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Culp KR. The Anticholinergic Drug Scale as a measure of drug-related anticholinergic burden: associations with serum anticholinergic activity. J Clin Pharmacol. 2006;46(12):1481-6.
42. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. BMJ. 2006;332(7539):455-9.
43. Han L, Agostini JV, Allore HG. Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. J Am Geriatr Soc. 2008;56(12):2203-10.
44. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. Arch Intern Med. 2008;168(5):508-13.
45. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of Anticholinergics on the Aging Brain: A Review and Practical Application. Aging Health. 2008;4(3):311-20.
46. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. J Neurol Neurosurg Psychiatry. 2010;81(2):160-5.

47. Sittironnarit G, Ames D, Bush AI, Faux N, Flicker L, Foster J, et al. Effects of anticholinergic drugs on cognitive function in older Australians: results from the AIBL study. Dement Geriatr Cogn Disord. 2011;31(3):173-8.
48. Salahudeen MS, Duffull SB, Nishtala PS. Anticholinergic burden quantified by anticholinergic risk scales and adverse outcomes in older people: a systematic review. BMC Geriatr. 2015;15:31.
49. Ramos H, Moreno L, Perez-Tur J, Chafer-Pericas C, Garcia-Lluch G, Pardo J. CRIDECO Anticholinergic Load Scale: An Updated Anticholinergic Burden Scale. Comparison with the ACB Scale in Spanish Individuals with Subjective Memory Complaints. J Pers Med. 2022;12(2).
50. Pasina L, Djade CD, Lucca U, Nobili A, Tettamanti M, Franchi C, et al. Association of anticholinergic burden with cognitive and functional status in a cohort of hospitalized elderly: comparison of the anticholinergic cognitive burden scale and anticholinergic risk scale: results from the REPOSI study. Drugs Aging. 2013;30(2):103-12.
51. Liang CK, Chou MY, Hsu YH, Wang YC, Liao MC, Chen MT, et al. The association of potentially inappropriate medications, polypharmacy and anticholinergic burden with readmission and emergency room revisit after discharge: A hospital-based retrospective cohort study. Br J Clin Pharmacol. 2023;89(1):187-200.
52. Grossi CM, Richardson K, Savva GM, Fox C, Arthur A, Loke YK, et al. Increasing prevalence of anticholinergic medication use in older people in England over 20 years: cognitive function and ageing study I and II. BMC Geriatr. 2020;20(1):267.
53. Hsu WH, Wen YW, Chen LK, Hsiao FY. Comparative Associations Between Measures of Anti-cholinergic Burden and Adverse Clinical Outcomes. Ann Fam Med. 2017;15(6):561-9.
54. Clark CM, Shaver AL, Aurelio LA, Feuerstein S, Wahler RG, Jr., Daly CJ, et al. Potentially Inappropriate Medications Are Associated with Increased Healthcare Utilization and Costs. J Am Geriatr Soc. 2020;68(11):2542-50.
55. Davies LE, Spiers G, Kingston A, Todd A, Adamson J, Hanratty B. Adverse Outcomes of Polypharmacy in Older People: Systematic Review of Reviews. J Am Med Dir Assoc. 2020;21(2):181-7.
56. Braithwaite E, Todd OM, Atkin A, Hulatt R, Tadrous R, Alldred DP, et al. Interventions for reducing anticholinergic medication burden in older adults-a systematic review and meta-analysis. Age Ageing. 2023;52(9).
57. Decaix T, Dumurgier J, Cognat E, Gotze K, Mouton-Liger F, Hugon J, et al. Dual risk of anticholinergic burden and CSF alzheimer's biomarkers in

older patients: a mortality follow-up study from daily medical practice. Alzheimers Res Ther. 2025;17(1):162.

58. Griffiths R, Lim S, Lin J, Bates A, Jones L, Ibrahim K. Deprescribing Anticholinergic Medications in Hospitalised Older Adults: A Systematic Review. Basic Clin Pharmacol Toxicol. 2025;137(4):e70103.
59. Jamieson H, Nishtala PS, Bergler HU, Weaver SK, Pickering JW, Ailabouni NJ, et al. Deprescribing Anticholinergic and Sedative Drugs to Reduce Polypharmacy in Frail Older Adults Living in the Community: A Randomized Controlled Trial. J Gerontol A Biol Sci Med Sci. 2023;78(9):1692-700.
60. Tiranaprakij P, Srisuma S, Putthipokin K, Ruangritchankul S. Anticholinergic burden and clinical outcomes among older adults admitted in a tertiary hospital: a prospective cohort study. PLoS One. 2025;20(9):e0332946.
61. Deglow B, Embree H, Chen J. Pharmacist-Driven Deprescribing to Reduce Anticholinergic Burden in Veterans With Dementia. Fed Pract. 2024;41(12):408-12.
62. Shawaqfeh B, Hughes CM, McGuinness B, Barry HE. Community pharmacists' knowledge of, and role in, managing anticholinergic burden among patients with dementia in primary care: a cross-sectional survey study. Int J Clin Pharm. 2025;47(2):345-53.

Chapter 2

Facilitating Cognitive Reserve in the Elderly Through Exercise-Induced Neuroplasticity Mechanisms

Burak BUĞDAY[1], Selman BÖLÜKBAŞI[2]

1. Introduction

Aging is characterized by a gradual decline across multiple cognitive domains, including memory, executive functions, and language. This process is a multifaceted phenomenon that affects individuals not only biologically but also psychosocially, profoundly impacting daily functioning. One of the most prominent consequences of aging is the slowing and deterioration of cognitive functions. As the global population ages, cognitive impairment has emerged as an increasingly pressing public health concern. Core mental abilities essential for daily life—such as attention, memory, executive functioning, and problem-solving—tend to diminish with advancing age. While this decline may progress mildly in some individuals, others may experience rapid cognitive deterioration and severe losses in functionality (Acharya et al., 2025; Lindenberger, 2014).

Despite being exposed to similar neurobiological changes, older individuals often exhibit varying levels of cognitive performance, a phenomenon explained by the concept of *cognitive reserve*. Cognitive reserve refers to the brain's capacity to compensate for pathological changes and maintain function through flexible neural mechanisms. It represents the cumulative result of an individual's lifetime intellectual, social, and physical experiences. Factors such as educational attainment, lifestyle, social engagement, and physical activity levels significantly influence the development and robustness of cognitive reserve (Cieri et al., 2025).

In this context, physical activity has increasingly gained attention in recent years as both a protective and supportive factor in healthy cognitive aging. It is now well-established that physical exercise benefits not only the musculoskeletal system but also the central nervous system. Physiological changes occurring in the brain during and after exercise contribute to the maintenance and enhancement of mental functions. Among older adults, regular

---

[1] Dr. Lecturer, Inonu University, Faculty of Health Sciences, Department of Gerontology,
Email: burak.bugday@inonu.edu.tr ORCID: 0000-0001-9806-291X
[2] Dr. Lecturer, Inonu University, Faculty of Health Sciences, Department of Gerontology,
Email: selman.bolukbasi@inonu.edu.tr | ORCID: 0000-0003-3771-4827

physical activity has been shown to slow cognitive decline and, in certain cases, even reverse it. A key biological mechanism underlying these effects is neuroplasticity—the brain's ability to reorganize itself by forming new neural connections, as well as strengthening or weakening existing synaptic pathways. Although neuroplasticity naturally decreases with age, it does not vanish entirely. Exercise acts as a potent environmental stimulus that supports neuroplastic restructuring, particularly in brain regions associated with learning and memory (Vint et al., 2024).

Physical exercise, cognitive training, and vitamin D supplementation are low-cost interventions that can enhance both cognitive functioning and mobility in older adults, especially those with Mild Cognitive Impairment (MCI). Although both aerobic and resistance exercises are associated with cognitive benefits, findings across studies are not always consistent. Aerobic activities that promote neuroplasticity—such as walking, dancing, and swimming—have been shown to enhance executive functioning by improving cerebral metabolism. Furthermore, dual-task exercises, which integrate motor and cognitive tasks, are effective in strengthening cognitive capacity. In this regard, combined aerobic and resistance training programs are hypothesized to yield superior cognitive outcomes compared to interventions focused solely on balance or toning. When supplemented with cognitive training and vitamin D, these programs may exert a synergistic effect on cognitive enhancement (Montero-Odasso et al., 2018; Franzén et al., 2019).

Exercise-based interventions designed to support cognitive health in older adults have now become integral components of contemporary physiotherapy practice. To mitigate cognitive decline, preserve independent living, and maintain quality of life, the incorporation of cognitively supportive exercise programs into physiotherapy regimens is increasingly imperative. The modern rehabilitation paradigm embraces the understanding that movement can invigorate not only the body but also the mind (Pieruccini-Faria et al., 2025).

This book chapter aims to examine the protective role of physical exercise against cognitive decline in older adults by exploring the underlying mechanisms of neuroplasticity. Additionally, it will discuss how cognitive reserve can be supported through exercise, identify the types of exercise most effective in this process, and offer insights on how current scientific approaches can be integrated into clinical practice.

2. What is Neuroplasticity?

Recent advances in our understanding of lifelong brain plasticity—supported by findings from behavioral research involving motor learning paradigms—have significantly reshaped assumptions regarding aging and the brain's capacity for learning and neuroplastic adaptation. These discoveries have prompted a re-evaluation of the long-standing belief that age-related motor decline is inevitable and irreversible. Neuroplasticity refers to the central nervous system's ability to undergo structural and functional changes in response to environmental stimuli, experiences, learning, trauma, or rehabilitation. In contrast to the traditional view of the brain as a fixed and immutable organ, the concept of neuroplasticity underscores the nervous system's lifelong capacity for dynamic reorganization and adaptation. This characteristic forms the foundation for both healthy development and recovery processes in disease contexts (Dai et al., 2024; Gooijers et al., 2024).

Neuroplastic mechanisms manifest at both microscopic and macroscopic levels. At the microscopic level, they include processes such as synaptic plasticity, neurogenesis, axonal growth, and dendritic pruning. At the macroscopic level, neuroplasticity involves functional cortical reorganization, changes in brain volume, and remodeling of connectivity networks. Importantly, these changes are not confined to childhood or young adulthood—they are also possible in older adults. In this regard, neuroplasticity holds substantial promise for the compensation of age-related neurological deterioration. Preserving and enhancing neuroplastic capacity in later life is therefore critical for the maintenance of cognitive functions. In an age group increasingly affected by neurodegenerative diseases, the brain's ability to form new connections directly contributes to the preservation of functional independence and quality of life. Thus, neuroplasticity has evolved beyond its origins as a neuroscientific concept into a therapeutic target for health maintenance and intervention (Pauwels, Chalavi, & Swinnen, 2018).

Neuroplasticity is generally categorized into two major forms: functional plasticity and structural plasticity. Functional plasticity refers to the strengthening or weakening of synaptic transmission pathways between neurons, often driven by learning and experiential input. Structural plasticity, on the other hand, involves morphological changes in neural cells, including the formation of new dendritic branches, increases in synapse numbers, or compensatory mechanisms for reduced connectivity. Both forms are complementary and have direct implications for behavioral outcomes.

To sustain neuroplasticity during aging, continuous environmental stimulation is essential. Among the most potent external stimuli is regular physical activity. Exercise promotes physiological changes such as increased cerebral blood flow, enhanced oxygenation, and elevated metabolic activity, all of which facilitate neuronal adaptation. Additionally, exercise stimulates the release of various neurotrophic factors in the brain—particularly Brain-Derived Neurotrophic Factor (BDNF). BDNF supports neuronal survival, promotes the formation of new synaptic connections, and regulates the synthesis of neural proteins essential for synaptic plasticity. In this context, BDNF is widely recognized as a biochemical marker of the neuroplastic effects of exercise (Boecker et al., 2024; Soshi et al., 2021).

Neuroimaging studies conducted among older adults have demonstrated that regular physical activity leads to both structural and functional brain changes, particularly in the hippocampus, prefrontal cortex, and cerebellum—regions critically involved in memory, attention, executive functioning, and motor coordination. Neuroplastic changes in these regions induced by exercise have been associated with improvements in both motor and cognitive performance. Additionally, findings such as increased cortical thickness, preservation of white matter integrity, and enhanced connectivity suggest that exercise may help mitigate the neurodegenerative processes associated with aging (Tarumi et al., 2022).

Neuroplasticity also manifests at the behavioral level. Improvements in new skill acquisition, problem-solving abilities, reaction time, and multitasking performance are indicative of an individual's functional neuroplastic capacity. In this regard, several factors—such as the type, duration, and intensity of exercise, as well as an individual's physical fitness level—modulate the extent of neuroplastic gains. In addition to aerobic exercises, coordination-based activities, dance, yoga, and dual-task training have also been shown to foster neuroplasticity. For older adults, neuroplasticity provides a robust biological foundation for slowing cognitive decline and preserving functional capacity. Maintaining and enhancing this capacity requires continuous cognitive stimulation and the integration of physical activity into daily life.

Approaches rooted in neuroplasticity are increasingly recognized as a priority not only by healthcare professionals but also within public health policies. In this context, physiotherapy practices are progressively aligning with the dynamic nature of the nervous system, aiming to support cognitive health in aging populations. The following table summarizes the key factors influencing neuroplasticity in older adults and their corresponding effects on brain structure, functionality, and behavioral outcomes (Bae et al., 2020).

Table 1. Factors Affecting Neuroplasticity in Aging and Their Outcomes

Category	Factor	Effect on Neuroplasticity	Related Brain Region	Behavioral Outcomes
Environmental Stimulation	Mental stimulation (learning, new skills, problem solving)	Formation of new synaptic pathways, synaptic strengthening	Prefrontal cortex, hippocampus	Improved cognitive performance, faster response times
Physical Activity	Regular aerobic exercise	Increased BDNF, enhanced cortical thickness and connectivity	Hippocampus, cerebellum, motor cortex	Memory improvement, enhanced executive functions
Motor Stimulation	Dance, yoga, coordination exercises	Sensorimotor integration, cerebellar plasticity	Cerebellum, parietal lobe	Better motor control, improved balance and coordination
Neurotrophic Factors	BDNF, IGF-1, VEGF	Increased synaptogenesis and neurogenesis	All cortical and subcortical areas	Synaptic flexibility, enhanced learning capacity
Structural Changes	Dendritic branching, increased number of synapses	Neural network reorganization	Prefrontal cortex, anterior cingulate	Improved multitasking, increased attention
Functional Reorganization	Cortical reorganization of underused regions	Compensation by other brain areas	Somatosensory cortex, motor areas	Functional recovery, adaptive behaviors
Neuroimaging Findings	Brain plasticity shown via fMRI, DTI	Increased brain volume, preserved white matter integrity	Hippocampus, frontal lobes	Slowed cognitive decline
Rehabilitation Inputs	Physiotherapy, neurophysiological exercises	Targeted plasticity, motor cortex reorganization	Motor cortex, premotor areas	Increased independence in daily living activities
Age-Related Potential	Persistence of neuroplastic capacity in later life	Sustained adaptive capacity	Entire central nervous system	Preserved functional independence, maintained quality of life
Sociocultural Factors	Education level, social interaction, cognitive stimulation	Increased cognitive reserve	Default Mode Network, prefrontal cortex	Reduced dementia risk, delayed cognitive deterioration

3. Cognitive Reserve and Aging

Cognitive reserve (CR) is a neuropsychological concept that describes an individual's capacity to maintain cognitive and functional abilities despite neurodegenerative processes or brain pathologies. Developed to explain the disconnect often observed between the severity of neurological damage and clinical symptoms, cognitive reserve offers a crucial framework for understanding individual variability in the aging process. In other words, among two individuals with similar degrees of structural brain damage, one may experience significant cognitive decline, while the other continues to function independently. This disparity reflects the accumulated neural resilience shaped by life-long experiences. A wide range of environmental and individual factors contribute to the formation and development of cognitive reserve. Among the most commonly identified are educational attainment, occupational complexity, frequency of social interaction, engagement in cognitively stimulating activities, and regular physical activity. Each of these variables has been shown to enhance synaptic efficiency and foster compensatory mechanisms by facilitating alternative neural pathways (Erickson et al., 2019; Litke, Garcharna, Jiwani, & Neugroschl, 2021).

Educational attainment is one of the earliest and most robust predictors of cognitive reserve. Beyond formal education, lifelong learning, problem-solving, and language-related activities contribute to strengthening neural plasticity and expanding reserve capacity. However, academic learning alone is not sufficient. Social engagement, creative activities, and occupational cognitive demands are also recognized as significant contributors. Cognitively enriching activities—such as reading, solving puzzles, playing musical instruments, or learning a new language—stimulate activation in the prefrontal cortex, hippocampus, and parietal regions, thus supporting both functional and structural aspects of reserve. These activities exert a neuroprotective effect even when performed in later life stages. Regular participation in such cognitively stimulating tasks has been associated with delayed onset or slower progression of dementia syndromes such as Alzheimer’s disease, as supported by various clinical observations (Lövdén et al., 2020; Riverol et al., 2024).

The influence of physical activity on cognitive reserve can also be explained at the neurobiological level. During aerobic exercise, increased cerebral blood flow and oxygenation promote neural tissue regeneration. Moreover, exercise enhances the release of neurotrophic factors in the brain, strengthening synaptic transmission and supporting the formation of new synaptic connections. These processes may enable individuals to preserve cognitive functioning despite age-related neuronal volume loss. Neuroimaging studies have shown that older adults who engage in regular physical activity demonstrate increased cortical thickness, more robust synaptic networks, and less impairment in memory, attention, and executive functions. The role of cognitive reserve in the aging process is not only protective, but also compensatory. Even when reductions in brain volume or synaptic integrity occur, individuals with high cognitive reserve can maintain functioning by recruiting alternative neural pathways. This phenomenon is conceptualized through mechanisms such as neural re-routing and compensatory activation. For instance, an individual typically reliant on the right hemisphere for a cognitive task may successfully shift that function to the left hemisphere if reserve capacity is high. This flexibility underscores the functional relevance of cognitive reserve (Baker et al., 2017; López et al., 2014).

Cognitive reserve has implications not only at the individual level but also at the societal level. Aging populations are associated with increased healthcare costs and caregiving burdens related to cognitive decline and dementia. Early interventions aimed at bolstering cognitive reserve hold strategic value in mitigating these costs. Across broad domains—from educational systems to public health policies—lifelong access to mental and physical stimulation must be promoted. Programs aimed at enhancing cognitive reserve should be expanded, especially for older adults. Cognitive reserve should be understood not merely as a protective buffer during aging but also as a mechanism of adaptation. Enhancing this reserve positively influences not only brain health, but also quality of life, independence, and an individual's capacity to contribute to society (Facal, Spuch, & Valladares-Rodriguez, 2022).

Accordingly, physiotherapy interventions, along with other health professional practices, should actively promote lifestyle changes that enhance cognitive reserve. This approach constitutes a cornerstone of holistic and preventive aging strategies.

4. Brain-Derived Neurotrophic Factor (BDNF)

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophic protein that plays a vital role in supporting synaptic plasticity, neurogenesis, cellular differentiation, and neuronal survival within both the central and peripheral nervous systems. It is synthesized in high concentrations particularly in brain regions associated with cognitive functions, such as the hippocampus, prefrontal cortex, and basal ganglia. By enhancing the quality of synaptic transmission, BDNF contributes significantly to the maintenance of executive functions including learning, memory, and attention (Mohammadi-Mahdiabadi-Hasani et al., 2025). BDNF facilitates the formation of new synaptic connections by promoting synaptic plasticity, a process mediated through long-term potentiation (LTP), which strengthens synaptic signaling. It also activates several intracellular signaling pathways involved in neural adaptability, including MAPK/ERK, PI3K/Akt, and PLCγ. These signaling cascades contribute to both functional and structural aspects of neuroplasticity (Zheng et al., 2025).

Among all known stimuli, physical exercise is considered one of the most potent enhancers of BDNF expression. Regular moderate-intensity aerobic exercise has been consistently associated with marked increases in BDNF levels in both the bloodstream and the central nervous system. This upregulation is known to slow age-related cognitive decline, preserve synaptic integrity, and support the formation of new neural connections (Fahimi et al., 2017). Naturally declining BDNF levels with age are linked to hippocampal atrophy and synaptic degeneration. Exercise-induced BDNF production is therefore a key mechanism to counteract these degenerative processes. Structural changes observed in the hippocampus, prefrontal cortex, and cerebellum as a result of physical activity are associated with improvements in attention, memory, executive functioning, and motor coordination.

However, to better understand the neurobiological mechanisms underlying these effects, future research should consider the temporal dynamics of exercise-induced plasticity. It is essential to assess both short- and long-term changes in growth factors, cerebral blood flow, brain volume, and memory. In addition, the use of microdialysis in animal models may offer important insights into how circulating growth factors relate to blood flow, hippocampal plasticity, and cognitive performance (Maass et al., 2016).

In physiotherapy practice, interventions that stimulate BDNF secretion are gaining increased relevance. Beyond aerobic exercise, activities such as coordination training, dance, balance exercises, dual-task interventions, and rhythmic movement have all been shown to enhance synaptic plasticity and support cognitive performance. BDNF also manifests at the behavioral level. Improvements observed after exercise in sustained attention, faster response times, enhanced problem-solving ability, and improved performance on multitasking tasks all reflect the clinical implications of this molecular mediator of neuroplasticity.

BDNF serves as a fundamental biological agent in preserving cognitive function in older adults. Exercise-based physiotherapy programs should center on strategies that stimulate BDNF expression, thereby promoting not only physical health but also neurocognitive well-being (Franzén et al., 2019).

5. Exercise and Neuroplasticity: Applied Approaches

5.1 Aerobic Exercises

Aerobic exercises are not only fundamental for systemic physiological activation but also directly influence brain plasticity. Through rhythmic and continuous movements that increase oxygen consumption, aerobic training promotes adaptive responses across both the nervous and biological systems (Radak, Chung, & Goto, 2008).

Animal studies have shown that the combination of physical activity and enriched sensory environments produces the most significant and sustainable effects on adult neuroplasticity. In human contexts, dance has been proposed as the analog to this combined intervention, as it places simultaneous demands on both physical and cognitive systems. Dance is not merely a physical activity but a neurointegrative experience that encompasses sensory, emotional, motor, and cognitive processes. Its rhythmic structure, multitasking nature, and integration with music activate brain areas such as the prefrontal cortex, cerebellum, and hippocampus. As a result, improvements are observed in motor and executive functions, as well as in learning and motivation. In older adults, dance programs have been shown to enhance balance and coordination, while also yielding positive effects on working memory, attention, and cognitive flexibility. Additionally, dance promotes social interaction, thereby contributing to psychosocial well-being (Rehfeld et al., 2018; Müller et al., 2017).

Interval walking—characterized by alternating periods of increased speed and intensity—supports executive functioning by stimulating prefrontal cortex activity. The elevated heart rate and oxygen uptake during this exercise are associated with increased BDNF levels, which, in turn, support synaptic plasticity. When cognitive tasks are integrated into walking routines, they reinforce both motor and cognitive development (Leckie et al., 2014).

Aerobic exercise exerts multifaceted benefits on brain health. It enhances neuronal function through mechanisms such as increased BDNF expression, hippocampal neurogenesis, reduced neuroinflammation, and suppression of oxidative stress. Improvements in endothelial function due to exercise lead to greater cerebral blood flow, which supports cortical thickness, white matter integrity, and neural connectivity. Neuroimaging studies have shown that aerobic exercise slows age-related brain atrophy and increases activity in brain regions involved in memory and executive functioning. Clinical guidelines recommend engaging in aerobic exercise 3–5 times per week, at 60–80% of maximum heart rate, for 30–45 minutes per session. Walking, dancing, cycling, and water-based exercises are all suitable options. Exercise routines should be tailored to individual physical and cognitive capacities and, when possible, enriched with concurrent cognitive tasks (Farhani et al., 2022).

5.2 Dual-Task Exercises

The ability to maintain posture or walk while simultaneously performing cognitive tasks is a critical skill for many daily life activities. When the demands of performing two tasks concurrently exceed cognitive capacity, performance in one or both tasks tends to decline. Dual-task exercises are an advanced training approach that involves performing a motor and a cognitive task simultaneously, aiming to enhance neuroplasticity. These exercises are particularly important for older adults as they help reduce fall risk, support executive functions, and improve motor-cognitive integration. During such exercises, multiple brain regions—including the prefrontal cortex, parietal lobe, and cerebellum—are activated concurrently. This activation supports the maintenance of cognitive functions through pathways associated with synaptic plasticity, such as the release of BDNF (Kahya et al., 2019).

Tasks such as counting while walking, counting backward, or talking while walking improve divided attention and information processing speed, thereby increasing adaptation to real-life multitasking situations. In physiotherapy practice, dual-task training is increasingly used to enhance postural balance, gait quality, and reaction time. For instance, exercises such as reacting to lights while standing on a balance board, solving arithmetic problems while climbing stairs, or storytelling while walking are designed to simultaneously challenge motor control and cognitive load. These methods have shown clinical value, particularly in neurological rehabilitation, geriatric physiotherapy, and conditions such as Parkinson's disease (Li et al., 2020).

Moreover, such exercises have been found to strengthen prefrontal cortical reserve, enhance attentional flexibility, and help prevent age-related cognitive decline. The level of task complexity should be progressively increased, from simple to more challenging, based on the individual's capacity. Dual-task exercises represent a holistic and functional intervention in physiotherapy practice, addressing not only physical capacity but also cognitive performance and environmental adaptability (Fritz et al., 2015).

5.3 Exergaming and Technological Approaches

Motor rehabilitation aims to restore functional motor abilities in order to improve patients' quality of life. Traditional rehabilitation approaches typically involve one-on-one therapy sessions with a physiotherapist or occupational therapist, focusing on range of motion, strength, coordination, and balance to help patients regain motor function (Farsi et al., 2025). The integration of technology into motor rehabilitation has enabled a data-driven approach, allowing for real-time progress tracking, individualized treatment adjustments, and evidence-based decision-making. Emerging technologies such as wearable devices, telerehabilitation platforms, and serious games have enhanced continuous monitoring, accessibility to therapy, and patient engagement. In recent years, extended reality (XR) technologies have also matured significantly and are now applicable across various domains, including motor rehabilitation (Lederman, 2010).

Exergaming and wearable technologies are among the most innovative applications of digitalization in motor and cognitive rehabilitation. Exergaming combines physical exercise with interactive, game-based environments, simultaneously targeting both motor skills and cognitive functions. This integration has been shown to improve attention span, motivation, and learning capacity, while also stimulating neuroplasticity in brain regions such as the prefrontal cortex and hippocampus. Neuroimaging studies indicate that exergaming activates more cortical regions than traditional exercise and leads to significant improvements in higher-order cognitive functions such as attention, decision-making, and working memory (Fiorenzato et al., 2025; Fritz et al., 2015).

Wearable technologies—including smartwatches, accelerometers, and EMG sensors—enable real-time monitoring of movement data, facilitating more objective and personalized rehabilitation processes. Additionally, immersive tools such as virtual reality (VR) and augmented reality (AR) enhance the exercise environment by making it more interactive and realistic, thereby increasing intrinsic motivation and promoting environmental awareness. These technological interventions have demonstrated high engagement and sustained improvements, particularly among older adults and individuals with neurological disorders such as stroke, Parkinson's disease, or multiple sclerosis.

Rather than simply improving the effectiveness of exercise, exergaming and wearable technologies foster active patient participation, thereby encouraging long-term behavioral change and lifestyle transformation. These digital solutions offer multi-dimensional support for neuroplasticity and represent evidence-based, person-centered interventions within physiotherapy practice (Farsi et al., 2025).

Tablo 2. Exercise and Neuroplasticity

Approach	Definition	Neuroplastic Effects	Neuroanatomical Contributions	Clinical Effects	Examples of Application
Aerobic Exercises	Rhythmic, continuous exercises that increase oxygen consumption (e.g., walking, dancing), activating both systemic and brain-level physiology.	Increased BDNF, hippocampal neurogenesis, synaptic plasticity, and reduced oxidative stress.	Prefrontal cortex, hippocampus, cerebellum, dorsolateral PFC.	Improvements in cognitive flexibility, attention, memory, balance, and coordination.	Dancing, interval walking, aquatic exercises, cycling.
Dual-Task Exercises	Exercises involving simultaneous cognitive and motor tasks to enhance cognitive-motor integration.	Stimulation of BDNF pathways, improved attention and processing speed, synaptic remodeling.	Prefrontal cortex, parietal lobe, cerebellum.	Enhanced executive functions, reduced fall risk, improved postural stability.	Walking while talking or counting backwards, balance tasks with cognitive stimuli.
Exergaming & Technological Approaches	Digital platforms combining physical activity with interactive gaming, supported by wearable technologies.	Activation of prefrontal and hippocampal areas, broader cortical network engagement, enhanced motivation and learning.	Frontal, parietal, and cerebellar regions, visual-auditory integration centers.	Increased rehabilitation adherence, cognitive-motor performance, and sustainable outcomes.	Xbox Kinect, VR-based navigation tasks, AR-enhanced walking, biofeedback-supported sessions.

6. Current Scientific Evidence

Maintaining a healthy brain is a critical factor in preserving quality of life and independence in older adults. Challenging the aging brain through cognitive training and physical exercise has been shown to be effective in mitigating age-related cognitive decline and associated disorders. Cognitive decline not only reduces individual well-being but also significantly increases the economic and social burden on healthcare systems. Consequently, preserving cognitive function in older adults has become a strategic priority in modern healthcare services. Recent scientific evidence highlights the potential of regular physical exercise to stimulate neuroplasticity and either prevent or decelerate age-related cognitive deterioration (Colcombe & Kramer, 2003; Maass et al., 2015).

The cognitive benefits of aerobic exercise are supported not only by behavioral outcomes but also by neuroimaging and neurobiological data. Physical activity that promotes hippocampal plasticity enhances synaptic connectivity, thereby improving memory processes, while also strengthening prefrontal cortical activation, which contributes to executive function (Stillman et al., 2018; Stillman et al., 2020). Increased BDNF levels induced by exercise support synaptogenesis and neurogenesis, thereby contributing to the sustainability of cognitive reserve (Szuhany et al., 2015; Kraft, 2012).

On a physiological level, exercise promotes cerebral blood flow, reduces oxidative stress, and suppresses inflammatory responses—effects that are particularly protective in pre-dementia populations. Davenport and colleagues (2025) demonstrated that after a 3-month dance-based aerobic program in individuals with amnestic mild cognitive impairment (aMCI), connectivity within the default mode network (DMN) improved significantly, suggesting that functional brain networks can be reorganized through physical activity (Davenport et al., 2025).

Furthermore, the neuroplastic effects of physical exercise are not limited to single-task routines. Dual-task exercises, which involve simultaneous cognitive and motor demands, have been shown to more effectively stimulate both synaptic plasticity and functional integration (Nishiguchi et al., 2015). Activation of both the frontal and parietal cortices during these exercises facilitates improvements in attention, executive functioning, and decision-making processes (Eggenberger et al., 2015).

Traditional exercise approaches are increasingly being supplemented or replaced by digitally driven interventions. Exergaming, defined as game-based physical activity, enhances motivation while activating multiple cognitive processes and increasing cortical engagement. Studies have demonstrated that exergaming can yield greater cognitive gains than traditional aerobic exercise (Anderson-Hanley et al., 2012).

The integration of wearable technologies further enhances the personalization of exercise interventions. These devices allow real-time monitoring of exercise responses, enabling more individualized and adaptive therapy. In both clinical and home-based rehabilitation settings, such technologies support the sustainability of neuroplasticity-focused physiotherapy interventions. The use of smart sensors and mobile applications to track physical activity levels provides healthcare professionals with objective data and empowers individuals to participate actively in their own treatment (Kraft, 2012).

Collectively, recent scientific findings suggest that aerobic exercise, dual-task protocols, and digital exercise technologies exert significant positive effects on cognitive functioning and the support of neuroplastic mechanisms in older adults. Physiotherapy is thus evolving from a musculoskeletal-focused discipline into a comprehensive health strategy aimed at promoting neurocognitive well-being (Stillman et al., 2020; Szuhany et al., 2015).

7. Conclusion and Future Perspectives

The preservation of cognitive and motor functions in the geriatric population directly influences not only individual quality of life but also the sustainability of healthcare systems. In light of recent neuroscientific evidence, regular physical exercise—particularly aerobic and dual-task approaches—has been strongly demonstrated to support declining neuroplastic capacity and enhance cognitive reserve in aging individuals. In this context, exercise is positioned in geriatric physiotherapy not merely as a means of improving mobility, but as a multidimensional neuromodulator that fosters synaptic plasticity, increases hippocampal volume, and strengthens executive functioning (Bamidis et al., 2014).

Current literature shows that structured exercise programs administered to older adults result in measurable structural changes, such as increased cortical thickness and improved white matter integrity. These adaptations are accompanied by significant improvements in memory, attention, and information processing speed. Importantly, such effects not only slow cognitive decline but may also enhance the capacity for independent living in older adults, potentially reducing the need for institutional care (Gomez-Pinilla & Hillman, 2013).

Looking forward, geriatric physiotherapy is expected to evolve into a more personalized and efficient model through the integration of artificial intelligence, neuroimaging, and biosensor technologies. Clinical decision support systems may soon offer tailored intervention protocols based on genetic and neurophysiological data, while virtual and augmented reality applications will further enhance patient motivation and therapeutic outcomes by making rehabilitation more engaging and effective (Denche-Zamorano et al., 2023).

Emerging evidence underscores the effectiveness of non-pharmacological interventions—centered on cognitive and physical training—in decelerating age-related cognitive decline and promoting healthy aging. A deeper understanding of the underlying neurobiological mechanisms will enable the development of more targeted and individualized intervention strategies in future clinical practice.

8. References

Acharya, V., Fan, K.-H., Snitz, B. E., Ganguli, M., DeKosky, S. T., Lopez, O. L., Feingold, E., & Kamboh, M. I. (2025). APOE Genotype-Stratified Meta-Analysis of Cognitive Decline Reveals Novel Loci for Language and Global Cognitive Function in Older Adults. International Journal of Molecular Sciences, 26(14), 6940. https://doi.org/10.3390/ijms26146940

Anderson-Hanley, C., Arciero, P. J., Brickman, A. M., et al. (2012). Exergaming and older adult cognitive function: A randomized clinical trial. American Journal of Preventive Medicine, 42(2), 109–119.

Bae, S., Harada, K., Lee, S., Harada, K., Makino, K., Chiba, I., Park, H., & Shimada, H. (2020). The Effect of a Multicomponent Dual-Task Exercise on Cortical Thickness in Older Adults with Cognitive Decline: A Randomized Controlled Trial. Journal of clinical medicine, 9(5), 1312. https://doi.org/10.3390/jcm9051312

Baker, L. M., Laidlaw, D. H., Cabeen, R., Akbudak, E., Conturo, T. E., Correia, S., Tate, D. F., Heaps-Woodruff, J. M., Brier, M. R., Bolzenius, J., Salminen, L. E., Lane, E. M., McMichael, A. R., & Paul, R. H. (2017). Cognitive reserve moderates the relationship between neuropsychological performance and white matter fiber bundle length in healthy older adults. Brain imaging and behavior, 11(3), 632–639. https://doi.org/10.1007/s11682-016-9540-7

Bamidis, P. D., Vivas, A. B., Styliadis, C., Frantzidis, C., Klados, M., Schlee, W., Siountas, A., & Papageorgiou, S. G. (2014). A review of physical and cognitive interventions in aging. Neuroscience and biobehavioral reviews, 44, 206–220. https://doi.org/10.1016/j.neubiorev.2014.03.019

Cieri, F., Cross, C. L., Francesco, G. D., & Caldwell, J. Z. K. (2025). Dynamic neurocognitive adaptation: A follow-up exposome investigation in aging. Alzheimer's & dementia (New York, N. Y.), 11(2), e70103. https://doi.org/10.1002/trc2.70103

Colcombe, S. J., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: A meta-analytic study. Psychological Science, 14(2), 125–130.

Dai, X., Liu, S., Li, Y., Long, S., Li, X., Chen, C., Yang, C., Zhang, J., Han, Z. R., Li, H., Wang, J., & Zhang, Z. (2024). White Matter Plasticity Underpins Cognitive Gains After Multidomain Adaptive Computerized Cognitive Training. The journals of gerontology. Series A, Biological sciences and medical sciences, 79(4), glae046. https://doi.org/10.1093/gerona/glae046

Davenport, M. H., et al. (2025). White matter integrity of default mode network after a 3-month aerobic dance program in patients with amnestic mild cognitive impairment: A secondary analysis of a randomized clinical trial. Quantitative Imaging in Medicine and Surgery, 15(10), 1460–1472.

Denche-Zamorano, A., Rodriguez-Redondo, Y., Barrios-Fernandez, S., Mendoza-Muñoz, M., Castillo-Paredes, A., Rojo-Ramos, J., Garcia-Gordillo, M. A., & Adsuar, J. C. (2023). Rehabilitation Is the Main Topic in Virtual and Augmented Reality and Physical Activity Research: A Bibliometric Analysis. Sensors, 23(6), 2987. https://doi.org/10.3390/s2306298

Eggenberger, P., Wolf, M., Schumann, M., & de Bruin, E. D. (2015). Exergame and balance training modulate prefrontal brain activity during walking and enhance executive function in older adults. Frontiers in Aging Neuroscience, 7, 66.

Erickson, K. I., Hillman, C., Stillman, C. M., Ballard, R. M., Bloodgood, B., Conroy, D. E., Macko, R., Marquez, D. X., Petruzzello, S. J., Powell, K. E., & FOR 2018 PHYSICAL ACTIVITY GUIDELINES ADVISORY COMMITTEE* (2019). Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines. Medicine and science in sports and exercise, 51(6), 1242–1251. https://doi.org/10.1249/MSS.0000000000001936

Facal, D., Spuch, C., & Valladares-Rodriguez, S. (2022). New Trends in Cognitive Aging and Mild Cognitive Impairment. Geriatrics (Basel, Switzerland), 7(4), 80. https://doi.org/10.3390/geriatrics7040080

Fahimi, A., Baktir, M. A., Moghadam, S., Mojabi, F. S., Sumanth, K., McNerney, M. W., Ponnusamy, R., & Salehi, A. (2017). Physical exercise induces structural alterations in the hippocampal astrocytes: exploring the role of BDNF-TrkB signaling. Brain structure & function, 222(4), 1797–1808. https://doi.org/10.1007/s00429-016-1308-8

Farhani, F., Shahrbanian, S., Auais, M., Hekmatikar, A. H. A., & Suzuki, K. (2022). Effects of Aerobic Training on Brain Plasticity in Patients with Mild Cognitive Impairment: A Systematic Review of Randomized Controlled Trials. Brain Sciences, 12(6), 732. https://doi.org/10.3390/brainsci12060732

Farsi, A., Cerone, G. L., Falla, D., & Gazzoni, M. (2025). Emerging Applications of Augmented and Mixed Reality Technologies in Motor Rehabilitation: A Scoping Review. Sensors, 25(7), 2042. https://doi.org/10.3390/s25072042

Fiorenzato, E., Zabberoni, S., Costa, A., Antonini, A., & Cona, G. (2025). Reply: Immersive virtual reality for cognitive training in Parkinson's disease - Future directions and neuroimaging perspectives. Journal of the neurological sciences, 476, 123636. https://doi.org/10.1016/j.jns.2025.123636

Fritz, N. E., Cheek, F. M., & Nichols-Larsen, D. S. (2015). Motor-Cognitive Dual-Task Training in Persons With Neurologic Disorders: A Systematic Review. Journal of neurologic physical therapy : JNPT, 39(3), 142–153. https://doi.org/10.1097/NPT.0000000000000090

Franzén, E., Johansson, H., Freidle, M., Ekman, U., Wallén, M. B., Schalling, E., Lebedev, A., Lövdén, M., Holmin, S., Svenningsson, P., & Hagströmer, M. (2019). The EXPANd trial: effects of exercise and exploring neuroplastic changes in people with Parkinson's disease: a study protocol for a double-blinded randomized controlled trial. BMC neurology, 19(1), 280. https://doi.org/10.1186/s12883-019-1520-2

Gomez-Pinilla, F., & Hillman, C. (2013). The influence of exercise on cognitive abilities. Comprehensive Physiology, 3(1), 403–428. https://doi.org/10.1002/cphy.c110063

Gooijers, J., Pauwels, L., Hehl, M., Seer, C., Cuypers, K., & Swinnen, S. P. (2024). Aging, brain plasticity, and motor learning. Ageing research reviews, 102, 102569. https://doi.org/10.1016/j.arr.2024.102569

Hernandez, M. E., Holtzer, R., Izzetoglu, M., & Motl, R. W. (2025). The Use of Augmented Reality on a Self-Paced Treadmill to Quantify Attention and Footfall Placement Variability in Middle-Aged to Older-Aged Adults with Multiple Sclerosis. Sclerosis, 3(1), 3. https://doi.org/10.3390/sclerosis3010003

Kahya, M., Moon, S., Ranchet, M., Vukas, R. R., Lyons, K. E., Pahwa, R., Akinwuntan, A., & Devos, H. (2019). Brain activity during dual task gait and balance in aging and age-related neurodegenerative conditions: A systematic review. Experimental gerontology, 128, 110756. https://doi.org/10.1016/j.exger.2019.110756

Kraft, E. (2012). Cognitive function, physical activity, and aging: Possible biological links and implications for multimodal interventions. Aging, Neuropsychology, and Cognition, 19(3), 248–263.

Leckie, R. L., Oberlin, L. E., Voss, M. W., Prakash, R. S., Szabo-Reed, A., Chaddock-Heyman, L., Phillips, S. M., Gothe, N. P., Mailey, E., Vieira-Potter, V. J., Martin, S. A., Pence, B. D., Lin, M., Parasuraman, R., Greenwood, P. M., Fryxell, K. J., Woods, J. A., McAuley, E., Kramer, A. F., & Erickson, K. I. (2014). BDNF mediates improvements in executive function following a 1-year exercise intervention. Frontiers in human neuroscience, 8, 985. https://doi.org/10.3389/fnhum.2014.00985

Lederman, E. (2010). 14—Neuromuscular rehabilitation: Summary. In Neuromuscular rehabilitation in manual and physical therapy (pp. 169–171). Churchill Livingstone/Elsevier.

Li, Z., Wang, T., Liu, H., Jiang, Y., Wang, Z., & Zhuang, J. (2020). Dual-task training on gait, motor symptoms, and balance in patients with Parkinson's disease: a systematic review and meta-analysis. Clinical rehabilitation, 34(11), 1355–1367. https://doi.org/10.1177/0269215520941142

Lindenberger, U. (2014). Human cognitive aging: corriger la fortune?. science, 346(6209), 572-578.

Litke, R., Garcharna, L. C., Jiwani, S., & Neugroschl, J. (2021). Modifiable Risk Factors in Alzheimer Disease and Related Dementias: A Review. Clinical therapeutics, 43(6), 953–965. https://doi.org/10.1016/j.clinthera.2021.05.006

López, M. E., Aurtenetxe, S., Pereda, E., Cuesta, P., Castellanos, N. P., Bruña, R., Niso, G., Maestú, F., & Bajo, R. (2014). Cognitive reserve is associated with the functional organization of the brain in healthy aging: a MEG study. Frontiers in aging neuroscience, 6, 125. https://doi.org/10.3389/fnagi.2014.00125

Lövdén, M., Fratiglioni, L., Glymour, M. M., Lindenberger, U., & Tucker-Drob, E. M. (2020). Education and Cognitive Functioning Across the Life Span. Psychological science in the public interest : a journal of the American Psychological Society, 21(1), 6–41. https://doi.org/10.1177/1529100620920576

Maass, A., Düzel, S., Goerke, M., et al. (2015). Vascular hippocampal plasticity after aerobic exercise in older adults. Molecular Psychiatry, 20(5), 585–593.

Maass, A., Düzel, S., Brigadski, T., Goerke, M., Becke, A., Sobieray, U., Neumann, K., Lövdén, M., Lindenberger, U., Bäckman, L., Braun-Dullaeus, R., Ahrens, D., Heinze, H. J., Müller, N. G., Lessmann, V., Sendtner, M., & Düzel, E. (2016). Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. NeuroImage, 131, 142–154. https://doi.org/10.1016/j.neuroimage.2015.10.084

Mohammadi-Mahdiabadi-Hasani, M. H., Farahmandfar, M., Zarrindast, M. R., Nasehi, M., Torkaman-Boutorabi, A., & Hassanzadeh, G. (2025). Therapeutic effects of resveratrol on memory deficits in offspring of sleep-deprived rats: Involvement of hippocampal BDNF-TrkB pathways. Journal of psychopharmacology (Oxford, England), 39(8), 870–882. https://doi.org/10.1177/02698811251334034

Montero-Odasso, M., Almeida, Q. J., Burhan, A. M., Camicioli, R., Doyon, J., Fraser, S., Li, K., Liu-Ambrose, T., Middleton, L., Muir-Hunter, S., McIlroy, W., Morais, J. A., Pieruccini-Faria, F., Shoemaker, K., Speechley, M., Vasudev, A., Zou, G. Y., Berryman, N., Lussier, M., Vanderhaeghe, L., … Bherer, L. (2018). SYNERGIC TRIAL (SYNchronizing Exercises, Remedies in Gait and Cognition) a multi-Centre randomized controlled double blind trial to improve gait and cognition in mild cognitive impairment. BMC geriatrics, 18(1), 93. https://doi.org/10.1186/s12877-018-0782-7

Müller, P., Rehfeld, K., Schmicker, M., Hökelmann, A., Dordevic, M., Lessmann, V., Brigadski, T., Kaufmann, J., & Müller, N. G. (2017). Evolution of Neuroplasticity in Response to Physical Activity in Old Age: The Case for Dancing. Frontiers in aging neuroscience, 9, 56. https://doi.org/10.3389/fnagi.2017.00056

Nishiguchi, S., Yamada, M., Tanigawa, T., et al. (2015). A 12-week structured cognitive-motor dual-task training program improves cognitive function and gait performance in older adults: A randomized controlled trial. Journal of the American Geriatrics Society, 63(8), 1535–1543.

Pauwels, L., Chalavi, S., & Swinnen, S. P. (2018). Aging and brain plasticity. Aging, 10(8), 1789–1790. https://doi.org/10.18632/aging.101514

Pieruccini-Faria, F., Son, S., Zou, G., Almeida, Q. J., Middleton, L. E., Bray, N. W., Lussier, M., Shoemaker, J. K., Speechley, M., Liu-Ambrose, T., Burhan, A. M., Camicioli, R., Li, K. Z. H., Fraser, S., Berryman, N., Bherer, L., & Montero-Odasso, M. (2025). Synergistic effects of exercise, cognitive training and vitamin D on gait performance and falls in mild cognitive impairment-secondary outcomes from the SYNERGIC trial. Age and ageing, 54(9), afaf242. https://doi.org/10.1093/ageing/afaf242

Radak, Z., Chung, H. Y., & Goto, S. (2008). Systemic adaptation to oxidative challenge induced by regular exercise. Free radical biology & medicine, 44(2), 153–159. https://doi.org/10.1016/j.freeradbiomed.2007.01.029

Rehfeld, K., Lüders, A., Hökelmann, A., Lessmann, V., Kaufmann, J., Brigadski, T., Müller, P., & Müller, N. G. (2018). Dance training is superior to repetitive physical exercise in inducing brain plasticity in the elderly. PloS one, 13(7), e0196636. https://doi.org/10.1371/journal.pone.0196636

Riverol, M., Ríos-Rivera, M. M., Imaz-Aguayo, L., Solis-Barquero, S. M., Arrondo, C., Montoya-Murillo, G., Villino-Rodríguez, R., García-Eulate, R., Domínguez, P., & Fernández-Seara, M. A. (2024). Structural neuroimaging changes associated with subjective cognitive decline from a clinical sample. NeuroImage. Clinical, 42, 103615. https://doi.org/10.1016/j.nicl.2024.103615

Soshi, T., Andersson, M., Kawagoe, T., Nishiguchi, S., Yamada, M., Otsuka, Y., Nakai, R., Abe, N., Aslah, A., Igasaki, T., & Sekiyama, K. (2021). Prefrontal Plasticity after a 3-Month Exercise Intervention in Older Adults Relates to Enhanced Cognitive Performance. Cerebral cortex.

Stillman, C. M., Esteban-Cornejo, I., & Brown, B. (2020). Effects of exercise on brain and cognition across age groups and health states. Trends in Neurosciences, 43(7), 533–543.

Stillman, C. M., Uyar, F., Huang, H., & Grove, G. A. (2018). Cardiorespiratory fitness is associated with enhanced hippocampal functional connectivity in healthy young adults. Hippocampus, 28(6), 541–549.

Szuhany, K. L., Bugatti, M., & Otto, M. W. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. Journal of Psychiatric Research, 60, 56–64.

Tarumi, T., Patel, N. R., Tomoto, T., Pasha, E., Khan, A. M., Kostroske, K., Riley, J., Tinajero, C. D., Wang, C., Hynan, L. S., Rodrigue, K. M., Kennedy, K. M., Park, D. C., & Zhang, R. (2022). Aerobic exercise training and neurocognitive function in cognitively normal older adults: A one-year randomized controlled trial. Journal of internal medicine, 292(5), 788–803. https://doi.org/10.1111/joim.13534

Vints, W. A. J., Šeikinaitė, J., Gökçe, E., Kušleikienė, S., Šarkinaite, M., Valatkeviciene, K., Česnaitienė, V. J., Verbunt, J., Levin, O., & Masiulis, N. (2024). Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. GeroScience, 46(4), 3971–3991. https://doi.org/10.1007/s11357-024-01110-6

Zheng, X. X., Wang, F., Ding, H., Li, H. T., Yang, X. J., Li, X. C., Dou, Z. W., Hu, W. C., Han, W. J., Li, Z. Z., Li, Y. C., Chu, W. G., Yuan, H., Wu, S. X., Xie, R. G., & Luo, C. (2025). cGMP-dependent protein kinase I in the dorsal hippocampus protects against synaptic plasticity and cognitive deficit induced by chronic pain. Pain, 166(10), 2355–2372. https://doi.org/10.1097/j.pain.0000000000003624

Chapter 3

Safety Of Ultrasound Exposure During Pregnancy

Demet CEYLAN[1]

Abstract

Ultrasound is a widely used imaging modality for assessing fetal development and detecting abnormalities during pregnancy. While generally considered safe, concerns have been raised about its potential long-term effects. This review examines the effects of ultrasound exposure on fetal growth, neurodevelopment, childhood malignancies, handedness, and thermal impact. Current literature suggests that ultrasound is safe when used appropriately, but unnecessary exposure should be minimized.

Key words: Ultrasonography, Pregnancy, Risk Assesment

1 Dr. Lecturer, Trabzon University, Tonya Vocational School, Department of Medical Services and Techniques, Medical Imaging Techniques Program, E-mail: demetceylan@trabzon.edu.tr,
ORCID: 0000-0002-2643-5630

INTRODUCTION

Ultrasonography (USG) is a widely used non-invasive imaging modality in prenatal care, significantly contributing to the assessment of fetal development (Abramowicz & Barnett, 2005). However, scientific debates persist regarding the long-term effects of frequent and repeated prenatal USG exposure on fetal biological systems. The thermal and mechanical effects of USG should be carefully evaluated concerning their potential biophysical consequences on fetal tissues (Miller, Brayman, & Abramowicz, 1998). While most studies affirm the safety of ultrasound, some suggest that prolonged and high-frequency exposure might pose certain risks (Newnham et al., 2004).

The aim of this study is to evaluate the potential adverse effects of prenatal USG exposure on the fetus and to discuss the clinical significance of these effects in light of existing scientific literature. Specifically, this study seeks to review the current evidence on USG safety, with a focus on fetal neurodevelopment, birth weight, and long-term health outcomes.

METHODOLOGY

This review was conducted as a narrative literature review. A comprehensive search of the scientific literature was performed in the following electronic databases: PubMed, Scopus, Web of Science, and Google Scholar. The search covered studies published between 1979 and 2025, with particular emphasis on articles from the last 10 years to capture the most recent evidence.

The following keywords: “prenatal ultrasound”, “obstetric ultrasonography” and (“safety” or “bioeffects” or “thermal effects” or “mechanical effects” or “neurodevelopment” or “fetal growth” or “handedness” or “childhood cancer” or “autism”).

Inclusion criteria:

- Original research articles (human and animal studies), systematic reviews, and meta-analyses addressing the safety or bioeffects of prenatal ultrasound.
- Publications in English or Turkish.
- Studies examining outcomes such as fetal growth, neurodevelopment, childhood malignancies, handedness, and biophysical effects of ultrasound.

Exclusion criteria:

- Conference abstracts, letters to the editor, or studies with insufficient methodological detail.

- Articles focusing solely on the diagnostic accuracy of ultrasound without reference to safety or bioeffects.
- Publications not available in full text.

Ultrasound Exposure During Pregnancy: Assessing Risks to the Fetus

Fetal Growth and Birth Weight

The potential effects of prenatal USG exposure on birth weight have been a subject of ongoing research. Grisso et al. (1994) conducted a study investigating the relationship between diagnostic USG during pregnancy and low birth weight. Their findings suggested a possible link between frequent USG exposure and reduced birth weight, indicating that repeated USG scans might contribute to intrauterine growth restriction (IUGR). This raises concerns about the frequency and necessity of multiple ultrasound examinations during pregnancy, particularly in low-risk cases.

Contrastingly, Newnham et al. (2004) conducted a large-scale study to assess the long-term effects of repeated prenatal USG examinations on fetal and childhood development. Their research concluded that multiple USG scans had minimal influence on fetal growth parameters, including birth weight. They argued that the observed variations in birth weight might be attributed to confounding factors rather than direct ultrasound exposure. This study suggests that while concerns exist, there is no definitive evidence linking prenatal USG to significant reductions in birth weight (Newnham 2004).

Following the implementation of an enhanced fetal growth ultrasound protocol at a single UK National Health Service (NHS) centre between 2014 and 2022, an overall increase in the detection of small for gestational age (SGA) babies was observed. However, this increase was largely due to false positive cases, with a more limited increase in the detection of babies who were truly small for gestational age. It was also found that there was a significant increase in the number of ultrasound scans and the use of Doppler following the implementation of the protocol, and that these increases were significantly and positively associated with the rate of small for gestational age baby diagnoses (Butterfield, 2024).

The conflicting findings in the literature highlight the need for further research to clarify the relationship between prenatal USG exposure and fetal growth outcomes. Future studies should focus on controlling for potential confounding factors and investigating the long-term implications of prenatal USG exposure on birth outcomes.

The aim of this study is to evaluate the potential adverse effects of prenatal USG exposure on the fetus and to discuss the clinical significance of these effects in light of existing scientific literature. Specifically, this study seeks to review the current evidence on USG safety, with a focus on fetal neurodevelopment, birth weight, and long-term health outcomes.

Neurodevelopmental Outcomes

Prenatal ultrasound exposure has raised concerns regarding its potential impact on neurodevelopment. Several studies have explored these risks, with varying findings (Ang 2006, Campbell 1993).

One study by Ang et al. (2006) examined the effects of prenatal ultrasound exposure in mice, showing that ultrasound waves could disrupt neuronal migration, a key process in brain development. This disruption has potential implications for the cognitive and neurological development of the fetus, suggesting that prenatal exposure to ultrasound may have a lasting effect on brain structure and function.

In humans, the relationship between prenatal ultrasound exposure and neurodevelopmental outcomes has been investigated in various studies. Campbell, Elford, and Brant (1993) conducted a case-control study focusing on the link between prenatal ultrasound exposure and delayed speech development in children. Their findings suggested that children who were exposed to ultrasound during pregnancy might experience delays in speech development, highlighting a potential impact of ultrasound on early language skills (Campbell 1993).

Today, the use of prenatal ultrasound is increasing, and the effects of screenings, especially in the first trimester, on fetal brain development are being discussed. Recent studies have examined possible relationships between ultrasound exposure and autism spectrum disorder (ASD). In this context, a large-scale case-control study found that children diagnosed with ASD were exposed to more ultrasound penetration depth (mean depth) in the prenatal period (Cranston et al., 2023). However, no significant difference was found between the ASD group and the control groups in terms of the total number or duration of ultrasounds.

Rosman el al. (2018) found that in their case-control study of 420 children, those with autism spectrum disorder were exposed to greater mean depth of ultrasonographic penetration during the first and second trimesters compared with typically developing children and during the first trimester compared with developmentally delayed children. Meanwhile no association between the number of scans or duration of ultrasound exposure and later autism spectrum disorder was found.

Animal models show that long-term ultrasound exposure can disrupt neuronal migration, cause cell disorganization in cortical layers, and negatively affect social behavior (Ang et al., 2006; McClintic et al., 2013). In addition, it is stated that ultrasound waves can cause thermal damage to surrounding soft tissues, especially by converting them into more energy in bone tissue. This may lead to microscopic changes in the fetal brain due to hyperthermia.

However, current human studies have not been able to establish a direct causal relationship between prenatal ultrasound and ASD. Nevertheless, the higher ultrasound depth observed in the ASD group necessitates further research on the safety of this technology. Due to the high acoustic power of Doppler, 3D and 4D ultrasound, it is particularly emphasized that the ALARA (as low as reasonably achievable) principle should be followed.

In this context, larger sample and prospective studies are needed to reveal the possible mechanical and thermal effects of prenatal ultrasound on brain development.

These studies collectively indicate that prenatal ultrasound exposure might have subtle yet significant effects on neurodevelopment, particularly with regard to neuronal migration, language development, and brain lateralization. Further research is needed to clarify these associations and better understand the mechanisms underlying these potential outcomes.

Handedness

The potential impact of prenatal ultrasound exposure on handedness has been a topic of research, particularly regarding its influence on the lateralization of brain function. Handedness, often considered an indicator of brain asymmetry, has been shown to be affected by prenatal factors, including ultrasound exposure (Hepper et al., 2005, Medland et al., 2009, Vuoksimaa et al., 2009).

Kieler et al. (2001) conducted a study investigating the relationship between prenatal ultrasound exposure and left-handedness in a cohort of young men. Their findings suggested a significant association between prenatal ultrasound exposure and an increased likelihood of left-handedness. This study hypothesized that ultrasound exposure during pregnancy may interfere with the natural process of brain lateralization, leading to a higher prevalence of left-handedness in individuals exposed to ultrasound in utero. The study concluded that prenatal ultrasound exposure could be a contributing factor to the development of non-right handedness, although the exact mechanisms remain unclear.

Further supporting these findings, Salvesen and Eik-Nes (1999) performed a meta-analysis examining the relationship between prenatal ultrasound exposure and non-right handedness in children. Their analysis pooled data from multiple studies and found a consistent association between prenatal ultrasound exposure and a higher incidence of left-handedness. This meta-analysis highlighted the potential link between ultrasound and altered brain lateralization, reinforcing the idea that prenatal ultrasound exposure may have subtle effects on the developing brain, particularly in terms of handedness.

Heikkilä et al. (2011) designed a study that aimed to investigate whether prenatal ultrasound exposure increases the likelihood of non-right-handedness (left- or mixed-handedness) in children, particularly boys. Data from the Helsinki Ultrasound Trial were analyzed using logistic regression, based on follow-up questionnaires on handedness completed by the parents of 4150 children aged 13–15 years. The odds ratios for non-right-handedness among children exposed to prenatal ultrasound were 1.16 (95% CI: 0.98–1.37) for all subjects, 1.12 (95% CI: 0.89–1.41) for boys, and 1.24 (95% CI: 0.97–1.58) for girls. Since the confidence intervals included 1.0, these associations were not statistically significant. In conclusion, prenatal ultrasound exposure was not found to be associated with handedness in children, and this finding was consistent regardless of the definition of handedness used.

Together, these studies suggest that prenatal ultrasound exposure may influence the lateralization of brain function, leading to an increased likelihood of left-handedness. However, while these findings are intriguing, the exact causal mechanisms remain speculative. Further research is needed to explore how prenatal ultrasound affects brain development and whether the observed associations with handedness are consistent across different populations and ultrasound protocols.

Childhood Malignancies

The potential link between prenatal ultrasound exposure and childhood malignancies has been a subject of concern, particularly regarding the risk of leukemia. Although the vast majority of studies suggest that prenatal ultrasound is safe, some research has raised questions about its potential association with an increased risk of childhood cancers, particularly leukemia (Naumburg et al., 2000, Rosman et al., 2018)

Naumburg et al. (2000) conducted a case-control study to examine the relationship between prenatal ultrasound exposure and the risk of childhood leukemia. Their study, which included data from Sweden, found a slight but statistically significant association between prenatal ultrasound examinations and an increased risk of leukemia in children. While the results indicated a potential link, the authors acknowledged the need for further studies to establish a definitive causal relationship. The study's findings suggested that the risk might be modest, but it raised concerns about the long-term effects of repeated prenatal ultrasound scans.

The International Commission on Non-Ionizing Radiation Protection (ICNIRP, 2017) has addressed concerns regarding the use of diagnostic devices that rely on non-ionizing radiation, such as ultrasound. The ICNIRP statement emphasized that while diagnostic ultrasound is generally considered safe, there are still ongoing debates about the potential health risks associated with exposure to non-ionizing radiation during prenatal development. Although the available evidence does not conclusively support a link between prenatal ultrasound and childhood malignancies, the ICNIRP highlighted the importance of adhering to safety guidelines and minimizing unnecessary exposure to ultrasound in pregnancy.

While these studies suggest a potential link between prenatal ultrasound exposure and childhood malignancies, particularly leukemia, the evidence remains inconclusive. The risk, if any, appears to be small, and most studies have found no substantial increase in cancer risk (Rajaraman et al. 2011). However, these findings underscore the importance of continued research to monitor and assess the long-term effects of prenatal ultrasound exposure on childhood health.

Thermal and Mechanical Effects of Ultrasound

Ultrasound is widely used in obstetric and gynecological practice due to its non-invasive nature and ability to provide valuable information about fetal development. However, the potential thermal and mechanical effects of ultrasound exposure on fetal tissues have been a subject of concern, especially with repeated or prolonged exposure (Dino et al. 1989, Liebeskind et al., 1979, Church 2007).

Miller, Brayman, and Abramowicz (1998) provided an in-depth biophysical analysis of the safety of obstetric ultrasonography, particularly focusing on the thermal and mechanical effects. They explained that ultrasound waves can produce two main types of bioeffects: thermal and mechanical. Thermal effects arise from the absorption of ultrasound energy by tissues, which leads to an increase in temperature. While these temperature increases are typically small, prolonged or excessive exposure could theoretically lead to thermal damage in sensitive tissues, including the developing fetus. The safety of ultrasound, therefore, depends on controlling the intensity and duration of exposure to avoid thermal effects that could harm fetal development.

Mechanical effects, on the other hand, are caused by the physical forces generated by the ultrasound waves. These forces can lead to cavitation, which is the formation of small bubbles within the tissue. If cavitation occurs at high intensities, it could potentially lead to tissue damage. However, under standard clinical conditions, the intensities used in obstetric ultrasonography are typically far below the levels that would cause harmful cavitation or mechanical damage. Miller et al. (1998) emphasized that the mechanical effects of ultrasound are less of a concern than the thermal effects, given the relatively low intensities used during clinical examinations.

Abramowicz and Barnett (2005) further examined ultrasound safety, providing an overview of the bioeffects and safety guidelines associated with diagnostic ultrasound. They emphasized that while the thermal and mechanical effects of ultrasound are potential concerns, current safety standards are designed to minimize the risk of harm. They noted that the intensity of ultrasound used in obstetrics is regulated to ensure that thermal and mechanical effects remain within safe limits. In their analysis, Abramowicz and Barnett highlighted the importance of adhering to established safety guidelines to mitigate the risk of potential bioeffects and to ensure that ultrasound remains a safe tool for prenatal care.

Overall, while the thermal and mechanical effects of ultrasound are valid considerations in terms of fetal safety, the current evidence suggests that when ultrasound is used according to established safety protocols, these risks are minimal. Nevertheless, ongoing research is necessary to continuously evaluate the long-term safety of ultrasound, especially in cases of repeated exposure.

Clinical Guidelines and Recommendations

Based on current evidence, prenatal ultrasound is considered safe when used appropriately. However, healthcare providers should adhere to the ALARA (As Low As Reasonably Achievable) principle, limiting exposure to the minimum necessary for accurate diagnosis. The following guidelines are recommended:

- Minimize Ultrasound Exposure: Ultrasound scans should be performed only when medically necessary, following the ALARA principle, to limit the duration and frequency of exposure (Abramowicz & Barnett, 2005).
- Caution with Doppler Ultrasound: Doppler ultrasound should be used cautiously, especially in the first trimester, as it produces greater energy than traditional scans. Use should be limited to situations where its benefits outweigh the risks (Miller et al., 1998).
- Limit 3D/4D Ultrasound to Medical Indications: The use of 3D/4D ultrasound should be restricted to medical reasons, such as detecting fetal abnormalities, rather than for non-medical purposes (Salvesen & Eik-Nes, 1999).

- Further Research: Additional studies are required to evaluate the long-term effects of ultrasound exposure, particularly with newer technologies like Doppler and 3D/4D imaging. Ongoing research will help clarify the potential risks and contribute to safer clinical practices.

CONCLUSION

Prenatal ultrasound remains one of the most widely utilized and valuable tools in modern obstetrics, providing essential information about fetal development and helping healthcare providers monitor maternal and fetal well-being. While the majority of studies affirm the safety of ultrasound when used appropriately, concerns regarding the potential long-term effects of excessive or unnecessary exposure persist. The evidence on the effects of prenatal ultrasound exposure, particularly in relation to fetal growth, neurodevelopment, childhood malignancies, handedness, and thermal impacts, suggests that while ultrasound is generally safe, its use should be carefully managed to minimize risks.

In conclusion, while prenatal ultrasound is an invaluable diagnostic tool, it is essential to continue researching its long-term effects to better understand its risks and ensure the safety of both mother and fetus. The ongoing evaluation of newer ultrasound technologies and adherence to established safety protocols will help maximize the benefits of ultrasound while minimizing potential risks, ultimately contributing to better maternal and fetal health outcomes.

REFERENCES

1. Abramowicz, J. S., & Barnett, S. B. (2005). Ultrasound safety and bioeffects. Ultrasound in Obstetrics & Gynecology, 25(6), 613–617.
2. Ang, E. S. B., Gluncic, V., Duque, A., Schafer, M. E., & Rakic, P. (2006). Prenatal exposure to ultrasound waves impacts neuronal migration in mice. Proceedings of the National Academy of Sciences, 103(34), 12903–12910.
3. Butterfield, E., & Skelton, E. (2024). The effect of an enhanced fetal growth ultrasound protocol on pregnancy outcomes: A retrospective service evaluation within a single UK National Health Service centre between 2014 and 2022. Ultrasound, 32(1), 1–10.
4. Campbell, J. D., Elford, R. W., & Brant, R. F. (1993). Case-control study of prenatal ultrasonography exposure in children with delayed speech. Canadian Medical Association Journal, 149(10), 1435–1440.
5. Church, C. C., & Miller, M. W. (2007). Quantification of risk from fetal exposure to diagnostic ultrasound. Progress in Biophysics and Molecular Biology, 93(1–3), 331–353.
6. Cranston, A., Poulton, A. S., Gold, W. A., McClintic, A. M., King, B. H., & Michel, G. F. (2023). Association between prenatal ultrasonographic depth and later autism spectrum disorder: A case-control study. JAMA Pediatrics, 177(3), 217–225.
7. Dinno, M. A., Dyson, M., Young, S. R., Mortimer, A. J., Hart, J., & Crum, L. A. (1989). The significance of membrane changes in the safe and effective use of therapeutic and diagnostic ultrasound. Physics in Medicine & Biology, 34(10), 1543–1552.
8. Grisso, J. A., Strom, B. L., Cosmatos, I., & Carson, J. L. (1994). Diagnostic ultrasound in pregnancy and low birthweight. American Journal of Perinatology, 11(4), 297–301.
9. Henderson, J., Willson, K., Jago, J. R., & Whittingham, T. A. (1995). A survey of the acoustic outputs of diagnostic ultrasound equipment in current clinical use. Ultrasound in Medicine & Biology, 21(5), 699–705.
10. Hepper, P. G., Wells, D. L., & Lynch, C. (2005). Prenatal thumb sucking is related to postnatal handedness. Neuropsychologia, 43(2), 313–315.
11. International Commission on Non-Ionizing Radiation Protection. (2017). ICNIRP statement on diagnostic devices using non-ionizing radiation: Existing regulations and potential health risks. Health Physics, 112(3), 305–320.
12. Heikkilä, K., Vuoksimaa, E., Oksava, K., Saari-Kemppainen, A., & Iivanainen, M. (2011). Handedness in the Helsinki Ultrasound Trial. Ultrasound in Obstetrics & Gynecology, 37(6), 638–642.

13. Kieler, H., Cnattingius, S., Palmgren, J., & Axelsson, O. (2001). Sinistrality—a side-effect of prenatal sonography: A comparative study of young men. Epidemiology, 12(6), 618–623.
14. Liebeskind, D., Bases, R., Mendez, F., Elequin, F., & Koenigsberg, M. (1979). Sister chromatid exchanges in human lymphocytes after exposure to diagnostic ultrasound. Science, 205(4411), 1273–1275.
15. Medland, S. E., Duffy, D. L., Wright, M. J., Geffen, G. M., Hay, D. A., Levy, F., van Beijsterveldt, C. E., Willemsen, G., Townsend, G. C., White, V., Hewitt, A. W., Mackey, D. A., Bailey, J. M., Slutske, W. S., Nyholt, D. R., Treloar, S. A., Martin, N. G., & Boomsma, D. I. (2009). Genetic influences on handedness: Data from 25,732 Australian and Dutch twin families. Neuropsychologia, 47(2), 330–337.
16. Miller, M. W., Brayman, A. A., & Abramowicz, J. S. (1998). Obstetric ultrasonography: A biophysical consideration of patient safety. American Journal of Obstetrics and Gynecology, 179(1), 241–254.
17. Naumburg, E., Bellocco, R., Cnattingius, S., Hall, P., & Ekbom, A. (2000). Prenatal ultrasound examinations and risk of childhood leukemia: Case-control study. BMJ, 320(7230), 282–283.
18. Newnham, J. P., Doherty, D. A., Kendall, G. E., Zubrick, S. R., Landau, L. I., & Stanley, F. J. (2004). Effects of repeated prenatal ultrasound examinations on childhood outcome. The Lancet, 364(9432), 2038–2044.
19. Rajaraman, P., Simpson, J., Neta, G., Berrington de Gonzalez, A., Ansell, P., Linet, M. S., Ron, E., & Roman, E. (2011). Early life exposure to diagnostic radiation and ultrasound scans and risk of childhood cancer: Case-control study. BMJ, 342, d472.
20. Rosman, N. P., Vassar, R., Doros, G., DeRosa, J., Froman, A., DiMauro, A., Santiago, S., & Abbott, J. (2018). Association of prenatal ultrasonography and autism spectrum disorder. JAMA Pediatrics, 172(4), 336–344.
21. Salvesen, K. A., & Eik-Nes, S. H. (1999). Ultrasound during pregnancy and subsequent childhood non-right handedness: A meta-analysis. Ultrasound in Obstetrics & Gynecology, 13(4), 241–246. https://doi.org/10.1046/j.1469-0705.1999.13040241.x
22. Vuoksimaa, E., Koskenvuo, M., Rose, R. J., & Kaprio, J. (2009). Origins of handedness: A nationwide study of 30,161 adults. Neuropsychologia, 47(5), 1294–1301.

Chapter 4

Uncontrolled Population Growth and Zoonous Infections With One Health Perspective

Derya KARATAŞ YENİ[1], Muhammed Can GÖKMEN[2], Asma ASHRAF[3]

Introduction

As living standards rose and infectious diseases became better controlled, the population growth rate exceeded 0.5% in the 19th century. This increase was particularly marked in Europe and overseas settlements. This period is widely regarded as the turbulent onset of the demographic transition (Cleland, 2013; Maddison, 2007). It is evident that until the mid-20th century, the growth rate increased only marginally, reaching 0.9%. However, since that period, it has undergone a substantial rise. The extensive implementation of contemporary preventive health measures in developing countries, coupled with the alleviation of local food shortages, has been instrumental in precipitating this surge. By 1960, the average life expectancy worldwide had risen to 48 years and continued to improve, reaching (Dyson, 2010).

In the contemporary era, characterised by accelerated population growth dynamics, urban expansion poses a significant threat to biodiversity, leads to the loss of agricultural land, and instigates climate change across diverse spatial scales (Cortinovis et al., 2022; Esipova et al., 2018). In consequence of the aforementioned events, which have been designated 'ecological changes', there has been a shift in the distribution and density of pathogen vectors, an alteration in the migratory patterns of wild animals, particularly birds, and an augmentation in the survival time of pathogens outside their hosts (Slingenbergh et al., 2004).

Consequently, the epidemiology of zoonotic infections is also undergoing change. Zoonotic infections are defined as infections that can be transmitted between animals and humans, either directly or via a vector (Yeni et al., 2025). Zoonotic infections have represented a major health problem for humanity since antiquity. Since the Neolithic period, zoonotic diseases have been among the

[1] Associate Professor Doctor, Necmettin Erbakan University Faculty of Veterinary Medicine
0000-0001-7261-1394.
[2] Research Assistant, Necmettin Erbakan University Faculty of Veterinary Medicine
0009-0003-6385-5495.
[3] Professor Doctor, Government College University Faisalabad Department of Zoology
0000-0001-7026-670X.

most significant factors contributing to the adaptation process of humanity (Stone, 2020). Recent studies have indicated that approximately 60% of new human infections are zoonotic in nature, with over 70% of these pathogens being of wildlife origin (Pacific, 2011).

It is evident that both domestic and wild animals of various species act as reservoirs for emerging or re-emerging diseases in humans. These diseases are caused by pathogens such as viruses, bacteria, fungi, and parasites originating from animals or animal products (Pal, 2005). Zoonotic infections are of significant public health importance for a number of reasons. A zoonotic agent is capable of causing infections that result in high morbidity and mortality rates in humans. In certain cases, these infections have the potential to evolve into epidemics, affecting large populations. Furthermore, zoonotic infections have the capacity to induce severe diseases in farm animals, thereby exerting an impact on the production of animal food (Cripps, 2000).

The interrelated concepts of population growth, urbanisation, and globalisation are reported to influence the emergence of zoonotic diseases. The correlation between population growth and increased consumption of animal protein has been well documented. This phenomenon has been demonstrated to result in the conversion of occupied rural land to livestock production, thereby increasing the likelihood of zoonotic diseases emerging. Urbanisation, increased population density and human interactions result in the accelerated transmission of zoonotic infections. In consequence of globalisation, the world's trade and travel networks are expanding, thus facilitating the international dissemination of pathogens (Antràs et al., 2023; Byrnes & Bumb, 2017; Wu et al., 2017). International travel and air transport, in particular, have led to the rapid intercontinental spread of epidemics such as Severe Acute Respiratory Syndrome (SARS) and influenza A (H1N1) (Mangili & Gendreau, 2005). Airports have also been demonstrated to play a role in the transmission of viruses through high-touch surfaces (Lake et al., 2009). Furthermore, an increase in global trade and food supply chains has been shown to facilitate the cross-border transport of foodborne pathogens. The rapid international dissemination of antibiotic-resistant bacterial strains, facilitated by factors such as medical tourism and migration, constitutes a grave threat to global health security (Hernando-Amado et al., 2019).

Consequently, due to rapid population growth, natural areas are being destroyed and uncontrolled urbanisation is becoming widespread. This destruction, carried out to meet the resource needs of the growing population, create new settlements, or for tourism purposes, increases the risk of transmission of zoonotic diseases. The epidemiology of zoonotic diseases is influenced by unplanned urbanisation and inadequate infrastructure, which are themselves consequences of rapid urbanisation. The One Health approach provides a critical framework for managing the risks associated with population growth, distorted urbanisation, and predatory settlement by addressing human, animal, and environmental health from a holistic perspective. The implementation of strategies grounded in One Health policy principles and the effective management of zoonotic diseases necessitates the involvement of a multidisciplinary array of professionals, including human physicians, veterinarians, dentists, pharmacists, nurses, ecologists, agricultural and environmental engineers, architects, and urban planners (Epiz, 2019).

The Relationship Between the Destruction of Natural Areas and Zoonoses

The expansion of land is driven by various factors, including population growth, urbanisation, tourism and mining activities. The process of land expansion has been demonstrated to result in deforestation and the destruction of both natural and agricultural areas (Esposito et al., 2023). The ongoing destruction of habitats, primarily driven by anthropogenic activities, globalisation, and species extinction, is a major contributing factor to the decline in biodiversity. Changes in biodiversity have been shown to result in ecosystem disturbances, which in turn can lead to alterations in the transmission patterns of infectious diseases, an accumulation of toxic pollutants, and the invasion of new or re-emerging infectious agents (Aguirre, 2017). It is evident that alterations in the movement patterns of animals and human interactions also have a significant impact on the level of exposure to zoonotic pathogens that may be transmitted by wildlife. As populations grow and urban areas expand, the fragmentation of natural habitats has resulted in wild animals approaching human settlements. This proximity facilitates direct interaction between animals and humans, thereby increasing the probability of transmission of zoonotic infectious pathogens. The alteration of biodiversity, coupled with the proliferation of mesopredators (e.g. red foxes, coyotes, raccoons, weasels, skunks, ferrets, domestic cats and stray dogs), has been shown to heighten the risk of the transmission of zoonotic infections. In addition, the interaction between domestic animals and harmful rodents has been demonstrated to increase the likelihood of pathogens being transmitted to humans and domestic animals (Scanes, 2018; Vinson et al., 2022).

Nipah virus outbreaks transmitted from bats to humans as a result of forest habitat degradation have emerged in Malaysia and Bangladesh (Hemamalani et al., 2025). Similarly, the Ebola virus emerges in Africa through recurring zoonotic transmission linked to deforestation and hunting (Olivero et al., 2017). Furthermore, coronaviruses such as SARS-CoV and SARS-CoV-2 are reported to have spread from bats and intermediate hosts to humans due to wildlife markets and habitat loss (Plowright et al., 2021). In addition to viral agents, the incidence of tick-borne infections such as *Francisella tularensis*, *Coxiella burnetii*, and *Borrelia burgdorferi* is increasing as a result of increased tick-human contact due to habitat fragmentation (Behzadi et al., 2021; Kilpatrick et al., 2017; Yeni et al., 2021). *Leptospira* spp. bacteria are increasingly infecting humans, particularly in agricultural irrigation areas and with the uncontrolled increase in rodent populations (Haake & Levett, 2014). Similarly, *Bacillus anthracis* spores are creating new contact areas with the expansion of livestock activities into forested areas, leading to the emergence of anthrax cases (Turner et al., 2013). Furthermore, *Salmonella enterica* and *Campylobacter* spp. are prominent foodborne zoonoses associated with intensive livestock farming and wildlife contact (Antunes et al., 2016). Consequently, these changes in land use increase interaction between humans and wildlife, significantly raising the risk of transmission of both viral and bacterial zoonotic pathogens.

The Relationship Between Wildlife and Zoonoses

The increased risk of zoonotic agents associated with wildlife entering intensive livestock production facilities as a result of the encroachment caused by the destruction of natural areas also has consequences for the transmission of wildlife-derived zoonotic agents (Bengis et al., 2004).

It is evident that wild animals play a pivotal role in the epidemiology of numerous bacterial, viral and parasitic zoonotic agents. Furthermore, these animals act as the primary reservoir for the transmission of such agents to domestic animals and humans (González-Barrio & Ruiz-Fons, 2019). Wildlife reservoirs have various transmission routes, including contact, bites, vectors, aerosols, and oral transmission (Altaf, 2020). Ticks, rodents, and wild mammals are the main animals that serve as reservoirs.

The main bacterial and viral zoonotic agents of wildlife origin, their wildlife hosts, and the infections they cause are listed in Table 1.

Table 1. Important zoonotic agents originating from wildlife

Infection	Agent	Primary Vector	Reference
BACTERIAL			
Lyme disease	*Borrelia burgdorferi*	Ixodes tick	(Helble et al., 2021)
Tularemia	*Francisella tularensis*	Rodents, Hares, Rabbits, Arthropoda, Tick	(Yeni et al., 2021)
Plauge	*Yersinia pestitis*	Wild Mammals, Rodents, Fleas	(Bevins et al., 2012)
Q fever	*Coxiella burnetii*	Ticks, Wild Mammals, Wild Birds	(Celina & Cerný, 2022)
Leptospirosis.	*Leptospira* spp	Rodents, Wild Mammals	(Fornazari et al., 2018)
Bartonellosis	*Bartonella* spp.	Rodents, Bats, Wild Carnivores	(Kosoy & Goodrich, 2019)
Salmonellosis	*Salmonella* spp.	Wild Birds, Wild Mammals	(Millán et al., 2004)
VIRAL			
Rabies	*Lyssavirus rabies*	Wild Carnivors	(Holmala & Kauhala, 2006)
West Nile fever	West Nile Virus	Arthropods, Wild Birds And Mammals	(Van der Meulen et al., 2005)
Hantavirus	*Orthohantavirus*	Rodents	(Chen et al., 2023)
Avian flu	*Alphainfluenzavirus influenzae*	Wild Birds	(Stallknecht & Brown, 2008)
Ebola	*Orthoebolavirus zairense*	Primats, Wild Mammals	(Leroy et al., 2004)
Monkeypox	*Monkeypox virus*	Rodents, Primats, Wild Mammals	(Song & Zheng, 2022)
Severe Acute Respiratory Syndrome	SARS coronavirus	Wild Mammals	(Shi & Hu, 2008)

Zoonoses Associated with Unplanned Urbanization and Inadequate Infrastructure

The general increase in the population of a settlement and the concomitant rise in the level of industrialization are referred to as urbanization. This phenomenon is characterised by the migration of individuals from rural to urban areas, resulting in an increase in the size and number of cities. Urbanisation is a process which has been proven to result in increased size and density of urban areas. The uncontrolled expansion of urban areas, a phenomenon that is particularly evident in developing countries, gives rise to a multitude of challenges. These include environmental degradation, the deterioration of water quality, excessive air pollution, and inadequate waste disposal (Özden & Enwere, 2012; Rex et al., 2017; Uttara et al., 2012).

The number and proportion of people living in urban areas is rapidly increasing. Between 1970 and 2000, there was a global increase of 5.8 million hectares in urban land area (Scanes, 2018). Today, as a result of the need for cities to expand, rural areas are also being urbanized to create new areas (Patterson et al., 2003). Despite the decline in the number of wildlife parasites in rural areas consequent to urbanisation, there is a possibility, albeit in some cases, that transmission among hosts that have adapted to the urban environment may increase. This phenomenon may have ramifications for rare wildlife species and species inhabiting areas outside urban areas (Bradley & Altizer, 2007). As a consequence of this phenomenon, biodiversity is declining, contact with wildlife species that act as reservoirs for zoonoses adapted to the urban environment is increasing, infections are spreading more rapidly due to dense populations, and changing climates due to urban structures facilitate the proliferation of vectors. Moreover, in unplanned urban growth areas, the absence of adequate water and sanitation infrastructure can result in the proliferation of waterborne diseases (Rahaman et al., 2023).

It has been observed that certain species of rodents have adapted to urban environments, demonstrating resilience and continued viability. These include the *Mus musculus* (house mouse), *Rattus norvegicus* (Norwegian rat), and the *Rattus rattus* species complex (black rat and its relatives), which are believed to have coexisted with humans for millennia. Nonetheless, despite their adaptation, the presence of rodents in urban areas imposes a significant economic burden and poses a substantial threat to human health (Blasdell et al., 2022).

Urban rodent infestations can lead to leptospirosis (*Leptospira* spp.), hantavirus infections (*Hantaviridae*), Lassa fever (*Lassa virus*), salmonellosis (*Salmonella enterica*), shigellosis (*Shigella* spp.), typhus (*Rickettsia* spp.), and plague (*Yersinia pestis*) (Azimi et al., 2024; Firth et al., 2014; Jahan et al., 2021; Karpagam & Ganesh, 2020; Ndenda et al., 2022).

The main zoonotic infections associated with unplanned urbanization and inadequate infrastructure are listed in Table 2.

Table 2. Main zoonotic infections associated with unplanned urbanization and inadequate infrastructure

Infections	Agent	Relationship with Inadequate Infrastructure	Referance
Leptospirosis	*Leptospira* spp.	Inadequate sewage systems, uncontrolled spread of flood and wastewater, contact with rodents	(Baharom et al., 2024)
Lassa fever	Lassa virus (*Arenaviridae*)	Inadequate food storage and hygiene, rodent contamination	(Reyna et al., 2024)
Enteritis	*Salmonella enterica*	Contaminated water and food sources, waste management deficiencies	(Mkangara, 2023)
Typhoid fever	*Salmonella Typhi*	Contaminated water and food sources, waste management deficiencies	(Vanderslott et al., 2023)
Shigellosis	*Shigella* spp.	Inadequate drinking water hygiene, limited sewage and handwashing facilities	(Hale & Keusch, 1996)
Typhus	*Rickettsia* spp.	Crowded living spaces, weak vector control	(Griffiths et al., 2022)
Plague	*Yersinia pestis*	Accumulation of garbage and increasing rodent and flea populations	(Bunker, 2008)
Dengue fever	Dengue virus (*Flaviviridae*)	Increased mosquito breeding grounds due to inadequate water storage and waste management	(Gibb et al., 2023)
Cholera	*Vibrio cholerae*	Inadequate drinking water infrastructure and sewerage deficiencies	(Jutla et al., 2013)
Yellow fever	Yellow fever virus (*Flaviviridae*)	Inadequate urban vector control, proliferation of Aedes mosquitoes	(Gubler, 2011)

One Health Approach and Control of Zoonoses

In 2022, the Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), the World Organization for Animal Health (WOAH), and the World Health Organization (WHO) developed the One Health Joint Action Plan (2022–2026). This plan, which employs a holistic approach that addresses human, animal, plant, and environmental health in unison, promotes an integrated and sustainable approach to health threats at the global, regional, and national levels. The One Health Approach is predicated on the understanding that human, animal, plant, and environmental health are interconnected. It provides an interdisciplinary framework for the control of zoonotic diseases. This approach is widely regarded as a highly effective strategy for the prevention and control of zoonoses (Quaresma et al., 2023). In particular, factors such as the increased interaction with wildlife and the destruction of habitat, in conjunction with climate change, have been identified as key elements that serve to increase the risk of the spread of zoonoses. In this context, strategies such as the One Health Approach, the strengthening of health systems, the establishment of early warning systems, the establishment of effective vaccination and biosecurity programs, and the encouragement of multisectoral collaboration should aim to effectively control zoonoses. This will facilitate more effective monitoring of the impact of population growth and rural settlements on zoonoses, reduce risks, and enhance the sustainability of public health strategies.

Conclusion

Uncontrolled population growth is regarded as one of the most significant factors endangering ecological balance and public health on a global scale. The rapid growth of the human population has resulted in a number of environmental issues, including the shrinkage of natural habitats, increased contact with wild and domestic animals, unsustainable practices in the food production chain, and, ultimately, the spread of zoonotic infections. This situation gives rise to a range of socioeconomic and health problems, affecting both developing and developed countries.

The interplay of zoonotic diseases, climate change, rapid urbanisation, uncontrolled migration, and inadequate veterinary and public health services creates a multifaceted risk. Recent epidemics, including SARS, MERS, and the ongoing global pandemic of the novel severe acute respiratory syndrome (SARS-CoV-2), have demonstrated the critical interconnection between uncontrolled population growth and the potential for the transmission of zoonotic diseases. Consequently, this predicament ought to be regarded not solely as a medical concern, but also as a social, economic, political and environmental phenomenon.

In this context, it is imperative to develop effective population planning strategies at the national level, to protect natural resources, and to adopt sustainable living models. The "One Health" approach, which addresses human, animal, and environmental health in unison, must be reinforced, and interdisciplinary collaborations must be augmented within this framework. The establishment of robust laboratory infrastructures, digital surveillance systems, and international data-sharing networks is of crucial importance for the early detection of zoonotic infections. Furthermore, awareness training on zoonoses, hygiene, animal contact, and environmental health should be provided for both the public and healthcare professionals. In addition, there is a necessity to implement infrastructural enhancements in both rural and urban regions, with the objective of fortifying sewage systems, optimising waste management, ensuring access to potable water, and enhancing the safety of the animal production chain. It is imperative that research on the ecology, transmission routes and control strategies of zoonotic agents is increased, and that the development of new vaccines and treatment methods is encouraged. In consideration of the borderless nature of zoonotic infections, the enhancement of coordination, information sharing, and collaborative intervention strategies among nations is imperative in this process.

In conclusion, there is a strong interaction between the environmental pressures created by uncontrolled population growth and the rise of zoonotic diseases. The solution to this problem is not confined to the health sector; rather, it necessitates an integrated approach encompassing environmental, agricultural, economic, and educational policies. Interdisciplinary approaches and global collaborations will be essential tools in combating both current and future zoonotic threats.

References

Aguirre, A. A. (2017). Changing patterns of emerging zoonotic diseases in wildlife, domestic animals, and humans linked to biodiversity loss and globalization. *ILAR journal*, *58*(3), 315-318.

Altaf, M. (2020). Wild animals as source of Zoonotic diseases-a review. *J Wildl*.

Antràs, P., Redding, S. J., & Rossi-Hansberg, E. (2023). Globalization and pandemics. *American Economic Review*, *113*(4), 939-981.

Antunes, P., Mourão, J., Campos, J., & Peixe, L. (2016). Salmonellosis: the role of poultry meat. *Clinical microbiology and infection*, *22*(2), 110-121.

Asia Pacific strategy for emerging diseases: 2010. WHO Regional Office for the Western Pacific. https://iris.who.int/handle/10665/207661 (accessed on 11 September 2025).

Azimi, T., Nasrollahian, S., Sabour, S., Hadi, N., Azimi, L., Fallah, F., & Pourmand, M. R. (2024). Detection of Yersinia enterocolitica, Shigella spp. and Salmonella spp. in Rattus norvegicus captured from Tehran, Iran. *Future Microbiology*, *19*(5), 377-384.

Baharom, M., Ahmad, N., Hod, R., Ja'afar, M. H., Arsad, F. S., Tangang, F., Ismail, R., Mohamed, N., Radi, M. F. M., & Osman, Y. (2024). Environmental and occupational factors associated with leptospirosis: a systematic review. *Heliyon*, *10*(1).

Behzadi, M. Y., Mostafavi, E., Rohani, M., Mohamadi, A., Ahmadinezhad, M., Moazzezy, N., Shams-Ghahfarokhi, M., & Razzaghi-Abyaneh, M. (2021). A review on important zoonotic bacterial tick-borne diseases in the Eastern Mediterranean region. *Journal of Arthropod-Borne Diseases*, *15*(3), 265.

Bengis, R., Leighton, F., Fischer, J., Artois, M., Morner, T., & Tate, C. (2004). The role of wildlife in emerging and re-emerging zoonoses. *Revue Scientifique et Technique-Office International des Epizooties*, *23*(2), 497-512.

Bevins, S. N., Baroch, J. A., Nolte, D. L., Zhang, M., & He, H. (2012). Yersinia pestis: examining wildlife plague surveillance in China and the USA. *Integrative zoology*, *7*(1), 99-109.

Blasdell, K. R., Morand, S., Laurance, S. G., Doggett, S. L., Hahs, A., Trinh, K., Perera, D., & Firth, C. (2022). Rats and the city: Implications of urbanization on zoonotic disease risk in Southeast Asia. *Proceedings of the National Academy of Sciences*, *119*(39), e2112341119.

Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in ecology & evolution*, *22*(2), 95-102.

Bunker, R. (2008). A plenitude, plethora or plague of plans? State strategic Plans, metropolitan strategies and infrastructure plans. *Built Environment*, *34*(3), 319-332.

Byrnes, B. H., & Bumb, B. L. (2017). Population growth, food production and nutrient requirements. In *Nutrient use in crop production* (pp. 1-27). CRC Press.

Celina, S. S., & Cerný, J. (2022). Coxiella burnetii in ticks, livestock, pets and wildlife: a mini-review. *Frontiers in veterinary science*, *9*, 1068129.

Centers for Disease Control and Prevention. (2025). *National One Health framework to address zoonotic diseases and advance public health preparedness in the United States*. U.S. Department of Health & Human Services. https://www.cdc.gov/one-health/media/pdfs/2025/01/354391-A-NOHF-ZOONOSES-508_FINAL.pdf

Chen, R.-X., Gong, H.-Y., Wang, X., Sun, M.-H., Ji, Y.-F., Tan, S.-M., Chen, J.-M., Shao, J.-W., & Liao, M. (2023). Zoonotic Hantaviridae with global public health significance. *Viruses*, *15*(8), 1705.

Cleland, J. (2013). World population growth; past, present and future. *Environmental and Resource Economics*, *55*(4), 543-554.

Cortinovis, C., Geneletti, D., & Haase, D. (2022). Higher immigration and lower land take rates are driving a new densification wave in European cities. *npj urban sustainability*, *2*(1), 19.

Cripps, P. J. (2000). Veterinary education, zoonoses and public health: a personal perspective. *Acta Tropica*, *76*(1), 77-80.

Dyson, T. (2010). *Population and development: the demographic transition*. Bloomsbury Publishing.

Epiz, R. (2019). One Health collaborations for zoonotic disease control in Ethiopia. *Rev. Sci. Tech. Off. Int. Epiz*, *38*(1), 51-60.

Esipova, N., Pugliese, A., & Ray, J. (2018). More than 750 million worldwide would migrate if they could. *World*, *13*, 14.

Esposito, M. M., Turku, S., Lehrfield, L., & Shoman, A. (2023). The impact of human activities on zoonotic infection transmissions. *Animals*, *13*(10), 1646.

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., Conte, J. M., Ng, J., Garcia, J., & Bhuva, N. P. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. *MBio*, *5*(5), 10.1128/mbio. 01933-01914.

Fornazari, F., Langoni, H., Marson, P. M., Nóbrega, D. B., & Teixeira, C. R. (2018). Leptospira reservoirs among wildlife in Brazil: Beyond rodents. *Acta Tropica*, *178*, 205-212.

Gibb, R., Colón-González, F. J., Lan, P. T., Huong, P. T., Nam, V. S., Duoc, V. T., Hung, D. T., Dong, N. T., Chien, V. C., & Trang, L. T. T. (2023). Interactions between climate change, urban infrastructure and mobility are driving dengue emergence in Vietnam. *Nature Communications*, *14*(1), 8179.

González-Barrio, D., & Ruiz-Fons, F. (2019). Coxiella burnetii in wild mammals: A systematic review. *Transboundary and emerging diseases*, *66*(2), 662-671.

Griffiths, J., Yeo, H. L., Yap, G., Mailepessov, D., Johansson, P., Low, H. T., Siew, C.-C., Lam, P., & Ng, L. C. (2022). Survey of rodent-borne pathogens in Singapore reveals the circulation of Leptospira spp., Seoul hantavirus, and Rickettsia typhi. *Scientific Reports*, *12*(1), 2692.

Gubler, D. J. (2011). Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Tropical medicine and health*, *39*(4SUPPLEMENT), S3-S11.

Haake, D. A., & Levett, P. N. (2014). Leptospirosis in humans. *Leptospira and leptospirosis*, 65-97.

Hale, T. L., & Keusch, G. T. (1996). Shigella. *Medical Microbiology. 4th edition*.

Helble, J. D., McCarthy, J. E., & Hu, L. T. (2021). Interactions between Borrelia burgdorferi and its hosts across the enzootic cycle. *Parasite immunology*, *43*(5), e12816.

Hemamalani, A., Thangam, T., Prakashini, R. S., Kumar, P. A., & Parthasarathy, K. (2025). Viral ecology in chiroptera: human-wildlife interactions and pandemic risk. *Veterinary Research Communications*, *49*(5), 1-14.

Hernando-Amado, S., Coque, T. M., Baquero, F., & Martínez, J. L. (2019). Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nature microbiology*, *4*(9), 1432-1442.

Holmala, K., & Kauhala, K. (2006). Ecology of wildlife rabies in Europe. *Mammal Review*, *36*(1), 17-36.

Jahan, N. A., Lindsey, L. L., & Larsen, P. A. (2021). The role of peridomestic rodents as reservoirs for zoonotic foodborne pathogens. *Vector-borne and zoonotic diseases*, *21*(3), 133-148.

Jutla, A., Whitcombe, E., Hasan, N., Haley, B., Akanda, A., Huq, A., Alam, M., Sack, R. B., & Colwell, R. (2013). Environmental factors influencing epidemic cholera. *The American journal of tropical medicine and hygiene*, *89*(3), 597.

Karpagam, K. B., & Ganesh, B. (2020). Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(5), 835-846.

Kilpatrick, A. M., Dobson, A. D., Levi, T., Salkeld, D. J., Swei, A., Ginsberg, H. S., Kjemtrup, A., Padgett, K. A., Jensen, P. M., & Fish, D. (2017). Lyme disease ecology in a changing world: consensus, uncertainty and critical gaps for improving control. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1722), 20160117.

Kosoy, M., & Goodrich, I. (2019). Comparative ecology of Bartonella and Brucella infections in wild carnivores. *Frontiers in veterinary science*, *5*, 322.

Lake, I., Gillespie, I., Bentham, G., Nichols, G., Lane, C., Adak, G., & Threlfall, E. (2009). A re-evaluation of the impact of temperature and climate change on foodborne illness. *Epidemiology and Infection*, *137*(11), 1538-1547.

Leroy, E. M., Rouquet, P., Formenty, P., Souquière, S., Kilbourne, A., Froment, J.-M., Bermejo, M., Smit, S., Karesh, W., & Swanepoel, R. (2004). Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science*, *303*(5656), 387-390.

Maddison, A. (2007). *Contours of the world economy 1-2030 AD: Essays in macro-economic history*. Oxford University Press.

Mangili, A., & Gendreau, M. A. (2005). Transmission of infectious diseases during commercial air travel. *The Lancet*, *365*(9463), 989-996.

Millán, J., Aduriz, G., Moreno, B., Juste, R. A., & Barral, M. (2004). Salmonella isolates from wild birds and mammals in the Basque Country (Spain).

Mkangara, M. (2023). Prevention and control of human Salmonella enterica infections: An implication in food safety. *International Journal of Food Science*, *2023*(1), 8899596.

Ndenda, J., Njagarah, J., & Shaw, S. (2022). Influence of environmental viral load, interpersonal contact and infected rodents on Lassa fever transmission dynamics: Perspectives from fractional-order dynamic modelling. *AIMS Math*, *7*(5), 8975-9002.

Olivero, J., Fa, J. E., Real, R., Márquez, A. L., Farfán, M. A., Vargas, J. M., Gaveau, D., Salim, M. A., Park, D., & Suter, J. (2017). Recent loss of closed forests is associated with Ebola virus disease outbreaks. *Scientific Reports*, *7*(1), 14291.

Özden, K., & Enwere, C. (2012). Urbanization and its political challenges in developing countries. *Eurasian Journal of Business and Economics*, *5*(10), 99-120.

Pacific, W. H. O. R. O. f. t. W. (2011). *Asia Pacific strategy for emerging diseases: 2010*. https://iris.who.int/handle/10665/207661

Pal, M. (2005). Importance of zoonoses in public health. *Indian Journal of Animal Sciences*, *75*(5), 586-591.

Patterson, M. E., Montag, J. M., & Williams, D. R. (2003). The urbanization of wildlife management: Social science, conflict, and decision making. *Urban Forestry & Urban Greening*, *1*(3), 171-183.

Plowright, R. K., Reaser, J. K., Locke, H., Woodley, S. J., Patz, J. A., Becker, D. J., Oppler, G., Hudson, P. J., & Tabor, G. M. (2021). Land use-induced spillover: a call to action to safeguard environmental, animal, and human health. *The Lancet Planetary Health*, *5*(4), e237-e245.

Quaresma, P. F., Martins-Duarte, E. S., & Soares Medeiros, L. C. (2023). One Health Approach in Zoonosis: strategies to control, diagnose and treat neglected diseases. In (Vol. 13, pp. 1227865): Frontiers Media SA.

Rahaman, M. A., Kalam, A., & Al-Mamun, M. (2023). Unplanned urbanization and health risks of Dhaka City in Bangladesh: uncovering the associations between urban environment and public health. *Frontiers in public health*, *11*, 1269362.

Rex, O. O., Yetunde, O., Grace, E. C., & Pearl, O. A. (2017). Sustainable Urbanization: Investigating Problems Encountered in Uncontrolled Urban Growth in Nyanya–A suburb of Abuja, Nigeria. *International Journal of Humanities, Arts and Social Sciences*, *3*(1), 13.

Reyna, R. A., Littlefield, K. E., Shehu, N., Makishima, T., Maruyama, J., & Paessler, S. (2024). The Importance of Lassa Fever and Its Disease Management in West Africa. *Viruses*, *16*(2), 266.

Scanes, C. G. (2018). Human activity and habitat loss: destruction, fragmentation, and degradation. In *Animals and human society* (pp. 451-482). Elsevier.

Shi, Z., & Hu, Z. (2008). A review of studies on animal reservoirs of the SARS coronavirus. *Virus research*, *133*(1), 74-87.

Slingenbergh, J., Gilbert, M., Balogh, K. d., & Wint, W. (2004). Ecological sources of zoonotic diseases. *Revue Scientifique et Technique-Office International des Epizooties*, *23*(2), 467-484.

Song, T.-Z., & Zheng, Y.-T. (2022). Monkeypox, wild animals, and potential big problem. *Zoological Research*, *43*(4), 612.

Stallknecht, D. E., & Brown, J. D. (2008). Ecology of avian influenza in wild birds. *Avian influenza*, 43-58.

Stone, A. C. (2020). Getting sick in the Neolithic. *Nature Ecology & Evolution*, *4*(3), 286-287.

Turner, W. C., Imologhome, P., Havarua, Z., Kaaya, G. P., Mfune, J. K., Mpofu, I. D., & Getz, W. M. (2013). Soil ingestion, nutrition and the seasonality of anthrax in herbivores of Etosha National Park. *Ecosphere*, *4*(1), 1-19.

Uttara, S., Bhuvandas, N., & Aggarwal, V. (2012). Impacts of urbanization on environment. *International Journal of Research in Engineering and Applied Sciences*, *2*(2), 1637-1645.

Van der Meulen, K., Pensaert, M., & Nauwynck, H. (2005). West Nile virus in the vertebrate world. *Archives of virology*, *150*(4), 637-657.

Vanderslott, S., Kumar, S., Adu-Sarkodie, Y., Qadri, F., & Zellweger, R. M. (2023). Typhoid control in an era of antimicrobial resistance: challenges and opportunities. Open Forum Infectious Diseases,

Vinson, J. E., Gottdenker, N. L., Chaves, L. F., Kaul, R. B., Kramer, A. M., Drake, J. M., & Hall, R. J. (2022). Land reversion and zoonotic spillover risk. *Royal Society open science*, *9*(6), 220582.

World Health Organization. Regional Office for the Western Pacific. (2011).

Wu, T., Perrings, C., Kinzig, A., Collins, J. P., Minteer, B. A., & Daszak, P. (2017). Economic growth, urbanization, globalization, and the risks of emerging infectious diseases in China: A review. *Ambio*, *46*(1), 18-29.

Yeni, D. K., Büyük, F., Ashraf, A., & Shah, M. S. U. D. (2021). Tularemia: a re-emerging tick-borne infectious disease. *Folia microbiologica*, *66*(1), 1-14.

Yeni, D. K., Gökmen, M. C., & Ashraf, A. Bartonella henselae and Zoonotic Significance. *Theoretical And Applied Perspectives In Health Sciences*, 1.

Chapter 5

Innovations in Equine Stable Design: Integrating Ethology, Enrichment, and Social Housing to Advance Horse Welfare

Fatih YILDIRIM[1]

Abstract

Equine physiology and behavior remain intrinsically linked to the adaptive patterns forged throughout their evolutionary history. Conventional stable management, however, often imposes environments that curtail the expression of fundamental equine needs, including voluntary movement, continuous foraging, and conspecific engagement. Such restrictions are correlated with an increased prevalence of stress-induced pathologies, the emergence of stereotypic behaviors, and compromised long-term health. This chapter explores the application of ethological principles to equine housing design, advocating for a paradigm shift toward models that prioritize welfare through environmental enrichment and strategic planning. Innovations central to this discussion encompass paced-nutrition delivery systems, circuit-based exercise areas, and architectural adaptations like communal contact zones that facilitate secure tactile communication. Findings from behavioral and cortisol-based studies are integrated with actionable design protocols addressing spatial configuration, fodder dispersal, and the logistics of maintaining stable social groups. Representative implementations across breeding, sanctuary, and competitive training operations demonstrate the successful translation of theory into practice. The analysis also addresses economic considerations, phased adoption strategies, and advancements in objective welfare assessment, positing that incremental, evidence-based modifications can produce significant measurable gains. In conclusion, the collective evidence argues compellingly for a departure from static confinement models and toward dynamic living systems that actively support innate behavioral repertoires, foster social cohesion, and enhance overall resilience in managed equine populations.

[1] Prof. Dr.; Atatürk University Faculty of Veterinary Medicine, Department of Animal Science. fatihyildirim@atauni.edu.tr ORCID No: 0000-0002-9402-4008 - Cell no: 0541 328 4418

1. Introduction

The horse is a grazing and foraging herbivore by evolution (Goodwin, 1999), having adapted to consume a diet primarily consisting of high-fibre, fresh forage (Harris and Arkell 1999). For numerous domesticated species, confinement systems create spatial limitations that restrict the performance of innate behaviours, including locomotion and social engagement. To evaluate the impact of these restrictions on behaviour and welfare, it is necessary to comprehend the fundamental motivational mechanisms that drive specific actions. A frequently employed method to gauge motivation involves offering the animal an opportunity to execute the behaviour and subsequently quantifying either its maintenance duration or its performance frequency (Christensen et al., 2002). Modern management practices (centered on single-stall stabling, high-concentrate rations, and restricted turnout) differ profoundly from these ancestral conditions. Over the past years, increasing evidence has documented the behavioral, physiological, and health-related costs of confinement, encouraging the adoption of stables that integrate enrichment and social opportunities (Marliani, 2023).

Domestication has improved horses' survival by providing care and protection, yet it has also introduced constraints that clash with their evolutionary heritage. Natural behaviours shaped by life as social prey animals (such as free movement, reproduction, and energy use for self-preservation) are often restricted in managed environments. While horses demonstrate tendencies that facilitate cooperation with humans, misinterpretations of dominance have historically distorted the understanding of their social systems. The progression of the horse–human relationship, from utilitarian use to companionship, continues to generate conflicts of interest, highlighting the need for management practices informed by ethological insights to safeguard equine welfare (Goodwin, 1999). When these behaviors are constrained in traditional stalls, a mismatch emerges between biological needs and living environments. This mismatch results in chronic stress and compromised welfare and is further reflected in measurable neuroendocrine alterations, particularly in stress-response systems, that may predispose animals to long-term health challenges (Alexander and Irvine, 1998). Consequently, there is both a scientific and ethical imperative to reframe equine housing through the lens of ethology and welfare science.

2. Behavioral, Psychological, and Health Consequences

Stable confinement, particularly under conditions of social isolation, disrupts the natural organization of equine groups, leading to heightened stress and the emergence of stereotypies. Behaviors such as weaving, crib-biting, and stall-walking are now widely interpreted as outward signs of internal frustration and psychological strain (Mason and Rushen, 2008; Cooper and Albentosa, 2005). Recent neuroendocrine findings further suggest that these stereotypic patterns are linked to alterations in dopaminergic and opioid pathways, pointing to a complex neurobiological basis for their persistence (McBride and Hemmings, 2009).

Physiological consequences of isolation have also been documented. Horses deprived of social contact frequently exhibit dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, with elevated baseline cortisol concentrations and maladaptive coping responses (Alexander and Irvine, 1998). Extended periods in enclosed environments further contribute to respiratory compromise due to prolonged exposure to dust, mold spores, and ammonia (Elfman et al., 2011). A lack of sustained locomotion reduces musculoskeletal resilience, impairs circulation, and heightens injury risk. Feeding management within stall-based systems (often based on infrequent, high-volume meals) has been strongly associated with digestive dysfunction, gastric ulceration, and colic (Van den Boom, 2022). The relationship between restricted feeding frequency, gastric pH instability, and squamous ulcer disease is particularly well established, underscoring the influence of management strategies on gastrointestinal health (Andrews et al., 2017).

3. Ethological foundations of stable design

The foundation of humane stable design lies in addressing core ethological needs. Three interconnected needs are paramount: (1) extended availability of forage through slow feeding, (2) opportunities for self-motivated locomotion and exercise, and (3) meaningful visual and physical social contact (Phelipon et al., 2024). When these needs are not met, horses often develop abnormal repetitive behaviours (stereotypies), digestive problems, and changes in stress-related physiology (Fureix et al., 2013).

Recent work also emphasizes the cognitive aspects of welfare: environments that offer complexity and opportunities for social learning can affect behavioral flexibility, the capacity to handle stress, and suitability for training (Henshall et al., 2022). Therefore, design changes that increase the range of possible behaviors can also create secondary benefits for handling, training, and the animal's functional lifespan.

Housing horses in open barns is associated with enhanced opportunities for social contact and voluntary locomotion, both of which are linked to improved welfare outcomes. Nevertheless, many owners remain concerned that such management systems may elevate the likelihood of injuries (Kjellberg et al., 2025).

4. Environmental enrichment: feeding, substrate, and sensory aproaches

Environmental enrichment for equines involves nutritional, physical, sensory, and cognitive modifications designed to stimulate natural behaviors and reduce frustration. Enrichment based on forage is the most extensively researched and consistently effective type.

Studies support that management systems designed to encourage near-constant movement and free-access foraging, such as track-based "paddock paradise" systems, offer significant benefits. One study observed a significant reduction in colic cases and an improvement in hoof health parameters, outcomes directly linked to the increased daily movement and natural grazing behaviors promoted by such environments (Jackson, 2006).

Multiple studies show that slow-feeding devices, haynets with small openings, and multiple, spread-out forage locations significantly extend feeding duration and reduce consumption speed, which helps lower risk factors for gastric ulcers and oral stereotypes (Glunk et al., 2014; Rochais et al., 2018; Ellis et al., 2015). Slow feeder systems are feed-delivery devices engineered to mechanically prolong feed intake time. They thus represent a potential tool for enhancing horse welfare by meeting their behavioral and physiological needs related to nutrition while also helping to manage obesity risks. However, there is limited data on how horse owners currently use slow feeders and the characteristics of horses that use them (Roig-Pons et al., 2025).

Beyond nutrition, objects that can be manipulated and pasture enrichment (e.g., balls, obstacles, and foraging puzzles) promote locomotion, play, and exploratory behavior (Jørgensen et al., 2011). Rotating these objects helps maintain novelty and ongoing interest.

Sensory enrichment (visual, auditory, olfactory) provides cost-effective additional benefits. Mirrors and calming sounds have been shown to reduce weaving and stress-related behaviors in stabled horses (McAfee et al., 2002). Olfactory stimulation using safe scents like lavender has demonstrated potential for short-term behavioral change (Bini de Lima et al., 2023).

Also, some researchers recommend grouping horses into classes according to certain measurements and housing them in groups rather than individual housing when adequate paddock space and correct management are used (Kjellberg et al., 2025).

5. Social housing: group turnout, social boxes, and management strategies

Social housing represents a second critical element of welfare-oriented stable design. Group turnout and stable group living arrangements are linked to better behavioral outcomes, including more mutual grooming, play, and more natural patterns of daily activity (Hartmann et al., 2012; Søndergaard and Ladewig, 2004). In equine management, individual stabling remains a common practice; however, this approach has been consistently associated with adverse consequences for animal welfare. Confinement of this kind is known to provoke a range of maladaptive behavioral responses, including the development of stereotypic actions, increased hostility toward humans, diminished environmental engagement, and manifestations of stress. Prolonged periods in solitary housing further intensify the risk of environmental withdrawal. Horses are often housed in individual stalls, a management approach associated with significant welfare issues. This form of isolation has been connected to various behavioral indicators of poor welfare, such as the development of stereotypic behaviors, increased aggression directed at handlers, a general state of apathy, and other stress-related conduct. Research suggests that prolonged solitary confinement may contribute to a horse's withdrawal from environmental stimuli. Consequently, enhancing welfare necessitates providing ample chances for unrestricted locomotion, interaction with other horses, and ad libitum access to forage, which aligns with the species' core behavioral and biological requirements (Ruet et al., 2019). Protecting equine welfare thus depends on implementing management strategies that facilitate self-directed movement, consistent social engagement with other horses, and continuous availability of roughage, supporting innate behavioral patterns and physical health. Other documented limitations in traditional stall designs include inadequately sized resting areas and narrow doorways, which can further compound welfare problems (Hartmann et al., 2012).

The successful implementation of social housing, however, is contingent upon variables such as group consistency, resource allocation, and environmental layout. Evidence demonstrates that carefully designed group environments equipped with ample feeding bins, water sources, and defined retreat areas can effectively minimize conflict (Dai et al., 2023; Puttkammer et al., 2024).

In situations where continuous group turnout is impractical, the 'social box' concept presents a scientifically validated compromise. Emerging research shows that these modified stalls, which enable safe tactile contact between adjacent horses, promote a marked increase in affiliative interactions among stallions and do not lead to a greater incidence of wounds when appropriate management protocols are followed (Zollinger et al., 2023).

6. Practical design recommendations and architectural blueprints

The architectural planning of equine facilities necessitates a holistic approach that integrates structural integrity, species-specific behavioral requirements, operational safety, and husbandry logistics. Scientific guidelines propose minimum spatial allocations, recommending stalls of approximately 4 x 4 meters for mature horses, dimensions of 3 x 3 or 3 x 2 meters for ponies, and a larger 4 x 5 meter area for foaling stalls to accommodate parturition (Özbeyaz and Akçapınar, 2010). Furthermore, the layout of group housing systems must be conceived to facilitate unimpeded access to outdoor paddocks or track systems. This is best achieved by incorporating several distinct entry and exit points within the design, which promotes voluntary movement and provides crucial opportunities for retreat during social interactions, thereby enhancing overall welfare.

Feeding infrastructure should focus on decentralized forage presentation: multiple hay racks, slow feeders, and foraging devices per group. A minimum of one feeding station per three horses helps reduce competition; more stations are better in groups with clear dominance structures (Glunk et al., 2014; Thorne et al., 2005).

Social-contact design features include partial walls, viewing windows, and adjustable panels that allow controlled proximity without complete separation. Social boxes should incorporate strong, smooth barriers and fixtures with no protrusions to minimize injury risk (Zollinger et al., 2023).

For turnout and track systems, designs should include variety in footing and terrain to encourage natural movement and hoof wear, while also ensuring good drainage and safe surfaces. Rotational grazing and track width are important: narrower tracks promote movement without creating areas where animals can be trapped.

7. Monitoring, evaluation, and staff engagement

Group-based outdoor housing is widely regarded as more advantageous for equine welfare than individual stabling, as it more closely resembles the ecological and social environment of free-ranging horses and enables the performance of key natural behaviors, including grazing, social interaction, and unrestricted movement. Nonetheless, ongoing debate remains concerning the challenges of sustaining optimal body condition in such systems and the heightened risk of injury associated with group living (Dalla Costa et al., 2016). Design improvements should be paired with consistent monitoring. The AWIN welfare assessment protocol offers validated, animal-focused measures (e.g., body condition, skin conditions, stereotypies) and evaluations of resources and management that are suitable for use on farms (Dalla Costa et al., 2016; Dai et al., 2023).

Equine behavior offers valuable insights into their subjective states and therefore serves as a meaningful indicator of welfare. Due to its complexity, however, reliable assessment requires objective, quantifiable, and evidence-based criteria. Since healthy and unstressed horses typically follow highly repetitive daily routines, analyzing the temporal distribution of their behavioral activities (time-budget analysis) can provide a robust tool for evaluating welfare status (Auer et al., 2021)

Positive modifications in housing management, such as free-range systems with access to stable, could enhance the welfare of breeding stallions. The observation that stallions became more friendly toward humans and less fearful indicates that consistent and careful handling can significantly improve the human-horse relationship. A positive relationship based on trust results in safer and more effective handling. This increased trust makes owners more willing to adapt their management practices to provide stallions with opportunities for free exercise. The results indicated a higher frequency of the indifferent and friendly responses in the stallions than in the mares. (Popescu et al., 2019).

8. Case studies and applied evidence

Case Study 1: Social boxes in a breeding stud. A controlled study involving Franches-Montagnes stallions housed in social boxes reported more affiliative interactions and better behavioral results during paired driving tests (Zollinger et al., 2023).

Case Study 2: Track system implementation in a rescue sanctuary. The use of track grazing systems has been linked to better management of body condition, improved hoof health, and reduced obesity at the herd level, while also increasing voluntary movement. Track grazing has been shown to promote greater weight loss in horses predisposed to obesity or metabolic disorders compared to traditional restricted grazing, without significant effects on behavior, social interaction, hoof health, or overall welfare. These results position track systems as a promising tool for weight management, warranting further research into the specific activity factors driving this effect (Cameron et al., 2025).

Case Study 3: A study involving twelve stabled horses investigated the effects of four different haynet designs on feeding behavior. Nets with reduced mesh size produced a comparable extension of feeding duration, adding approximately five minutes. Regardless of the haynet type, all forage was completely consumed by 22:30. However, the smaller-mesh haynets promoted a higher frequency of distinct feeding bouts during the nighttime period. (Ellis et al., 2015).

Case Study 4: In a study examining how different roughage feeding devices used in horse feeding affect horse behavior, researchers found that feeding roughage using a slow feeder reduced stereotyped behaviors and increased horse friendliness toward humans. They also noted that hay bags can be a source of frustration, and that slow feeders can reduce hay consumption in horses. (Rochais et al., 2018).

Case Study 5: In another study of horses at a shelter, blanketed horses consumed less hay than unblanketed horses. Blanketed horses may reduce energy expenditure to prevent weight loss. Owners may save money by reducing hay and labor costs due to blanketed horses (DeBoer et al., 2020).

9. Economic considerations: Costs, benefits, and adoption pathways

Equine business investments carry substantial risks due to the high costs of specialized startup equipment, which is difficult to repurpose if the venture fails Ongoing expenses for horse maintenance vary widely, influenced by the type of facility and training provided (Lima and Cintra, 2016). Economic downturns heighten these risks, as services like horse boarding and training are often viewed as discretionary. Current cost estimation tools lack the adaptability and global relevance needed to address the equine industry's complexities, particularly in nations where it holds significant economic value, revealing a critical gap in research and application (Alves et al., 2024).

Economic factors play a major role in horse breeding and the adoption of horses by people. Simple modifications to equine housing, such as using haynets, introducing rotating enrichment objects, or installing partial barriers, entail minimal upfront expenses and deliver rapid enhancements in animal welfare by promoting natural foraging and reducing stereotypic behaviors (Baxley, 2025).

Large-scale investments (such as the installation of track systems or the renovation of stabling facilities) require substantial upfront financial resources. Nevertheless, these expenditures can result in notable long-term economic advantages. Such benefits typically arise from reduced veterinary expenditures, a diminished dependence on pharmaceuticals for the management of stress-induced conditions, and improved overall performance and usability of horses.

For effective implementation, a phased and evidence-driven strategy is advisable. The recommended approach includes: (1) initiating small-scale, low-cost projects with demonstrable impact; (2) systematically documenting and evaluating the outcomes; (3) expanding the initiatives gradually while ensuring continuous education and training for staff; and (4) conducting a thorough cost–benefit analysis after the first year to assess the investment’s effectiveness and long-term sustainability.

10. Limitations and future research directions

Significant research deficiencies persist in equine science. In particular, there is a shortage of long-term randomized controlled trials that systematically compare musculoskeletal and metabolic health outcomes of horses kept in track-based systems with those housed in conventional paddocks. Equally underexplored are standardized approaches for regulating group composition in facilities characterized by frequent animal turnover. Moreover, comprehensive economic assessments that evaluate the costs and benefits of different management models across diverse equine sectors (including racing, sport, and leisure) remain limited.

At the same time, methodological progress provides new opportunities for advancing equine welfare research. Developments such as automated behavioral detection through machine learning and the deployment of long-term sensor technologies hold considerable promise for generating detailed, large-scale datasets that can capture welfare dynamics with unprecedented precision.

11. Conclusion

Equine housing systems must evolve beyond traditional confinement to effectively safeguard health and welfare. Research across behavioral science, physiology, and applied management consistently shows that restricting movement, social interaction, and foraging opportunities compromises both physical and psychological well-being. By contrast, stables designed with ethological principles—integrating slow-feeding technologies, sensory and cognitive enrichment, group housing, and track systems—promote resilience, reduce stereotypic behaviors, and enhance human–horse relationships. Importantly, meaningful improvements need not rely solely on large-scale investments; incremental adaptations, coupled with systematic welfare monitoring and staff training, can deliver significant outcomes. Economic analyses further reveal that welfare-oriented design not only reduces long-term veterinary and management costs but also enhances performance and usability across different equine sectors. Diminished equine welfare has been linked to management practices such as limited forage availability, restricted feeding duration, social deprivation, and insufficient opportunities for free exercise. It is suggested that enhancing the knowledge and practical competencies of horse owners and caretakers may alleviate or partially prevent such welfare concerns. Furthermore, improving the dissemination of relevant information requires a deeper understanding of the motivations, knowledge base, and behavioral patterns of those engaged with horses (Visser and Van Wijk-Jansen, 2012). Looking forward, advancing equine welfare will depend on combining scientific evidence with practical design innovation, ensuring that stables function not merely as shelters but as environments that actively sustain the horse's natural biology and behavioral repertoire.

References

Alexander, S. L., & Irvine, C. H. G. (1998). The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. Journal of endocrinology, 157(3), 425-432. https://doi.org/10.1677/joe.0.1570425

Alves, L., Pairis-Garcia, M., Veloso, B., dos Santos, H., Silveira, N., Lopes, I., & Raineri, C. (2024). Economic feasibility and cost modeling for horse training centers: a financial perspective from Brazil. International Journal of Agricultural Extension, 12(3), 335-345. https://doi.org/10.33687/ijae.012.003.5369

Andrews, F. M., Larson, C., & Harris, P. (2017). Nutritional management of gastric ulceration. Equine Veterinary Education, 29(1), 45-55. https://doi.org/10.1111/eve.12495

Auer, U., Kelemen, Z., Engl, V., & Jenner, F. (2021). Activity time budgets—a potential tool to monitor equine welfare?. Animals, 11(3), 850. https://doi.org/10.3390/ani11030850

Baxley, B. (2025). Thinking outside the box: Ideas for enrichment to improve horse welfare. https://pathintl.org/resources/thinking-outside-the-box-ideas-for-enrichment-to-improve-horse-welfare/ Online Access: 10.09.2025

Bini de Lima, A. C., Sebastião da Fé, V. C., Palermo Hernandes, M. S., & Oliveira dos Santos, V. M. (2023). Olfactory stimulation as environmental enrichment for domestic horses—A review. Animals, 13(20), 3180. https://doi.org/10.3390/ani13203180

Cameron, L., Challinor, M., Armstrong, S., Kennedy, A., Hollister, S., & Fletcher, K. (2025). Tracking the Track: The Impact of Different Grazing Strategies on Managing Equine Obesity. Animals, 15(6), 874. https://doi.org/10.3390/ani15060874

Christensen, J. W., Ladewig, J., Søndergaard, E., & Malmkvist, J. (2002). Effects of individual versus group stabling on social behaviour in domestic stallions. Applied Animal Behaviour Science, 75(3), 233-248. https://doi.org/10.1016/S0168-1591(01)00196-4

Cooper, J. J., & Albentosa, M. J. (2005). Behavioural adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behaviour. Livestock Production Science, 92(2), 177-182. https://doi.org/10.1016/j.livprodsci.2004.11.017

Dai, F., Dalla Costa, E., Minero, M., & Briant, C. (2023). Does housing system affect horse welfare? The AWIN welfare assessment protocol applied to horses kept in an outdoor group-housing system: The ‘parcours’. Animal Welfare, 32, e22. https://doi.org/10.1017/awf.2023.9

Dalla Costa, E., Dai, F., Lebelt, D., Scholz, P., Barbieri, S., Canali, E., ... & Minero, M. (2016). Welfare assessment of horses: The AWIN approach. Animal welfare, 25(4), 481-488. https://doi.org/10.7120/09627286.25.4.481

DeBoer, M., Konop, A., Fisher, B., & Martinson, K. (2020). Dry matter intake, body weight, and body condition scores of blanketed and nonblanketed horses in the upper midwest. Journal of Equine Veterinary Science, 94, 103239. https://doi.org/10.1016/j.jevs.2020.103239

Elfman, L., Wålinder, R., Riihimäki, M., & Pringle, J. (2011). Air quality in horse stables. In Chemistry, emission control, radioactive pollution and indoor air quality. IntechOpen. https://doi.org/10.5772/18228

Ellis, A. D., Fell, M., Luck, K., Gill, L., Owen, H., Briars, H., ... & Harris, P. (2015). Effect of forage presentation on feed intake behaviour in stabled horses. Applied Animal Behaviour Science, 165, 88-94. https://doi.org/10.1016/j.applanim.2015.01.010

Fureix, C., Benhajali, H., Henry, S., Bruchet, A., Prunier, A., Ezzaouia, M., ... & Jego, P. (2013). Plasma cortisol and faecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: do stereotypic horses cope better with poor environmental conditions?. BMC veterinary research, 9(1), 3. https://doi.org/10.1186/1746-6148-9-3

Glunk, E. C., Hathaway, M. R., Weber, W. J., Sheaffer, C. C., & Martinson, K. L. (2014). The effect of hay net design on rate of forage consumption when feeding adult horses. Journal of Equine Veterinary Science, 34(8), 986-991. https://doi.org/10.1016/j.jevs.2014.05.006

Goodwin, D. (1999). The importance of ethology in understanding the behaviour of the horse. Equine Veterinary Journal, 31(S28), 15-19. https://doi.org/10.1111/j.2042-3306.1999.tb05150.x

Goodwin, D. H. P. B., Davidson, H. P. B., & Harris, P. (2002). Foraging enrichment for stabled horses: effects on behaviour and selection. Equine Veterinary Journal, 34(7), 686-691. https://doi.org/10.2746/042516402776250450

Harris, P. A., & Arkell, K. (1999). How understanding the digestive process can help minimise digestive disturbances due to diet and feeding practices. Harris, PA, Gomarsall, GM, Davidson HPB, Green, RE (Eds.), proceedings of the BEVA Specialist Days on Behaviour and Nutrition. Newmarket, Equine Veterinary Journal 45, 49.

Hartmann, E., Søndergaard, E., & Keeling, L. J. (2012). Keeping horses in groups: A review. Applied Animal Behaviour Science, 136(2-4), 77-87. https://doi.org/10.1016/j.applanim.2011.10.004

Henshall, C., Randle, H., Francis, N., & Freire, R. (2022). Habit formation and the effect of repeated stress exposures on cognitive flexibility learning in horses. Animals, 12(20), 2818. https://doi.org/10.3390/ani12202818

Jackson, J. (2006). Paddock paradise: A guide to natural horse boarding (p. 121). Star Ridge Publishing.

Jørgensen, G. H. M., Liestøl, S. H. O., & Bøe, K. E. (2011). Effects of enrichment items on activity and social interactions in domestic horses (Equus caballus). Applied animal behaviour science, 129(2-4), 100-110. https://doi.org/10.1016/j.applanim.2010.11.004

Kjellberg, L., Dahlborn, K., Roepstorff, L., & Morgan, K. (2025). Frequency and nature of health issues among horses housed in an active open barn compared to single boxes-A field study. Equine veterinary journal, 57(1), 54-61. https://doi.org/10.1111/evj.14054

Lima, R. A. D. S., & Cintra, A. G. (2016). Revisão do estudo do complexo do agronegócio do cavalo. Brasília: MAPA.

Marliani, G. (2023). Multidisciplinary approach to horse welfare in different management systems. Dottorato di ricerca in Scienze veterinarie, 34 Ciclo. https://doi.org/10.48676/unibo/amsdottorato/10500

Mason, G., & Rushen, J. (Eds.). (2008). Stereotypic animal behaviour: fundamentals and applications to welfare. 2nd Edition. Cabi. ISBN: 978-1-84593-465-1

McAfee, L. M., Mills, D. S., & Cooper, J. J. (2002). The use of mirrors for the control of stereotypic weaving behaviour in the stabled horse. Applied Animal Behaviour Science, 78(2-4), 159-173. https://doi.org/10.1016/S0168-1591(02)00086-2

McBride, S., & Hemmings, A. (2009). A neurologic perspective of equine stereotypy. Journal of Equine Veterinary Science, 29(1), 10-16. https://doi.org/10.1016/j.jevs.2008.11.008

Özbeyaz, C., & Akçapınar, H., (2010). Horse breeding: Lecture notes. Ankara University Faculty of Veterinary Medicine, Department of Animal Science Türkiye.

Phelipon, R., Hennes, N., Ruet, A., Bret-Morel, A., Górecka-Bruzda, A., & Lansade, L. (2024). Forage, freedom of movement, and social interactions remain essential fundamentals for the welfare of high-level sport horses. Frontiers in Veterinary Science, 11, 1504116. https://doi.org/10.3389/fvets.2024.1504116

Popescu, S., Lazar, E. A., Borda, C., Niculae, M., Sandru, C. D., & Spinu, M. (2019). Welfare quality of breeding horses under different housing conditions. Animals, 9(3), 81. https://doi.org/10.3390/ani9030081

Puttkammer, N., Hildebrandt, F., Krieter, J., & Czycholl, I. (2024). How should one design and position straw feeders in group-housed horses? A case study on occupancy and agonistic interactions at straw feeders in a large group of horses. Applied Animal Behaviour Science, 280, 106423. https://doi.org/10.1016/j.applanim.2024.106423

Rochais, C., Henry, S., & Hausberger, M. (2018). “Hay-bags” and “Slow feeders”: Testing their impact on horse behaviour and welfare. Applied Animal Behaviour Science, 198, 52-59. https://doi.org/10.1016/j.applanim.2017.09.019

Roig-Pons, M., Bachmann, I., & Freymond, S. B. (2025). Slow-feeding dispensers for horses: Who, how and why?. Journal of Veterinary Behavior, 79, 7-18. https://doi.org/10.1016/j.jveb.2025.01.006

Ruet, A., Lemarchand, J., Parias, C., Mach, N., Moisan, M. P., Foury, A., ... & Lansade, L. (2019). Housing horses in individual boxes is a challenge with regard to welfare. Animals, 9(9), 621. https://doi.org/10.3390/ani9090621

Søndergaard, E., & Ladewig, J. (2004). Group housing exerts a positive effect on the behaviour of young horses during training. Applied Animal Behaviour Science, 87(1-2), 105-118. https://doi.org/10.1016/j.applanim.2003.12.010

Thorne, J. B., Goodwin, D., Kennedy, M. J., Davidson, H. P. B., & Harris, P. (2005). Foraging enrichment for individually housed horses: Practicality and effects on behaviour. Applied Animal Behaviour Science, 94(1-2), 149-164. https://doi.org/10.1016/j.applanim.2005.02.002

Van den Boom, R. (2022). Equine gastric ulcer syndrome in adult horses. The Veterinary Journal, 283, 105830. https://doi.org/10.1016/j.tvjl.2022.105830

Visser, E. K., & Van Wijk-Jansen, E. E. (2012). Diversity in horse enthusiasts with respect to horse welfare: An explorative study. Journal of Veterinary Behavior, 7(5), 295-304. https://doi.org/10.1016/j.jveb.2011.10.007

Zollinger, A., Wyss, C., Bardou, D., & Bachmann, I. (2023). Social box: a new housing system increases social interactions among stallions. Animals, 13(8), 1408. https://doi.org/10.3390/ani13081408

Chapter 6

Landscape Planning as a Framework for Sustainable Food Systems

Fatma Özsel ÖZCAN[1], Mahmut TUĞLUER[2]

INTRODUCTION

One of the key problems of our century is that today's food systems fail to remain sustainable in the long term. Population growth, rapid urbanization, climate uncertainty, and the overuse of natural resources make it necessary to rethink how we produce and manage food, considering its ecological and social impacts as well as its quantity (IPCC, 2022).

Industrial food systems often focus on maximizing production efficiency, while the negative impacts on the environment, growing social inequalities, and the loss of cultural diversity are largely overlooked (Lang & Barling, 2012). It is estimated that activities along the food chain contribute to more than a quarter of global greenhouse gas emissions (Poore & Nemecek, 2018). At the same time, the fact that hundreds of millions of people suffer from hunger while billions of tons of food are wasted every year clearly shows how unsustainable the current system is (FAO, 2023; HLPE, 2014).

Ensuring the sustainability of food systems is not limited to reducing environmental impacts. It also requires approaches that promote social inclusion, economic resilience, access to nutritious food, and the autonomy of local food systems (UN, 2015; IPES-Food, 2016).

The relationship between food production and landscape dates back to the earliest human settlements and has played a key role in shaping cultural landscapes. However, urbanization and land-use changes that accelerated after the Industrial Revolution pushed agricultural areas away from city peripheries. This not only created pressure on ecosystem balance but also disrupted the social fabric of rural areas (Antrop, 2004). Unplanned urban sprawl, the fragmentation of production areas, and the conversion of farmland into built-up areas have caused not only the loss of physical space, but serious threats to cultural continuity, food security, and landscape identity as well. (Plieninger & Bieling, 2012).

[1] Asst. Prof, Haliç University, Department of Nutrition and Dietetics, https://orcid.org/0000-0002-4668-5880
[2] Asst. Prof. Kahramanmaraş Sütçü İmam University, Department of Landscape Architecture, https://orcid.org/0000-0002-4357-9599

Landscape planning initially emerged as a tool to protect natural areas and agricultural lands. Over time, it has evolved into a comprehensive approach aiming to shape urban and rural settlements sustainably and to maintain environmental integrity (Forman, 1995). Today, this approach contributes to the integration of sustainable food systems with spatial planning through practices such as the inclusion of urban agriculture areas, the creation of agroecological corridors, the development of edible landscape designs, and the adoption of green infrastructure strategies (Viljoen & Bohn, 2014). Landscape planning stands out as an important tool for establishing the spatial foundations of sustainable food systems, aligning ecological processes with social needs, and strengthening the interaction between urban and rural areas. This study explores how sustainable food systems can be integrated with landscape planning.

In addition, the study presents planning recommendations based on international examples and local approaches, focusing on food production, consumption, and circularity.

Sustainable Food Systems and Their Components

According to the Food and Agriculture Organization of the United Nations (FAO), a sustainable food system is defined as one that meets the need for safe and healthy nutrition in today's societies while contributing to the protection of natural resources, economic sustainability, and social inclusion. This approach focuses on the long-term use of ecological assets such as soil, water, air, energy, and biodiversity (FAO, 2014). PES-Food (2016) defines sustainability through a broad perspective that links environmental issues with social structures, cultural values, and inequalities at the policy level. In response to the ecological and structural challenges created by industrial food production, ideas such as agroecological transition, local production models, and food sovereignty have become increasingly important. The United Nations Sustainable Development Goals (SDGs), particularly Goal 2 (Zero Hunger), Goal 12 (Responsible Consumption and Production), and Goal 13 (Climate Action), provide a global framework for promoting sustainable food systems (UN, 2015).

Food systems consist of interconnected and interactive stages from production to consumption. This structure represents a multifaceted system that includes many components such as agricultural activities, processing technologies, logistics infrastructure, consumption habits, and waste management. Each step has environmental impacts as well as social and economic implications. The production process covers practices such as soil preparation, seed selection, planting, fertilization, irrigation, harvesting, and livestock production. Instead of traditional high-input methods, sustainable production approaches such as agroecology, organic farming, permaculture, and integrated pest management have come to the forefront due to their lower environmental impact (Gliessman, 2015).

At the processing stage, techniques including pasteurization, fermentation, canning, and packaging are applied to extend the shelf life of food and ensure safe consumption. However, these processes need careful evaluation from a sustainability perspective due to factors like high energy demand, waste generation, and the use of chemicals (Van der Goot et al., 2021).

The distribution process covers transportation, storage, and retail operations from production areas to consumption centers. Products transported over long distances increase the carbon footprint, while local supply chains offer an alternative by reducing environmental impact (Kneafsey et al., 2013).

At all stages of the food system, a significant amount of waste and loss occurs. According to FAO (2013), about 30% of the food produced globally is lost or wasted before it reaches consumers. To minimize these losses, the expansion of practices including composting organic waste, redistributing food surpluses, and integrating circular systems is recommended.

Environmental Footprint of Food Systems

The environmental impacts of food systems are multifaceted. These impacts are mainly observed in the form of greenhouse gas emissions, soil degradation, depletion of water resources, loss of biodiversity, and waste generation. Food systems account for approximately 26% of global emissions, with meat production and rice cultivation being major sources of methane release (Poore & Nemecek, 2018) The intensive use of fossil fuels, along with the widespread application of synthetic fertilizers and pesticides, causes severe degradation in soil and water ecosystems. Industrial agriculture, in particular, leads to a decline in soil organic matter, loss of biodiversity, and disruption of ecosystem services (Rockström et al., 2009). On the other hand, the post-consumption stage of the food chain also represents a significant environmental burden. According to FAO data, around one-third of all food produced globally is wasted each year, and this waste contributes substantially to methane emissions into the atmosphere (FAO, 2021). In this regard, the concept of the “environmental footprint” provides a holistic framework for measuring the overall impact of food systems on nature. The carbon footprint represents the greenhouse gases emitted throughout the production-to-consumption process, while the water footprint refers to the total amount of water used, both directly and indirectly, within the production chain (Hoekstra & Mekonnen, 2012). In addition, indicators such as land use and biodiversity footprints have become increasingly important for assessing the spatial impacts of food production on ecosystems. The sustainable landscape planning approach plays a key role in reducing these environmental pressures. Supporting local production systems, establishing short supply chains, and promoting organic and low-input farming methods stand out as strategies that can reduce the overall environmental footprint of food systems (Tilman & Clark, 2014; Foley et al., 2011).

Edible landscape designs and urban agricultural models help reduce carbon emissions while strengthening food literacy and environmental awareness within society (Figure 1.)

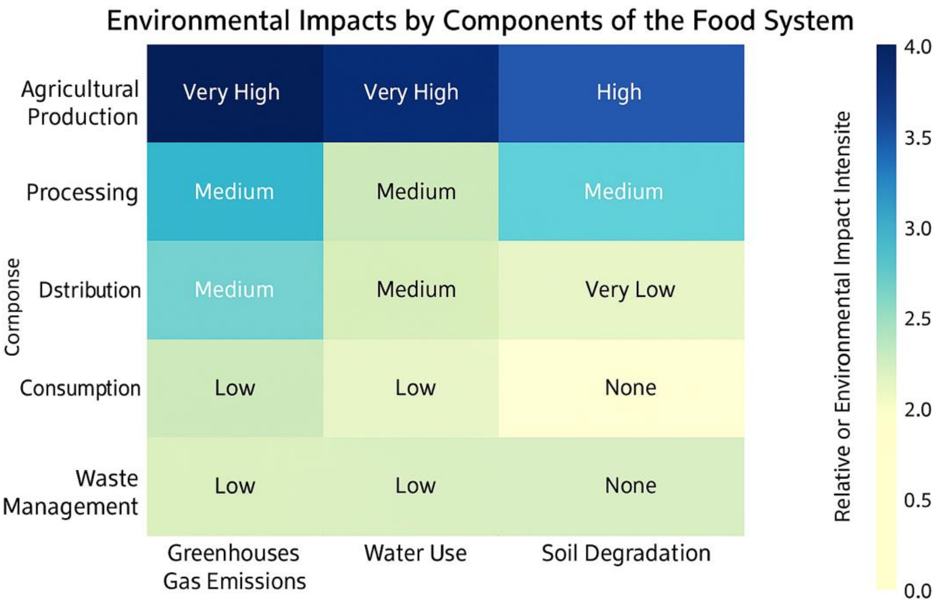


Figure 1. Environmental impact intensity relative scoring according to the components of Food Systems

The Relationship Between Landscape Planning and Food Systems

Landscape planning emerged in the 19th century, grounded in public open space projects, and over time evolved into a multidimensional approach that is sensitive to ecological processes, sustainable, data-driven, and inclusive of rural areas (Weller, 2008). While nature conservation and rural area management have come to the fore since the mid-20th century, these approaches have been replaced by new planning paradigms focused on ecosystem services and food security in the 21st century (Arı, 2024; Poppy, et al., 2014).

With the ever-accelerating urbanization, food production areas have been pushed out of urban centers, leading to the formation of food deserts, transportation systems, and supply chains with high carbon footprints. While the reintegration of food systems with the urban environment has become a necessity not only in terms of sustainability but also in terms of social and spatial equality, in recent years, the focus of solutions has shifted to a holistic and integrated planning approach covering urban and rural areas (Tabrez, 2024; Yarış & Ayalp, 2023). Urban agriculture, edible landscapes, and community-based production areas have become important themes in contemporary landscape planning Dönmez, et al., 2016). .

The shrinking of agricultural areas under urbanization pressure leads to serious problems such as the decline of food production and biodiversity. Landscape planning develops strategic decisions for the protection and efficient use of agricultural land, contributing to food security (Çakır & Gül, 2024; Işıklar & Yalçıntaş, 2023; Hossain, et al., 2020, Tuğluer & Çakır, 2019). In addition, green infrastructure practices play a critical role in ensuring the sustainability of ecosystem services. Through functions such as water management, soil protection, carbon storage, and habitat conservation, green infrastructure helps maintain the health of both agricultural production and natural landscapes (Ogwu & Kosoe, 2025). In this way, landscape planning establishes a direct link between ecological resilience and food systems.

The relationship between landscape planning and food systems includes both ecological and social dimensions. Agricultural production areas planned around cities improve access to fresh and healthy food for local communities and serve as an important tool for reducing social inequalities. Within the idea of "food sovereignty," this approach encourages local people to engage in food production and consumption processes, linking social justice with sustainability goals. (Horst, et al., 2024). Urban agriculture and community gardens are increasingly becoming part of landscape planning, allowing city residents to take an active role in food production.

The growing impacts of climate change, along with the loss of fertile soil, decline in biodiversity, and reduction of carbon sinks, threaten agricultural production and make the connection between landscape planning and food systems even more critical (FAO, 2025, Tuğluer & Çakır, 2021). Rising temperatures, changing water resources, and extreme weather events caused by climate change pose significant risks to agricultural productivity. Landscape planning serves as an important tool in adaptation processes to these risks. Ecosystem-based adaptation strategies and green infrastructure solutions help improve the resilience of agricultural areas by supporting the sustainable management of water resources. In spatial planning, factors such as biodiversity conservation and soil health improvement are vital for the sustainability of food systems (Kim & Suh, 2016; Fastré, et al.,2021; Muthee, et al, 2021).

The integration of food systems into landscape planning goes beyond the protection of production areas and plays an important role in food distribution and accessibility. In cities, food deserts have emerged in low-income and disadvantaged neighborhoods, limiting access to healthy food. This issue can be mitigated through landscape planning and urban design strategies that promote local food markets, community gardens, and urban agriculture (Horst, et al., 2024; Walker, et al., 2010). In this way, by considering the principles of spatial justice, access to adequate and healthy food for all can be supported.

The relationship between landscape planning and food systems requires a multidimensional integration of both environmental sustainability and social justice. The protection of agricultural areas, green infrastructure practices, climate adaptation strategies, and social accessibility are considered key components of this integration. Addressing food systems comprehensively within planning processes can lay the foundation for sustainable, resilient, and equitable urban and rural environments.

Implementation Approaches for Sustainable Food Systems in Landscape Planning

Implementing sustainable food systems in landscape areas requires a holistic planning approach that supports ecological processes, strengthens local production, and enhances societal resilience. These approaches consider food production not only as an economic activity but also as a component of ecosystem services and spatial sustainability (Foley et al., 2011). The main application models are discussed below;

Urban agriculture is a practice that strengthens local food cycles through production activities carried out within or around cities. These forms of production can take place in community gardens, green roofs, or public open spaces. Edible landscape designs combine productivity with aesthetics, enhancing both urban ecology and social cohesion. (Guitart, Pickering, & Byrne, 2012).

Organic Production and Ecological Management Approaches: Organic production systems are forms of agriculture that reduce chemical inputs and preserve soil health and biodiversity. These forms of production also play an important role in mitigating the effects of climate change by increasing carbon storage capacity (Reganold & Wachter, 2016). At the landscape level, organic farming practices support maintaining ecological balance and reducing the environmental footprint.

Short Supply Chains and Local Production Areas: Short supply chains reduce the distance between producers and consumers, reducing carbon emissions and strengthening the local economy. These systems redefine the relationships between rural and urban areas in sustainable landscape planning and contribute to local food security (Ilbery & Maye, 2005).

Integrated Landscape Planning Approaches: The integration of sustainable food systems with landscape planning seeks to reduce environmental impacts while linking production, consumption, and waste management cycles. Strategies developed at the landscape scale support goals like maintaining soil fertility, managing water resources, and reducing the carbon footprint. (Foley et al., 2011).

Approaches to sustainable food systems in landscape planning offer a multidimensional framework that considers not only environmental sustainability but also economic vitality and societal well-being. Strengthening ecological and socioeconomic connections between urban and rural areas, supporting local production networks, and integrating nature-based solutions into planning processes are of paramount importance. Thus, food production becomes more than just an agricultural activity, but an essential component in the design of sustainable landscapes, enhancing ecological integrity, resilience, and quality of life.

Sustainable Food and Landscape Integration in the Light of International Practices

Around the world, various policies and practices have been implemented to integrate food systems with landscapes in order to achieve a more sustainable environment. One example is Freiburg in Germany, which stands out as a model of sustainable urban development. The city has integrated permaculture principles into its urban fabric, promoting urban agriculture through the planting of edible species in public spaces and supporting local ecosystems (Beatley, 2011).

With a project of the City of Paris, "Parisculteurs" has emerged, aiming to create 100 hectares of agricultural green space by 2020. The roofs and walls in the city were used for agricultural vertical production, so that production was included in the landscape of the city center (Mairie de Paris, 2019).

In the Oosterwold district of Almere, a project has been implemented requiring each household to allocate a specific portion of land for food production before any urban construction begins. Oosterwold covers a 4,300-hectare rural-urban development area, where the construction of 15,000 new homes is planned.

According to Almere’s urban policies, the area aims to produce 10% of the city’s future food needs (Jansma ve ark., 2024).

As a solution to the food crisis that developed after the collapse of the Soviet Union in Cuba, agroecological urban agriculture was expanded in empty areas. With systems called "Organopónicos", production based on ecological principles was provided in the city center (Koont, 2011).

Curitiba aimed to prevent food insecurity by establishing urban gardens in low-income areas. A similar application is tried to be created in Kadıköy with edible landscaping applications and neighborhood gardens project (Rabinovitch & Leitman 1996; Kadıköy belediyesi, 2021) (Table 1.)

Table 1. Some Examples of Food and Landscape Oriented Application Areas in the World

Country, City	**Food+Landscape Oriented Applications**	
Freiburg, Germany	Permaculture neighborhoods, edible public green spaces	
Paris, France	Parisculteurs: agriculture on green roofs, public vertical gardens	
Almere, Holland	Food-first urban planning (Oosterwold)	

Havana, Cuba	Transformation of vacant spaces into agroecological agriculture	
Curitiba, Brazil	Integrating food access and green infrastructure	
Kadıköy, Türkiye	Neighborhood gardens and edible landscaping applications	

Conclusion and Recommendations

One of the most pressing challenges of the twenty-first century is the unsustainable food systems that fail to ensure long-term ecological balance and social equity. Rapid population growth, accelerating urbanization, climate instability, and the overuse of natural resources clearly expose the structural fragility of conventional industrial food production models. These systems, while aiming to maximize efficiency, contribute to ecological degradation, deepen social inequalities, and weaken cultural diversity.

Current data indicate that more than one-quarter of global greenhouse gas emissions originate from food-related activities. Meanwhile, the fact that roughly one-third of all food produced is wasted, while millions of people suffer from hunger, highlights the unsustainable nature of the current system. This situation underscores the need for a transition toward sustainable food systems that integrate ecological resilience, economic viability, and social inclusion.

Landscape planning emerges as a key tool in this transformation, combining ecological, spatial, and socio-political dimensions. It has evolved beyond a conservation-oriented perspective into a holistic approach that strengthens food sovereignty and supports local autonomy through urban agriculture, agroecological corridors, edible landscapes, and green infrastructure strategies.

International examples demonstrate the transformative potential of this integration: permaculture-based neighborhoods in Freiburg (Germany), the *Parisculteurs* initiative in Paris, food-oriented urban planning in Almere (the Netherlands), agroecological *organopónicos* systems in Havana, and neighborhood gardens and rural corridor planning in Turkey all illustrate how food security, ecological resilience, and social justice can be interconnected within a single framework.

Strengthening short supply chains, promoting organic and low-input production systems, adopting circular waste management practices, and enhancing food literacy are essential strategies for achieving both ecological and social sustainability.

In conclusion, integrating sustainable food systems within the framework of landscape planning should be seen not only as an environmental necessity but as a normative paradigm for building resilient, just, and sustainable urban and rural environments. Expanding short supply chains, encouraging organic and low-input production, and integrating local food areas through edible landscape practices are of great importance. In addition, developing alternative waste management solutions such as composting and biogas, improving food literacy, and supporting conscious consumer behavior will make significant contributions to the establishment of sustainable and resilient food systems.

References

Antrop, M. (2004). Landscape change and the urbanization process in Europe. *Landscape and Urban Planning, 67*(1–4), 9–26. https://doi.org/10.1016/S0169-2046(03)00082-4.

Arı, Y. (2024). İnsansız korumadan biyokültürel korumaya: son 150 yılda dünyada doğa koruma paradigmalarının değişimi. *International Journal of Geography and Geography Education*(*53*), 298-317. https://doi.org/10.32003/igge.1492106

Beatley, T. (2011). Biophilic cities: Integrating nature into urban design and planning. Island Press.

Çakır, M., & Gül, A. (2024). Urban Biodiversity Performance Determining Model (UrBioPDeM): The Case of Isparta, Türkiye. *BioResources*, *19*(4).

Dönmez, Ş., Çakır, M., & Kef, Ş. (2016). Bartın'da yetişen bazı tıbbi ve aromatik bitkilerin peyzaj mimarlığında kullanımı. *Journal of Architectural Sciences and Applications*, *1*(2), 1-8.

Evola, R. S., Peira, G., Varese, E., Bonadonna, A., & Vesce, E. (2022). Short Food Supply Chains in Europe: Scientific Research Directions. *Sustainability*, *14*(6), 3602. https://doi.org/10.3390/su14063602

FAO. (2013). *Food wastage footprint: Impacts on natural resources*. Food and Agriculture Organization of the United Nations. https://www.fao.org/3/i3347e/i3347e.pdf

FAO. (2014). *Building a common vision for sustainable food and agriculture*. Rome: Food and Agriculture Organization of the United Nations. https://www.fao.org/3/i3940e/i3940e.pdf

FAO. (2021). The State of Food and Agriculture 2021: Making agri-food systems more resilient to shocks and stresses. Rome: Food and Agriculture Organization of the United Nations.

FAO. (2023). *The State of Food Security and Nutrition in the World 2023*. Rome: FAO, IFAD, UNICEF, WFP, WHO. https://doi.org/10.4060/cc3017en

FAO. (2025). *From climate-smart agriculture to climate-smart landscapes*. Food and Agriculture Organization of the United Nations. Retrieved July 3, 2025, from https://www.fao.org/climate-smart-agriculture-sourcebook/concept/module-a3-landscapes/chapter-a3-2/en/

Fastré, C., van Zeist, W. J., Watson, J. E. M., & Visconti, P. (2021). Integrated spatial planning for biodiversity conservation and food production. *One Earth*, *4*(11), 1635-1644.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., Mueller, N. D., O'Connell, C., Ray, D. K., West, P. C., Balzer, C., Bennett, E. M., Carpenter, S. R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., & Zaks, D. P. M. (2011). Solutions for a cultivated planet. Nature, 478(7369), 337–342.

Forman, R. T. T. (1995). *Land Mosaics: The Ecology of Landscapes and Regions*. Cambridge University Press.

Gliessman, S. R. (2015). Roots of resistance to industrialized food systems. Agroecology: A transdisciplinary, participatory and action-oriented approach, 23.

Guitart, D., Pickering, C., & Byrne, J. (2012). Past results and future directions in urban community gardens research. Urban Forestry & Urban Greening, 11(4), 364–373. https://doi.org/10.1016/j.ufug.2012.06.007

Hoekstra, A. Y., & Mekonnen, M. M. (2012). The water footprint of humanity. Proceedings of the National Academy of Sciences, 109(9), 3232–3237.

Horst, M., McClintock, N., & Hoey, L. (2024). The intersection of planning, urban agriculture, and food justice: A review of the literature. *Planning for equitable urban agriculture in the United States: future directions for a new ethic in city building*, 89-120.

Hossain, A., Krupnik, TJ, Timsina, J., Mahboob, MG, Chaki, AK, Farooq, M., ... & Hasanuzzaman, M. (2020). Tarımsal arazi bozulması: gelecekteki gıda güvenliğini baltalayan süreçler ve sorunlar. *Çevre, iklim, bitki ve bitki örtüsü büyümesi* (s. 17-61). Cham: Springer International Publishing.

Ilbery, B., & Maye, D. (2005). Food supply chains and sustainability: Evidence from specialist food producers in the Scottish/English borders. Land Use Policy, 22(4), 331–344. https://doi.org/10.1016/j.landusepol.2004.06.002

IPCC. (2022). *Climate Change and Land: Summary for Policymakers*. Intergovernmental Panel on Climate Change. https://www.ipcc.ch/srccl/

IPES-Food. (2016). *From uniformity to diversity: A paradigm shift from industrial agriculture to diversified agroecological systems*. International Panel of Experts on Sustainable Food Systems. http://www.ipes-food.org/_img/upload/files/UniformityToDiversity_FULL.pdf

Işıklar, Ö., & Yalçıntaş, E. (2023). Kentsel Tarımın Mekânsal Olarak Düzenlenmesi; Tarım Arsası Kavramı. *Türkiye Arazi Yönetimi Dergisi*, *5*(1), 1-8. Işıklar, Ö., & Yalçıntaş, E. (2023). Kentsel Tarımın Mekânsal Olarak Düzenlenmesi; Tarım Arsası Kavramı. *Türkiye Arazi Yönetimi Dergisi*, *5*(1), 1-8.

Jansma, J. E. & Wertheim-Heck, S. C. (2024). A city of gardeners: What happens when policy, planning, and populace co-create the food production of a novel peri-urban area? Environment and Planning B: Urban Analytics and

City Science, 51(3), 705- 720. https://doi.org/10.1177/23998083231193802.

Kim, J. O., & Suh, J. H. (2016). A review of climate change adaptation policies applied to landscape planning and design in Korea. *Landscape and ecological engineering*, *12*, 171-177.

Koont, S. (2011). *Sustainable Urban Agriculture in Cuba.*

Lang, T., & Barling, D. (2012). Food security and food sustainability: Reformulating the debate. *The Geographical Journal, 178*(4), 313–326. https://doi.org/10.1111/j.1475-4959.2012.00480.x.

Mairie de Paris. (2019). *Les Parisculteurs – Rapport annuel*. Ville de Paris.

Muthee, K., Duguma, L., Nzyoka, J., & Minang, P. (2021). Ecosystem-based adaptation practices as a nature-based solution to promote water-energy-food nexus balance. *Sustainability*, *13*(3), 1142.

Nassauer, J. I. (1995). Messy ecosystems, orderly frames. *Landscape journal*, *14*(2), 161-170.

Ogwu, M. C., & Kosoe, E. A. (2025). Integrating Green Infrastructure into Sustainable Agriculture to Enhance Soil Health, Biodiversity, and Microclimate Resilience. *Sustainability*, *17*(9), 3838.

Plieninger, T., & Bieling, C. (Eds.). (2012). *Resilience and the Cultural Landscape: Understanding and Managing Change in Human-Shaped Environments*. Cambridge University Press.

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science, 360*(6392), 987–992. https://doi.org/10.1126/science.aaq0216.

Poppy, G. M., Chiotha, S., Eigenbrod, F., Harvey, C. A., Honzák, M., Hudson, M. D., ... & Dawson, T. P. (2014). Food security in a perfect storm: using the ecosystem services framework to increase understanding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1639), 20120288.

Rabinovitch, J., & Leitman, J. (1996). *Urban Planning in Curitiba.*

Reganold, J. P., & Wachter, J. M. (2016). Organic agriculture in the twenty-first century. Nature Plants, 2(2), 15221. https://doi.org/10.1038/nplants.2015.221

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H. J., Nykvist, B., de Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P. K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R. W., Fabry, V. J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., & Foley, J. (2009). A safe operating space for humanity. Nature, 461(7263), 472–475.

Tabrez, Z. (2025). Sustainable cities: enhancing food systems with urban agriculture. *Discover Food*, *5*(1), 1-12.

Tilman, D., & Clark, M. (2014). Global diets link environmental sustainability and human health. Nature, 515(7528), 518–522.

Tuğluer, M., & Çakır, M. (2019). Ufore modeli'nin kent ekosistemine hizmet eden bileşenlerinin irdelenmesi. *Journal of Architectural Sciences and Applications*, *4*(2), 193-200.

Tuğluer, M., & Çakır, M. (2021). *Ecological importance of urban trees and their role in sustainable cities*. In Ş. Ertaş Beşir, M. B. Bingül Bulut, & İ. Bekar (Eds.), *Architectural sciences and sustainability* (pp. 81–96). İksad Publishing House. ISBN 978-625-8061-43-7

UN. (2015). *Transforming our world: The 2030 Agenda for Sustainable Development*. United Nations. https://sdgs.un.org/2030agenda

Van der Goot, A. J., Pelgrom, P. J. M., Berghout, J. A. M., Geerts, M. E. J., Jankowiak, L., Hardt, N. A., Keijer, J., Schutyser, M. A. I., Nikiforidis, C. V., & Boom, R. M. (2016). Concepts for further sustainable production of foods. *Journal of Food Engineering, 168*, 42–51. https://doi.org/10.1016/j.jfoodeng.2015.07.010

Viljoen, A., & Bohn, K. (2014). *Second Nature Urban Agriculture: Designing Productive Cities*. Routledge.

Walker, R. E., Keane, C. R., & Burke, J. G. (2010). Disparities and access to healthy food in the United States: A review of food deserts literature. *Health & place*, *16*(5), 876-884.

Weller, R. (2008). Landscape (sub) urbanism in theory and practice. *Landscape journal*, *27*(2), 247-267.

Yarış, A., & Ayalp, E. K. (2023). Kentsel Gıda Planlaması için Çıkarımlar: Gıda'nın Kent-Bölgesi'ni Tanımlamak. *Sketch: Journal of City and Regional Planning*, *5*(01), 46-60.

Chapter 7

Orthodontic Treatment in Periodontally Compromised Patients

Ferit DADAŞLI[1]

Introduction

Adults with a history of periodontitis often seek orthodontic care to correct pathologic migration, restore function, and improve esthetics. Their biology differs from that of adolescents: attachment loss shifts centers of resistance apically, embrasures are wider, and soft tissues may be thin or scarred. When inflammation is controlled and light, well-directed forces are used, tooth movement does not damage a reduced but healthy periodontium and can support rehabilitation across specialties (Feu, 2020; Geisinger et al., 2014). The therapeutic aim is not an ideal occlusion in isolation. The aim is to reduce traumatic loading, create cleansable tooth positions, recover esthetics where anatomy allows, and provide a scaffold for durable prosthetic solutions, while maintaining periodontal stability (Erbe et al., 2023). These goals are best met within a coordinated periodontal–orthodontic–restorative plan that begins with control of inflammation, proceeds with measured mechanics, and is sustained by long-term supportive periodontal care (SPC). This chapter offers a practical roadmap-diagnosis and case selection; biomechanics and appliance choices, including clear aligners; sequencing with periodontal surgery; and retention, grounded in contemporary consensus statements, systematic reviews, and controlled clinical data.

Biology and Biomechanics in the Reduced Periodontium

Attachment loss alters both the mechanical behavior of teeth and the biologic response to force. With apical displacement of the center of resistance, the same force tends to produce greater tipping and less translation; safe movement therefore demands lighter forces coupled with higher moments to maintain root control (Erbe et al., 2023). Under non-inflamed conditions the periodontium retains capacity to remodel: controlled intrusion can reduce probing depths around elongated incisors; extrusion (forced eruption) can level isolated

[1] Uzm.Dt, Private Practice, fdadasli@gmail.com, 0009-0009-0803-1905

infrabony defects; and molar uprighting reduces plaque stagnation and traumatic contacts (Wagenberg et al., 1980). Conversely, persistent plaque and bleeding transform otherwise benign mechanics into jiggling trauma, increasing the risk of recession, attachment loss, or root resorption. Periodontal endpoints should be established before orthodontic activation, and supportive periodontal care (SPC) is maintained throughout treatment. SPC is provided at risk-based 6–12-week intervals and includes professional control of supra- and subgingival biofilm, reinforcement of home care, and re-instrumentation or local antimicrobials when indicated. Ongoing monitoring of bleeding on probing, pocket depths, mobility, and systemic risk factors (e.g., smoking) guides the timing and intensity of mechanics. Under this protocol, forces are applied only to a quiescent periodontium; if inflammation recurs, activation is paused or adjusted. The same surveillance continues into the retention phase.

Diagnosis and Risk Assessment

Diagnosis is comprehensive and should be shared with the patient. Full-mouth charting, probing pocket depths, clinical attachment levels, and bleeding on probing together with targeted periapical radiographs and, when indicated, cone-beam computed tomography, maps horizontal and vertical bone loss, checks root proximity, estimates dehiscence risk, and evaluates crown-to-root ratios (Zhong et al., 2025). Occlusal analysis records fremitus, mobility, functional pathways, and signs of parafunction. The periodontal phenotype (keratinized-tissue width and gingival thickness) and tooth form are noted; triangular crowns are prone to black triangles even after ideal alignment. Systemic modifiers such as tobacco use, diabetes, and medications are addressed, and hygiene capacity is demonstrated before appliances are placed. Equally important is clarity about goals: whether the priority is hygiene-oriented repositioning, removal of traumatic contacts, space redistribution for prosthetics, or anterior esthetic improvement will guide the mechanics and treatment sequence (Pini Prato & Chambrone, 2020).

Indications and Case Selection

Orthodontic treatment is indicated when moving teeth can reliably improve periodontal health, function, or prosthetic planning, rather than being performed solely for the sake of tooth movement.

When considering orthodontic treatment for adults with a history of periodontitis, it is important to identify specific situations where tooth movement can offer clear clinical benefits. The following are common scenarios in which orthodontic intervention can improve periodontal health, function, or restorative outcomes:

First, pathologic migration with anterior flaring, spacing, and elongation is common after posterior support is lost. Here, intrusion with root control and staged retraction can reduce mobility and fremitus, shorten the appearance of clinical crowns, and when performed in a plaque-free field, can improve probing depths. Interproximal enamel reduction (IPR) and apicalization of contact points temper open gingival embrasures, although residual "black triangles" may persist where interproximal bone has resorbed (Zhong et al., 2025).

Second, secondary occlusal trauma arising from mesially tilted molars, supra-eruption of antagonists, and uncoordinated arches invites targeted uprighting and vertical control. Correcting axial inclinations broadens the zone of stable contacts, improves cleansability at mesial defects, and prepares sites for implants or fixed prostheses (Papageorgiou et al., 2024).

Third, pre-prosthetic site development is often decisive. Orthodontics can upright and parallel roots to open implant corridors, extrude subcrestal fractures to gain ferrule without resective surgery, and redistribute space for papilla-friendly pontics. Movement through regenerated bone is feasible once healing endpoints are confirmed, using gentle force systems and close periodontal surveillance (Pinho et al., 2012).

Fourth, management of vertical defects can be orthodontic. Slow, controlled extrusion of a tooth with a one- or two-walled intrabony defect reshapes the attachment apparatus coronally; paired with fiberotomy and soft-tissue re-contouring, it can reduce pocketing and preserve tooth structure where resection would be destructive (Paolone & Kaitsas, 2018).

Fifth, alignment for maintenance, limited orthodontics to relieve crowding that blocks interdental access, or to eliminate food-trap contacts has value even when broader occlusal corrections are not pursued. The common thread across these indications is inflammation control, realistic goals, and mechanics that respect the altered envelope of bone and soft tissues (Kwon et al., 2024).

Interdisciplinary Sequencing and Timing

Treatment sequence follows biology. Initial periodontal therapy and risk-factor control come before any orthodontic activation. Persistent vertical defects can be managed with resective or regenerative surgery, depending on defect morphology. Orthodontic treatment starts only after endpoints are met and proceeds in step with supportive periodontal care. In selected, well-healed augmented sites, early light forces may be used under periodontal supervision. Expectations should be discussed openly: the aim is periodontal stability with functional and esthetic improvement, not a textbook occlusion at the expense of soft tissues (Kwon et al., 2024; Zhong et al., 2025).

Appliance Selection, Hygiene, and Anchorage

Fixed appliances remain unmatched for three-dimensional control, especially when mechanics are segmented, statically determinate, and supported by temporary anchorage devices (TADs). Their drawback is biologic: brackets and ligatures increase biofilm retention and demand intensive hygiene coaching and professional debridement. Wire sequences are designed to deliver light, continuous forces throughout treatment. Early control of root torque is important to help protect the periodontal tissues. In certain situations, removing the orthodontic brackets from specific teeth can make it easier for patients to clean areas that are at higher risk for periodontal problems. These strategies help reduce irritation and support better oral hygiene during orthodontic therapy in patients with a history of periodontitis (Feu, 2020).

Clear aligners offer hygienic advantages by allowing full removal for brushing and interdental cleaning. Across comparative studies, aligner cohorts tend to show lower plaque accumulation and gingival bleeding than fixed-appliance cohorts, provided wear and tray hygiene are adequate. However, aligners are not automatically better at preventing plaque: they can trap biofilm under the trays, the tray edges may irritate the gums, and complex tooth movements like root torque, expansion, or vertical changes often still need extra fixed appliances or TADs. In periodontally compromised patients, aligners are well suited to alignment, space redistribution, and controlled intrusion when staging is conservative and anchorage is thoughtfully supplemented (Dipalma et al., 2025; Giannini et al., 2025).

Anchorage is planned with reduced support in mind. TADs placed in cleansable, non-inflamed sites can spare compromised dental units from reciprocal forces; osseointegrated implants or provisional crowns may serve as anchorage once stable within a restorative plan. Whatever the appliance, SPC intervals are synchronized with orthodontic visits to monitor BOP, PPD, and hygiene and to pause mechanics if indices worsen (Erbe et al., 2023).

Movement-Specific Considerations

Intrusion of migrated incisors requires light continuous intrusive forces and generous moment-to-force ratios to avoid labial tipping. TAD-assisted intrusion limits reciprocal posterior extrusion and facilitates pocket reduction when inflammation is under control (Cardaropoli et al., 2001).

Forced eruption (orthodontic extrusion) is indicated for isolated vertical defects and subgingival fractures. Slow extrusion (≈1 mm/month) with fiberotomy minimizes relapse and, coupled with coronal re-contouring, can improve bony architecture without sacrificing support (Erbe et al., 2023; Zhong et al., 2025).

Molar uprighting and **protraction** improve access for hygiene in mesial defects and restore posterior support. With TADs, molars can be uprighted while minimizing unwanted extrusion in areas with reduced support (Feu, 2020).

Transverse correction in adults with thin buccal plates should be cautious and limited to what the bone can support. Teeth moved too far buccally can cause dehiscence, fenestration, or recession. If expansion is needed, use slow, root-controlled movements and avoid crown tipping. The bony envelope should be checked with CBCT or targeted periapicals before and during treatment. When the required gain exceeds the alveolus, segmental mechanics or surgically assisted expansion (e.g., MARPE/SARPE) should be planned. Tissues should be assessed regularly for the signs of breakdown (Feu, 2020).

Making sure the roots are parallel and managing contact points helps reduce black triangles. Interproximal reduction (IPR) and adjusting the axis of the teeth can move the contact point closer to the gumline. If the tooth shape leaves a gap, composite material can be added to reshape the crown and close the space (Feu, 2020).

Clear Aligners in Periodontally Compromised Patients: Evidence and Protocol

The hygienic promise of aligners is supported by both experimental and systematic evidence. In a split-arch clinical experiment with 90 adults treated by one orthodontist, upper arches with aligners were contrasted with lower arches with fixed appliances: plaque index increased with fixed appliances, while aligner arches showed better plaque control and more favorable microbial profiles, though outcomes still depended critically on patient hygiene (Giannini et al., 2025). A PRISMA-guided systematic review focusing on periodontitis patients concluded that, compared with fixed appliances, aligners generally produced lower plaque and bleeding indices and supported better periodontal comfort and compliance, albeit with heterogeneous evidence and limitations (Dipalma et al., 2025).

In practice, aligner protocols for reduced periodontium emphasize conservative staging, with modest per-aligner steps and limited simultaneous movements. These protocols also call for minimal and strategically placed attachments away from the gingival margin, frequent short recall with tray inspection, and hybridization with sectional fixed appliances or TADs for movements that exceed aligner biology. The goal is the same as with fixed appliances: move teeth within the periodontal envelope, keep forces light and well controlled, and synchronize SPC to the aligner schedule.

Periodontal Maintenance During Orthodontics

Maintenance keeps the tissues healthy, so orthodontic treatment works well. SPC every 6-12 weeks (or individualized to risk) includes disclosing, mechanical debridement, and reinforcement of brushing and interdental cleaning; short courses of chemotherapeutic adjuncts may be used during flare-ups but never replace biofilm control. For fixed appliances, powered brushes, interdental brushes sized to embrasures, and daily interdental irrigation are emphasized. For aligners, daily mechanical cleaning of trays and avoidance of hot, pigmented beverages during wear prevent irritation and staining. Any recurrence of bleeding or suppuration prompts a pause in activation and a return to periodontal stabilization before mechanics resume (Pini Prato & Chambrone, 2020).

Finishing, Esthetics, and Black Triangle Management

Because interproximal bone peaks are often blunted, open gingival embrasures may remain after alignment despite excellent root parallelism. The management of black triangles follows a systematic approach. First, it is important to verify the position of the roots and assess the height of the contact points. Conservative interproximal reduction (IPR) and careful adjustment of torque are then utilized to move the contact area closer to the gumline. Soft-tissue grafting or papilla augmentation is reserved for selected, stable cases. Pre-treatment counseling is essential so patients understand that black triangles are a sequel of prior disease, not a treatment failure.

Retention and Long-Term Stability

Retention is compulsory in the reduced periodontium. Device choice is individualized. Fixed lingual retainers support alignment where diastema closure or torque control was substantial but demand impeccable hygiene and professional maintenance. Removable vacuum-formed retainers simplify cleaning but rely on compliance. Fiberotomy may aid stability after rotational corrections or diastema closure. Beyond device selection, stability depends on continued SPC and periodic joint reviews by periodontist and orthodontist to track CAL, PPD, BOP, recession, and radiographic bone levels over time (Erbe et al., 2023).

Complications and Risk Mitigation

The principal hazards, gingival recession during labial movement across a thin phenotype, attachment loss under persistent inflammation, root resorption from heavy or jiggling forces, irritation at TAD or attachment sites, and esthetic dissatisfaction from residual black triangles are predictable and largely preventable. Mitigation includes phenotype modification where indicated, disciplined light mechanics with root control, staged movement of limited units, synchronized SPC, and frank, ongoing consent about trade-offs and limits (Erbe et al., 2023; Zhong et al., 2025).

Conclusion

Orthodontic treatment in periodontally compromised adults succeeds when biology leads mechanics. With inflammation controlled and forces kept light and precise, tooth movement within a reduced but healthy periodontium can reduce trauma, improve cleansability, and enable durable restorative solutions. Fixed appliances remain indispensable for complex control, whereas clear aligners, when staged conservatively and combined with meticulous hygiene and SPC, offer hygienic and patient-friendly pathways to alignment and space management. The hallmark of success is not the absence of residual anatomic sequelae but the presence of periodontal stability

Keywords: Periodontitis, Orthodontic tooth movement, Supportive periodontal care (SPC), Pathologic tooth migration

REFERENCES

Cardaropoli, D., Re, S., Corrente, G., & Abundo, R. (2001). Intrusion of migrated incisors with infrabony defects in adult periodontal patients. *American Journal of Orthodontics and Dentofacial Orthopedics*, *120*(6), 671–675. https://doi.org/10.1067/mod.2001.119385

Dipalma, G., Inchingolo, A. D., Fiore, A., Balestriere, L., Nardelli, P., Casamassima, L., Di Venere, D., Palermo, A., Inchingolo, F., & Inchingolo, A. M. (2025). The Differential Impact of Clear Aligners and Fixed Orthodontic Appliances on Periodontal Health: A Systematic Review. *Children*, *12*(2), 138. https://doi.org/10.3390/children12020138

Erbe, C., Heger, S., Kasaj, A., Berres, M., & Wehrbein, H. (2023). Orthodontic treatment in periodontally compromised patients: A systematic review. *Clinical Oral Investigations*, *27*(1), 79–89. https://doi.org/10.1007/s00784-022-04822-1

Feu, D. (2020). Orthodontic treatment of periodontal patients: Challenges and solutions, from planning to retention. *Dental Press Journal of Orthodontics*, *25*, 79–116. https://doi.org/10.1590/2177-6709.25.6.079-116.sar

Geisinger, M. L., Abou-Arraj, R. V., Souccar, N. M., Holmes, C. M., & Geurs, N. C. (2014). Decision making in the treatment of patients with malocclusion and chronic periodontitis: Scientific evidence and clinical experience. *Seminars in Orthodontics*, *20*(3), 170–176. https://doi.org/10.1053/j.sodo.2014.06.006

Giannini, L., Galbiati, G., Tartaglia, F. C., Grecolini, M. E., Maspero, C., & Biagi, R. (2025). Orthodontic Treatment with Fixed Appliances Versus Aligners: An Experimental Study of Periodontal Aspects. *Dentistry Journal*, *13*(2), 70. https://doi.org/10.3390/dj13020070

Kwon, T. H., Salem, D. M., & Levin, L. (2024). Periodontal considerations in orthodontic treatment: A review of the literature and recommended protocols. *Seminars in Orthodontics*, *30*(2), 80–88. https://doi.org/10.1053/j.sodo.2024.01.011

Paolone, M. G., & Kaitsas, R. (2018). Orthodontic-periodontal interactions: Orthodontic extrusion in interdisciplinary regenerative treatments. *International Orthodontics*, *16*(2), 217–245. https://doi.org/10.1016/j.ortho.2018.03.019

Papageorgiou, S. N., Antonoglou, G. N., Eliades, T., Martin, C., & Sanz, M. (2024). Orthodontic treatment of patients with severe (stage IV) periodontitis. *Seminars in Orthodontics*, *30*(2), 123–134. https://doi.org/10.1053/j.sodo.2024.01.004

Pinho, T., Neves, M., & Alves, C. (2012). Multidisciplinary management including periodontics, orthodontics, implants, and prosthetics for an adult.

American Journal of Orthodontics and Dentofacial Orthopedics, *142*(2), 235–245. https://doi.org/10.1016/j.ajodo.2010.10.026

Pini Prato, G. P., & Chambrone, L. (2020). Orthodontic treatment in periodontal patients: The use of periodontal gold standards to overcome the "grey zone." *Journal of Periodontology*, *91*(4), 437–441. https://doi.org/10.1002/JPER.19-0306

Wagenberg, B. D., Eskow, R. N., & Langer, B. (1980). Orthodontic Procedures that Improve the Periodontal Prognosis. *The Journal of the American Dental Association*, *100*(3), 370–373. https://doi.org/10.14219/jada.archive.1980.0099

Zhong, W., Zhou, C., Yin, Y., Feng, G., Zhao, Z., Pan, Y., Bai, Y., Jin, Z., Xu, Y., Fang, B., Liu, Y., He, H., Chen, F., Li, W., Ge, S., Li, A., Ding, Y., Chen, L., Yan, F., & Song, J. (2025). Expert consensus on orthodontic treatment of patients with periodontal disease. *International Journal of Oral Science*, *17*(1), 27. https://doi.org/10.1038/s41368-025-00356-w

Chapter 8

Survival Analyses in Veterinary Epidemiological Studies

Furkan Çağrı BEŞOLUK[1]

Introduction

In veterinary medicine, understanding the time elapsed from the onset of a disease to recovery, from treatment to recurrence, or from infection to death is critically important for clinical decision-making and herd health management (1). Questions such as how long a dog will live after a cancer diagnosis, when a cow will be culled from the herd following mastitis, or how long a vaccine will provide protection are frequently encountered by veterinarians in daily practice. Traditional statistical methods are inadequate for analyzing such time-to-event data because not all animals may experience the event of interest during the study period. For instance, in a study lasting three years, some animals may not die, may die from a different cause, or may be removed from the herd, and including the data from these animals in simple average calculations yields misleading results.

Survival analyses are a set of statistical methods developed to handle this specific data structure and are increasingly utilized in veterinary epidemiological research (2,3). The fundamental characteristic of these methods is their ability to incorporate data from individuals who have not experienced the event during the study follow-up. Survival analyses are employed in numerous areas, including life expectancy prediction after cancer treatment in oncology, assessment of vaccine protection duration in infectious diseases, modeling of reproductive performance and disease elimination times in herd health management, and identifying prognostic factors in chronic diseases (4-7). The number of publications utilizing survival analyses in the veterinary literature has significantly increased over the last two decades, and these methods have become standard tools in veterinary epidemiology.

[1] Research Assistant ORCID:0000-0002-1967-9418
E-Mail: furkan.besoluk@selcuk.edu.tr
Department of Biostatistics, Faculty of Veterinary Medicine, Selcuk

What is Survival Analysis?

Survival analysis is a set of statistical methods that examine the time elapsed from a starting point until a specific event occurs (8). The starting point can be a clear time, such as diagnosis, initiation of treatment, birth, date of surgery, or entry into a farm, while the event can refer to various outcomes like death, disease development, recurrence, conception, or culling. Although the term is "survival" analysis, the method can actually be used to analyze any time-to-event relationship. The time from a cow's first insemination to conception, the time until a cat develops a disease after vaccination, or the time until a dog develops a recurrence after surgery can all be examined using survival analyses.

The accurate recording of each individual's follow-up time and event status is of critical importance. In a study, some animals may be followed for a long time while others are followed for a shorter period. Some animals may experience the event, while others may not experience it by the time the study concludes. Taking these situations into account, survival analyses are designed to handle this complex data structure.

The most important feature that distinguishes survival analyses from other statistical methods is the concept of censoring. A censored observation means that the event of interest did not occur within the study period (9). Censoring is a very common occurrence in studies, and the correct handling of this data is essential for the validity of the results. Censoring can occur in various forms. The most common type is right censoring, where individuals who have not experienced the event by the time the study ends are censored (10).

For example, when examining the survival time after treatment in a canine lymphoma study, we may encounter the following scenarios: Some dogs die during the study period, resulting in a complete observation. Some dogs do not die during the study period and are censored because their true survival time is longer than the study duration, but we do not know how much longer. Some dogs are taken to a different clinic by their owners and are lost to follow-up; these are also censored. Some dogs die from an unrelated cause, such as a traffic accident; these are also censored because we cannot observe the effect of the lymphoma. All these situations constitute censoring, and survival analyses correctly account for them.

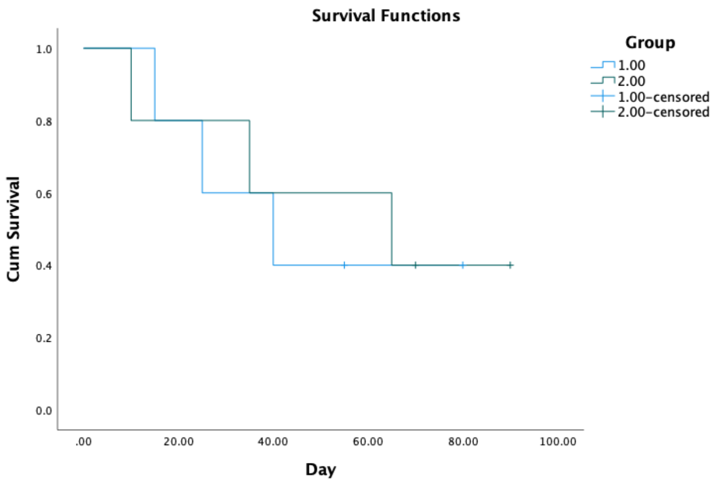
Left censoring occurs when the event took place before the start of observation, but the exact time is unknown. This is less common in veterinary medicine, but if the time an animal contracted a disease is unknown, and it is only known to be sick when brought to the clinic, left censoring is present. Interval censoring refers to situations where the event is known to have occurred within a specific time interval, but the exact time is unknown (11). For example, when a tumor metastasis is detected during a regular check-up, it is known that the metastasis occurred at some point between the two check-ups, but the exact time is unknown.

It is also important to clarify what survival analysis is not. This analysis is not a simple calculation of the mean; thus, calculating the average time only for those who experienced the event while ignoring censored data is incorrect and leads to bias in the results. Excluding censored observations systematically underestimates the true outcome, as individuals who survive for longer periods are often included in the censored group. Additionally, despite its potentially misleading name, it is not solely an analysis of death but can be used for any event. Finally, it is not a single technique but a family of methodologies that includes different methods such as Kaplan-Meier, Cox regression, and parametric models. Each method has its unique assumptions, advantages, and disadvantages.

Fundamental Concepts and Mathematical Foundations

To understand survival analyses, several fundamental concepts must be known. The survival function defines the probability that an individual remains event-free up to a specific time (12). It is denoted by the formula $S(t)=P(T>t)$,where T represents the random variable for the event time. This function starts at 1, as everyone is alive or event-free at baseline, and decreases over time, approaching 0. For example, a value of $S(180) = 0.65$ indicates that 65% of the patients are still alive within 180 days after treatment. This also implies that 35% have died within this period.

The graphical representation of the survival function is called the survival curve, which is the most frequently encountered graph in the veterinary literature. These curves generally have a step-like shape because the curve drops at each event time. The steepness of the curve indicates how frequently events occur. A steep curve suggests frequent events, while a slowly descending curve indicates rare events or a longer survival time.



Cumulative Survival Plot by Group

The hazard function, on the other hand, defines the instantaneous rate of experiencing the event at a given time, conditional on having remained event-free up to that time (13). This function is a more abstract concept but is crucial for understanding the natural course of a disease. The hazard function can change over time and reflects the dynamics of the disease. For example, in some cancer types, there is a high hazard in the first six months after surgery because micrometastases transition into clinical disease during this period, after which the hazard stabilizes. In some diseases, the hazard increases with age, while in others, it decreases over time. Understanding the shape of the hazard function is important for planning treatment strategies and designing follow-up programs.

The cumulative hazard function shows the total hazard accumulated from the start up to a specific time and is mathematically related to the survival function. This function is particularly used for comparing different groups and for model diagnostics.

The median survival time is the time at which the survival probability drops to 0.5, or 50%. This value is a standard output in veterinary oncology studies and is more robust than the mean because it is less affected by extreme values (14). In clinical practice, using median survival time when communicating with owners provides more realistic expectations. For example, a median survival time of 6 months for a treatment means that half of the animals will live less than 6 months, and half will live longer than 6 months. This information is more meaningful to owners than the mean, which can be affected by a few animals that live very long or very short periods and can be misleading.

The Kaplan-Meier Method and the Log-Rank Test

The Kaplan-Meier estimator is the non-parametric estimate of the survival function with censored data and is the most widely used method in veterinary research (12). Developed by Edward Kaplan and Paul Meier in 1958, this method has been used for over sixty years and constitutes the cornerstone of survival analysis. The method is a non-parametric approach, meaning it does not assume that the data follow a specific distribution. This characteristic makes the method highly flexible and broadly applicable.

The Kaplan-Meier method recalculates the survival probability at each event time, producing a step-function curve. The calculation is based on the number of individuals at risk at that event time, i.e., individuals who have not yet experienced the event and have not been censored. Censored observations are marked on the curve but do not cause the curve to drop, as the event did not occur in these individuals. One of the most important advantages of the method is the ability to calculate 95% confidence intervals (CIs), which indicate the uncertainty of the estimates. Confidence intervals are typically calculated using the Greenwood formula and widen as the sample size decreases or the number of events is small.

Kaplan-Meier curves are also used for comparisons between groups. For example, the survival curves of groups that received and did not receive treatment, different treatment protocols, or different disease stages can be displayed on the same graph. This visual comparison provides valuable information for clinicians and researchers.

The curve showed a sharp decline in the first three months post-treatment, followed by a flatter trajectory. This study demonstrated the aggressive nature of hemangiosarcoma and its propensity for early metastasis. The Kaplan-Meier curve also indicated that the one-year survival rate was only around 10%, which was used for realistic communication with owners.

The log-rank test is used to statistically test whether the differences between groups are significant. The log-rank test is a non-parametric method that assesses whether the survival curves of two or more groups are statistically different (15). The test compares the observed and expected number of events at each event time. The test statistic follows a chi-square distribution, and the p-value is calculated to evaluate the statistical significance of the difference between groups.

The key assumptions of the test include a random censoring mechanism and the assumption that the hazard ratio remains constant over time. The assumption of a constant hazard ratio is known as the proportional hazards assumption and presumes that the relative risk between groups does not change throughout the test period. This assumption may not always hold, and alternative tests can be used when the assumption is violated. The Wilcoxon test, which gives more weight to early events, can serve as an alternative to the log-rank test.

Cox Proportional Hazards Regression Model

Cox regression is the most commonly used method for multivariate analysis of survival data (13). Developed by Edward Cox in 1972, this semi-parametric model has made it possible in veterinary epidemiology to simultaneously evaluate the effect of multiple variables—such as age, treatment, stage, breed, and sex—on survival. The method is termed semi-parametric because it makes no assumptions about the form of the baseline hazard function but models the effect of the covariates parametrically. This characteristic grants the method both flexibility and interpretability.

The basic structure of the model is $h(t|X)=h_0(t)\times \exp(\beta_1X_1+\beta_2X_2+...\beta_PX_P)$. Here, $h(t|X)$ is the hazard at time t given the covariates X, $h_0(t)$ is the baseline hazard function (the hazard when all covariates are zero), the β values are the regression coefficients, and the X values represent the covariates. The exponential function indicates that the effect of the covariates is multiplicative; that is, a change in a covariate multiplies the hazard by a specific ratio.

The Hazard Ratio (HR) indicates how a one-unit increase in a covariate changes the hazard. It is calculated as $HR=\exp(\beta)$, and its interpretation is highly meaningful for clinicians. If $HR=1$, the covariate has no effect and is not associated with survival. If $HR>1$, the covariate increases the hazard and is a poor prognostic factor, meaning animals with that characteristic experience the event sooner. If $HR<1$, the covariate reduces the hazard and is a protective factor, meaning animals with that characteristic experience the event later or not at all. For example, finding an $HR = 0.43$ indicates that the treatment reduces the risk of death by 57% because $1\text{-}0.43=0.57$ (16).

The most important assumption of the model is the proportional hazards (PH) assumption, which states that the hazard ratio remains constant over time (17). This assumption implies that the ratio of the hazard functions for any two individuals is independent of time. Mathematically, if the HR for a covariate is 2, this ratio should be 2 on the first day of treatment as well as on the last day. However, in real life, this assumption may not always hold. For example, the effect of a treatment might decrease or increase over time. The risk of surgical complications might be high in the early period and decrease over time, while the risk of chronic diseases might increase over time.

Methods such as the Schoenfeld residuals test and log-log plots are used to check the assumption. Schoenfeld residuals are calculated separately for each covariate, and when plotted against time, no trend is expected. If a trend exists, the assumption is violated. Log-log plots are obtained by plotting log(-log(S(t))) against log(t), and the curves should be parallel. These graphical methods provide visual assessment and help detect assumption violations.

When the assumption is violated, time-dependent covariates can be used, a stratified Cox model may be preferred, or researchers may switch to parametric models. Time-dependent covariates allow the effect of the covariate to change over time. The stratified model uses the variable that violates the assumption as a stratification variable and defines a separate baseline hazard function for each stratum, but the effects of the other covariates are assumed to be the same across all strata.

Variable selection in the Cox regression model is also a critical issue. If too many variables are included, the risk of overfitting increases, and the model performs poorly on new data. Too few variables, on the other hand, can lead to missing important factors. The general rule is to have at least 10-15 events per covariate. Variable selection should be based on literature knowledge, biological plausibility, and univariate analyses. Automatic stepwise selection methods should be used with caution as they can lead to overfitting and false positive findings.

Parametric Survival Models

Parametric models assume that the survival time follows a specific probability distribution (18). In contrast to Cox regression, these models make an assumption about the baseline hazard function, making them a more structured but also more restrictive approach. Parametric approaches are preferred when there is strong prior knowledge about the baseline hazard function, when extrapolation (i.e., prediction beyond the observation period) is required, or when working with smaller sample sizes. One advantage of parametric models is that they provide smooth estimates and a better measure of uncertainty.

One of the most commonly used parametric distributions is the Weibull distribution. The Weibull distribution can model the hazard as monotonically increasing, decreasing, or constant, and is frequently used in studies due to this flexibility (19). The distribution is defined by two parameters: the shape parameter gamma and the scale parameter lambda. If the shape parameter gamma equals 1, the Weibull distribution reduces to the exponential distribution, implying a constant hazard. If gamma is greater than 1, the hazard increases over time; if it is less than 1, the hazard decreases over time. In veterinary studies, the risk of early complications after surgery generally decreases over time, while the risk of cancer increases with aging.

An advantage of parametric models is that they provide coefficients interpreted as the time ratio (TR), offering results that are more easily understood by clinicians. The time ratio is calculated as $TR = \exp(\beta)$ and indicates how much a one-unit increase in a covariate changes the median survival time. If $TR > 1$, the covariate prolongs the survival time; if $TR < 1$, it shortens it. For example, finding $TR = 2.3$ suggests that the treatment extends the median survival time by 2.3 times.

Other parametric distributions include the exponential, log-normal, log-logistic, and Gompertz distributions. The exponential distribution is the simplest and relies on the constant hazard assumption. The log-normal and log-logistic distributions are suitable for situations where the hazard function first increases and then decreases. The Gompertz distribution is a specific distribution used in aging processes, where the hazard increases exponentially. Model selection is performed using information criteria like AIC and BIC (Bayesian Information Criterion), as well as graphical methods. Cox-Snell residuals and Q-Q plots are used to check the distributional assumption.

Advanced Analysis Methods and Special Circumstances

Certain special circumstances in veterinary epidemiology necessitate methods beyond standard survival analyses. The first of these is recurrent events. In some veterinary research, an animal may experience the event of interest multiple times. Situations such as repeated mastitis cases, epileptic seizures, urinary tract infections, or bladder stone recurrence require recurrent event analyses (20). Standard survival analyses assume only one event per animal, but this assumption is violated with recurrent events. Specialized modeling approaches such as the Andersen-Gill model, the PWP model (Prentice, Williams, and Peterson), or frailty models are used for these situations.

The Andersen-Gill model assumes dependence between events and uses robust standard errors. The PWP model defines a separate baseline hazard for each event and takes the order of events into account. Frailty models account for unobserved heterogeneity among individuals (21). The term 'frailty' is included in the model as an individual-specific random effect and typically follows a gamma or log-normal distribution. This term explains the tendency for events within the same individual to be similar. Frailty models are particularly important in clustered data—for example, animals from the same farm or offspring from the same dam—because these individuals may share similar risk profiles, and this clustering affects standard error calculations.

The second special circumstance is competing risks. Competing risks refer to situations where an animal is at risk of multiple distinct events, and the occurrence of one event precludes the occurrence of the others (22). Examples include death from heart failure versus death from cancer in dogs, death from renal failure versus death from other causes in cats, or culling due to disease versus voluntary culling in dairy cows. Traditional Kaplan-Meier and Cox regression models provide biased estimates in this scenario because they treat the competing risk as censoring. However, a competing risk is not true censoring because the individual can no longer experience the event of interest once the competing risk has occurred.

Specialized methods have been developed for competing risks. The Cumulative Incidence Function (CIF) calculates the cumulative probability of occurrence for each event in the presence of competing risks. The Fine-Gray model, known as the subdistribution hazard model, provides regression analysis in the presence of competing risks. This model can be set up separately for each competing risk and evaluates the effect of covariates on that specific risk. Competing risk analyses are increasingly utilized, particularly in multi-cause mortality studies.

The third special circumstance is time-dependent covariates. Some variables change over time, and this dynamic structure must be incorporated into the model (23). Changes in treatment, recurrent infections, body weight variations, or biomarker levels can change over time. Standard Cox regression assumes that covariates are measured at baseline and remain constant thereafter, which is not always realistic. The Extended Cox model handles time-dependent covariates, and the value of the covariate at each time point is included in the model. An example of this approach is the addition of somatic cell count (SCC) as a time-dependent covariate when evaluating mastitis treatment success in dairy cows. The monthly measured SCC affects treatment success and culling risk, and this dynamic relationship can be captured with time-dependent modeling (24).

Methodological Considerations and Common Errors

In survival analyses, the sample size depends on the number of events rather than the total number of individuals. This is an important distinction because if only 20 events occur in a study with a thousand animals, the statistical power is low. Cox regression requires at least 10-15 events per covariate, a rule known as Events Per Variable (EPV) (32). For instance, if five covariates are planned and an EPV of 10 is used, 50 events are needed. If an event rate of 60% is expected, a total of 84 individuals should be enrolled in the study. For power analysis, the powerSurvEpi package in R, the stpower command in Stata, or the Freedman and

Schoenfeld formulae can be used. Sample size calculations must consider the expected effect size, alpha error, beta error, and the censoring rate.

The fundamental assumption of Cox regression, the proportional hazards (PH) assumption, must be tested. The hazard ratio is expected to remain constant over time. The Schoenfeld residuals test is applied separately for each covariate, and the assumption is met if the p-value is greater than 0.05. Log-log plots are obtained by plotting log(-log(S(t))) against log(t), and the curves should be parallel. These graphical methods provide a visual assessment. When the assumption is violated, there are several options. Time-dependent covariates can be added, and interaction terms such as covariate multiplied by log-time or covariate ×time can be created. The stratified Cox model can be used, with the variable that violates the assumption designated as the stratification variable using the strata function. Alternatively, researchers can switch to parametric models or use non-assumption-dependent methods such as the Restricted Mean Survival Time (RMST).

The assumption that the censoring mechanism is random and independent of the event time is crucial. Informative censoring, where sicker animals are withdrawn early, causes severe bias. For example, owners deciding on early euthanasia for animals with poor prognoses or animals not responding to treatment being sent to another clinic creates informative censoring. Detailed recording of the reasons for censoring and comparing the characteristics of censored versus uncensored groups help detect this problem. Suspicion of informative censoring arises if the censored group contains animals that are older, sicker, or in a more advanced stage. Sensitivity analysis can be performed by testing different censoring scenarios. For instance, checking whether the results change when early censored observations are excluded.

Immortal time bias is a common error in survival analyses. This error occurs when a period during which the event cannot possibly occur is incorrectly included in the risk period. For example, including the time elapsed before treatment initiation in the treatment group creates immortal time bias, as it is impossible to die before treatment begins, providing an unfair advantage to the treatment group. Landmark analysis can be used as a solution. In landmark analysis, a specific time point is chosen, e.g., 30 days after diagnosis, and only individuals who are alive up to that point are included in the analysis. Alternatively, time-dependent covariates can be used to code the treatment group starting from the moment the treatment was initiated.

Overfitting occurs when there are too many covariates and too few events, and the model performs poorly on new data. The risk of overfitting increases when the EPV is less than 10. The solution involves adhering to the EPV rule, performing a priori variable selection, and relying on literature knowledge and biological plausibility. Automatic stepwise selection methods should be used with caution as they can lead to p-value fishing and false positive findings. Penalized regression methods such as LASSO or Ridge regression can help prevent overfitting. Internal validation using bootstrapping can be performed to calculate the optimism-corrected performance of the model. If possible, external validation with a different cohort should be performed to test the generalizability of the model.

P-hacking is the error of trying different analyses until a significant p-value is found. This approach is contrary to scientific rigor and leads to erroneous conclusions. The solution involves pre-specifying the analysis plan and, if possible, performing pre-registration. Registering the study protocol beforehand on platforms like ClinicalTrials.gov or PROSPERO ensures transparency. Multiple testing correction should be applied using methods like Bonferroni or False Discovery Rate. Publishing negative results also reduces publication bias.

Reporting Standards and Good Practices

For observational studies, the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines, and for animal studies, the ARRIVE (Animal Research Reporting of In Vivo Experiments) guidelines must be followed (25,26). These guidelines specify the necessary elements for transparent and reproducible reporting and enhance the quality of scientific publications. STROBE provides a 22-item checklist and offers detailed guidance on reporting study design, participants, variables, data sources, bias, statistical methods, and results. ARRIVE 2.0 contains 21 items for transparency and reproducibility in animal studies.

It is critical that the definitions of baseline and event are clear and operational. The starting point—whether it is the date of diagnosis, surgery, or treatment initiation—must be clearly stated. If the event definition relies on clinical, laboratory, or radiological criteria, these criteria must be explained in detail. The types and rates of censoring must be specified. Details such as how many animals were right-censored, how many due to study end, and how many due to loss to follow-up or unrelated death, should be provided. Reporting the follow-up time as both the median and interquartile range is important because the mean follow-up time can be influenced by extreme values. The number and percentage of events should be reported separately for each group.

Kaplan-Meier curves must be presented along with a risk table. The risk table shows the number of individuals at risk at each time point, providing the reader with information about the reliability of the curve. As the number at risk decreases toward the end of the curve, estimates become more uncertain, and the risk table demonstrates this uncertainty. Censoring marks should be displayed on the curve. 95% confidence intervals should be plotted. If p-values were calculated using the log-rank test, this should be specified. Median survival times and their 95% CIs should be presented in a table.

Cox regression results should be reported with the Hazard Ratio (HR), 95% confidence intervals, and p-values. Univariate and multivariate results should be presented in separate tables. The variables included in the multivariate model and the variable selection strategy must be explained. Model performance should be reported with the C-index or concordance index. The C-index ranges from 0.5 to 1.0, where 0.5 represents random prediction and 1.0 represents perfect prediction. Values generally above 0.7 are considered good. The results of the proportional hazards assumption test must be reported. The p-values of the Schoenfeld residuals test should be provided for each covariate. If the assumption was violated, the steps taken to address it must be explained.

The limitations section must be honest and comprehensive. In retrospective studies, missing data, selection bias, and unmeasured confounding should be discussed. Limitations such as small sample size, short follow-up duration, or single-center study status should be noted. The generalizability (external validity) of the results must be evaluated. It should be discussed whether the study population is representative of the general population or a selected subgroup.

Future Perspectives and Novel Approaches

The integration of machine learning (ML) methods into survival analysis is rapidly evolving and opening up new horizons in veterinary epidemiology. Methods such as Random Survival Forests (RSF) and DeepSurv can automatically detect non-linear relationships and complex interactions (27,28). Traditional Cox regression assumes linear relationships and proportional hazards, but in real life, relationships may be non-linear, and interactions can be complex. RSF is a tree-based method that creates thousands of decision trees for prediction. Each tree is trained on a random subset of the data, and the results are aggregated to achieve robust estimates. The method provides variable importance scores, indicating which variables are most crucial. It is tolerant of missing data and works well with categorical variables.

DeepSurv is a deep learning-based method that performs survival analysis using neural networks. The potential of these methods is high, particularly in genomic data and image analysis. The integration of radiological and histopathological images directly into survival models could enable prognosis prediction without human intervention. Convolutional Neural Networks (CNNs) can extract prognostic features from radiology images, and these features can be provided as input to the survival model. Features such as tumor morphology, cell density, and necrosis area can be automatically measured in pathology images for prognosis prediction.

Precision medicine or personalized treatment approaches necessitate dynamic prognosis models (29). Static models provide a single prediction at the start of treatment, which remains unchanged. However, in reality, treatment response, biomarker changes, and complications require the prognosis to be updated. Risk scores updated after each treatment cycle will provide real-time decision support for clinicians. For example, in canine lymphoma treatment, risk reclassification could be performed after each CHOP cycle using Minimal Residual Disease (MRD) testing. Treatment intensity could be increased or alternative protocols adopted for animals with high MRD positivity. Conversely, the treatment burden could be reduced for animals with low MRD negativity. The Joint Modeling approach combines longitudinal biomarker data with survival outcomes, providing updated predictions at every time point.

The consolidation of electronic veterinary health records will provide data from millions of animals, enabling robust analyses even for rare diseases (30). Large electronic record systems, such as PetCare Data in the US, collect data from millions of animals. This Big Data provides Real-World Evidence (RWE) and offers valuable insights in situations where randomized controlled trials are infeasible. Sufficient sample size can be achieved for rare diseases, post-marketing surveillance of drug safety can be performed, and geographical and temporal trends can be analyzed. However, Big Data also presents challenges. Missing data management is complex, censoring mechanisms are heterogeneous, data quality and standardization issues can arise, and ethical and privacy concerns are paramount.

Federated learning enables collaborative training of models without merging the data from different clinics. Each clinic trains the model on its own data and sends only the model parameters to a central server. The central server aggregates these parameters to create a global model. This approach allows for the advantage of a large sample size while preserving privacy. Federated learning can be a solution when legal and ethical barriers hinder data sharing in multi-center veterinary studies.

Causal inference methods are increasingly used for confounding control in observational studies. In observational studies, systematic differences may exist between treatment groups, which can affect the results. Propensity score methods have been developed to balance these differences. The propensity score represents the probability of an individual receiving treatment and is calculated from baseline covariates. Groups can be balanced using the propensity score through methods like matching, stratification, weighting, or covariate adjustment. Instrumental variables utilize natural experiments to estimate causal effects. Marginal structural models are used in the presence of time-dependent confounding and estimate the causal effect in complex scenarios where treatment changes over time.

Multi-omics integration involves incorporating genomic, transcriptomic, proteomic, and metabolomic data into survival analyses. As the cost of next-generation sequencing technologies decreases, this data will become more accessible. Pathway-based survival analysis examines the relationship between biological pathways and survival. Gene set enrichment approaches evaluate the prognostic value of specific gene sets. These approaches will be crucial in veterinary oncology for understanding tumor biology and developing targeted therapies.

Conclusion and Recommendations

Survival analyses are an indispensable tool for the analysis of time-to-event data in veterinary epidemiological research. The correct handling of censored data, the simultaneous evaluation of the effects of multiple variables, and the modeling of time-dependent dynamics are the most significant advantages of these methods. A broad methodological spectrum, ranging from Kaplan-Meier curves and Cox regression to parametric and advanced models, offers researchers the opportunity to find answers to diverse clinical questions.

The practical value of survival analyses for veterinarians is immense. The identification of prognostic factors guides treatment decisions. Risk scoring systems facilitate realistic communication with owners. The comparison of treatment protocols strengthens evidence-based medicine practices. Vaccine efficacy studies provide a scientific foundation for public health policies. Economic analyses in herd health management guide decision-makers.

Recommendations for researchers include: First, consider the requirements of survival analyses during the study design phase. Clearly define the baseline and event, plan the censoring mechanisms, and conduct a power analysis for adequate sample size. Second, during the data collection phase, use standardized forms, record censoring reasons in detail, and minimize missing data. Third, during the analysis phase, test assumptions, select the appropriate method, and perform sensitivity analysis. Fourth, during the reporting phase, adhere to the STROBE and ARRIVE guidelines, provide all necessary information, and honestly discuss limitations.

Recommendations for clinicians include: Interpret survival analyses in the literature correctly. Pay attention to median survival times and 95% confidence intervals. Understand the Hazard Ratio values and distinguish between clinical importance and statistical significance. Consult the risk table on Kaplan-Meier curves and recognize the uncertainty towards the end of the curve. Evaluate whether the study population is representative of your own patients.

It is anticipated that survival analyses will become even more widespread and developed in veterinary medicine in the future. The integration of machine learning, personalized treatment approaches, big data analytics, and multi-omics studies will accelerate developments in this field. Despite these technological advancements, the importance of understanding the fundamental methodological principles, checking assumptions, and transparent reporting will never diminish.

The accurate, ethical, and transparent use of these methods plays a critical role in the adoption of evidence-based medicine principles in veterinary science. Survival analysis is not just a statistical technique; it is a powerful tool for generating scientific evidence to improve the lifespan and quality of life for animals. Within the framework of the One Health approach, veterinary epidemiology also contributes to human and environmental health. Veterinary survival analyses provide important information on topics such as the control of zoonotic diseases, food safety, and antimicrobial resistance.

Finally, scientific rigor, ethical values, and animal welfare must always be the priority. Sample size calculations should be conducted carefully to avoid unnecessary animal use, the 3R principles (replacement, reduction, refinement) should be implemented, and humane endpoints should be clearly defined. Conflicts of interest must be reported transparently, and publication bias must be combated. Negative results should also be published to contribute to the literature. Open science practices, such as data and code sharing, should be encouraged. Pre-registration of study protocols enhances scientific integrity.

Survival analyses have made significant progress in veterinary epidemiology over the last two decades and have become a standard analytical method. It is hoped that this progress will continue in the coming years and that new methodological developments will improve animal health and welfare across all areas of veterinary medicine.

References

1. Collett D. Modelling survival data in medical research. 3rd ed. Boca Raton: Chapman and Hall/CRC; 2015.
2. Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. 2nd ed. Charlottetown: VER Inc.; 2009.
3. Sergeant ES, Perkins NR. Epidemiology for field veterinarians: An introduction. Wallingford: CABI; 2015.
4. Withrow SJ, Vail DM, Page RL. Withrow and MacEwen's small animal clinical oncology. 6th ed. St. Louis: Elsevier; 2019.
5. Thiry E, Addie D, Belák S, et al. Feline herpesvirus infection. ABCD guidelines on prevention and management. J Feline Med Surg. 2009;11(7):547-555.
6. Gröhn YT, Rajala-Schultz PJ. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci. 2000;60-61:605-614.
7. O'Neill DG, Church DB, McGreevy PD, Thomson PC, Brodbelt DC. Longevity and mortality of owned dogs in England. Vet J. 2013;198(3):638-643.
8. Klein JP, Moeschberger ML. Survival analysis: Techniques for censored and truncated data. 2nd ed. New York: Springer; 2003.
9. Leung KM, Elashoff RM, Afifi AA. Censoring issues in survival analysis. Annu Rev Public Health. 1997;18:83-104.
10. Lawless JF. Statistical models and methods for lifetime data. 2nd ed. Hoboken: Wiley-Interscience; 1981.
11. Lindsey JC, Ryan LM. Methods for interval-censored data. Stat Med. 1998;17(2):219-238.
12. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53(282):457-481.
13. Cox DR. Regression models and life-tables. J R Stat Soc Series B Stat Methodol. 1972;34(2):187-220.
14. Swindell WR. Accelerated failure time models provide a useful statistical framework for aging research. Exp Gerontol. 2009;44(3):190-200.
15. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep. 1966;50(3):163-170.
16. Boerman I, Selvarajah GT, Nielen M, Kirpensteijn J. Prognostic factors in canine appendicular osteosarcoma - a meta-analysis. BMC Vet Res. 2012;8:56.
17. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 1994;81(3):515-526.

18. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis Part II: Multivariate data analysis - an introduction to concepts and methods. Br J Cancer. 2003;89(3):431-436.
19. Carroll KJ. On the use and utility of the Weibull model in the analysis of survival data. Control Clin Trials. 2003;24(6):682-701.
20. Amorim LD, Cai J. Modelling recurrent events: A tutorial for analysis in epidemiology. Int J Epidemiol. 2015;44(1):324-333.
21. Duchateau L, Janssen P. The frailty model. New York: Springer; 2008.
22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94(446):496-509.
23. Andersen PK, Gill RD. Cox's regression model for counting processes: A large sample study. Ann Stat. 1982;10(4):1100-1120.
24. Gröhn YT, Eicker SW, Ducrocq V, Hertl JA. Effect of diseases on the culling of Holstein dairy cows in New York State. J Dairy Sci. 1998;81(4):966-978.
25. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. Lancet. 2007;370(9596):1453-1457.
26. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000410.
27. Ishwaran H, Kogalur UB, Blackstone EH, Lauer MS. Random survival forests. Ann Appl Stat. 2008;2(3):841-860.
28. Katzman JL, Shaham U, Cloninger A, Bates J, Jiang T, Kluger Y. DeepSurv: Personalized treatment recommender system using a Cox proportional hazards deep neural network. BMC Med Res Methodol. 2018;18(1):24.
29. van Houwelingen HC. Dynamic prediction by landmarking in event history analysis. Scand J Stat. 2007;34(1):70-85.
30. O'Neill DG, Brodbelt DC, Hodge R, Church DB, Meeson RL. Epidemiology and clinical management of elbow joint disease in dogs under primary veterinary care in the UK. Canine Med Genet. 2020;7:1.

Chapter 9

Establishing Causal Relationships in Veterinary Medicine: Statistical Approaches

Furkan Çağrı BEŞOLUK[1]

Introduction

In the field of veterinary medicine, the understanding of disease etiology and the development of effective treatment methods largely depend on the accurate determination of cause-and-effect relationships. Evidence-based practices are becoming increasingly prevalent in modern veterinary medicine, posing a significant challenge to researchers: Is an observed association truly causal or merely a spurious correlation (1,2)? Statistical methods offer valuable tools for making this critical distinction and achieving reliable conclusions.

Establishing causal relationships in veterinary epidemiology brings with it certain difficulties that differ from human medicine. The heterogeneity of animal populations, environmental factors, financial constraints, and ethical limitations that often preclude randomized controlled trials (RCTs) push researchers toward observational study designs (3,4). This context is important for the selection and application of statistical analysis methods. For example, in an investigation into the factors affecting the incidence of mastitis in a dairy cattle operation, both individual animal-level characteristics and herd-level management practices are crucial, and this hierarchical structure must be accounted for in the analyses.

This chapter will address the fundamental statistical methods used to elucidate cause-and-effect relationships in veterinary health research. Topics to be examined include the epidemiological foundations of causality, causal inferences from study designs, regression analysis methods, control of confounding factors, and the analysis of mediating and moderating variables. Throughout the chapter, concrete examples from the veterinary field will be used to demonstrate how theoretical knowledge can be applied in practice.

[1] Research Assistant ORCID:0000-0002-1967-9418
E-Mail: furkan.besoluk@selcuk.edu.tr
Department of Biostatistics, Faculty of Veterinary Medicine, Selcuk

The Concept of Causality and Epidemiological Criteria

Causality is one of the fundamental goals of scientific research, yet it remains a complex concept. Since David Hume's classical critique, it has been accepted that causality cannot be directly observed; only the constant conjunction of events can be observed (5). Therefore, systematic criteria are needed in research to determine whether a factor genuinely causes a disease.

The most widely used framework for investigating causality in epidemiological studies is the criteria proposed by Sir Austin Bradford Hill in 1965. Hill's nine criteria offer an approach for assessing the probability that an association is causal (6). The most important of these criteria is the strength of the association. The greater the difference in disease risk between the exposed and unexposed groups, the higher the likelihood that the association is causal. For example, a ten-fold higher risk of disease among veterinary practitioners working in brucellosis-infected herds compared to the general population suggests a strong causal link between the profession and the disease (7).

The second important criterion is consistency. Obtaining similar results across different populations, by different researchers, and using different methodologies supports the existence of a causal relationship (8). In the veterinary literature, for instance, the association between *Cryptosporidium* and calf diarrhea has been consistently demonstrated in studies conducted across various geographical regions worldwide. The criterion of specificity refers to the situation where the risk associated with a cause is linked to only a single disease. However, this criterion is weaker in epidemiology compared to others because many causes can lead to multiple diseases, and many diseases have a multifactorial etiology (9).

The temporality criterion dictates that the cause must precede the effect. Cohort studies and randomized controlled trials provide evidence for assessing this criterion, but determining temporality is often not possible in cross-sectional studies (10). Biological gradient (dose-response relationship) refers to the increasing risk of disease with increasing exposure. For example, a linear relationship between high milk yield and the risk of ketosis in dairy cows supports this criterion.

Biological plausibility refers to the requirement that the observed association aligns with existing biological knowledge. However, this criterion must be interpreted cautiously, as the body of existing knowledge is constantly evolving (11). The criterion of experimental evidence questions whether intervention on the cause in controlled experimental studies alters the outcome. In the veterinary field, for instance, the reduction of disease risk by a vaccine strongly supports the causal relationship between the agent and the disease. The analogy criterion suggests that the fact that similar causes lead to similar effects may increase the probability that the relationship under evaluation is causal.

Study Designs and the Strength of Causal Inference

The power of causal inference varies greatly among the different study designs used in veterinary epidemiology. At the pinnacle of the evidence pyramid are Randomized Controlled Trials (RCTs), which maximize control over confounding factors (12,13). Randomization maximizes the likelihood that observed effects are genuinely attributable to the intervention. In the veterinary field, RCTs are crucial for situations such as testing the efficacy of a new vaccine or comparing different treatment protocols.

However, conducting RCTs in veterinary practice is not always feasible. For ethical reasons, exposing animals to a known harmful factor or withholding a potentially beneficial treatment for research purposes may be unacceptable (14). Furthermore, very large sample sizes may be required for rare diseases, and studies on diseases with long latent periods can take years to complete. In these situations, economic costs also pose a significant limitation. Consequently, researchers are often compelled to resort to observational study designs.

Among observational studies, Cohort Studies stand out as a strong design for evaluating cause-and-effect relationships (15,16). In prospective cohort studies, disease-free individuals are classified according to the risk factor, and the development of the disease is monitored over time. The most important advantage of this design is that it meets the temporality criterion and allows for the direct calculation of disease risk. In retrospective cohort studies, past records are utilized to assess exposures and outcomes retrospectively. In the veterinary context, for example, a typical cohort study to identify risk factors for lameness in a dairy cattle operation would involve monitoring healthy cows and comparing the incidence of lameness across groups with different housing conditions, feeding programs, and genetic traits.

Case-Control Studies are an effective design, especially for investigating rare diseases (17,18). In these studies, cases and controls are identified, and their past exposures are compared. The most significant advantage of case-control studies is their feasibility with small sample sizes, even for rare diseases. However, this design also has disadvantages. Disease incidence cannot be calculated, and temporality is often uncertain. In the veterinary field, for example, a typical case-control study for investigating risk factors for a rare type of tumor would compare the past exposures of animals diagnosed with the tumor with those of healthy controls.

Cross-Sectional Studies, also known as prevalence studies, measure exposure and outcome simultaneously at a single point in time. Since temporality cannot be established with this design, its power to make causal inferences is limited (19). However, cross-sectional studies can be useful for assessing disease burden and identifying potential associations, and they are often used in the hypothesis-generating phase for more comprehensive studies. Ecological Studies are conducted at the group level rather than the individual level and are unsuitable for making causal inferences due to the risk of ecological fallacy.

Regression Analysis Methods

Regression analysis is a widely used statistical method in veterinary epidemiology for the quantitative evaluation of cause-and-effect relationships (20,21). Fundamentally, it aims to determine the effect of one or more independent variables (risk factors, exposures) on a dependent variable (disease, outcome). The main advantage of regression analysis is its ability to simultaneously assess the effects of multiple factors and control for confounding variables.

Different regression models are chosen based on the type of outcome variable. Methods include linear regression for continuous outcome variables, logistic regression for binary outcomes, negative binomial or Poisson regression for count data, and Cox regression for time-to-event analysis (22). Among regression analyses, logistic regression is one of the most frequently used methods in veterinary research. This is because the outcome variable in many veterinary epidemiological studies is binary: treatment success or failure, death present or absent, disease present or absent, etc.

In the logistic regression model, the logarithm of the odds of an event occurring is modeled as a linear combination of the independent variables. The results of logistic regression analysis are typically interpreted using the Odds Ratio (OR). The exponentiation of the coefficient for each independent variable indicates how many times a one-unit increase in that variable changes the odds. For instance, logistic regression can be used in a study investigating the effect of different risk factors on the risk of metritis in dairy cows. Variables such as dystocia, twinning, parity, and body condition score may be included in the research model. If the analysis result yields an OR of 3.2 for dystocia, this is interpreted as cows experiencing dystocia having a 3.2 times higher risk of metritis (23).

Cox regression, or the Cox proportional hazards model, is a regression method used for time-to-event analysis and is suitable for analyzing the time until disease development or time until death (24,25). This model is particularly common in cohort studies. The fundamental assumption of the Cox model is that the hazard ratios remain constant over time, which is known as the proportional hazards assumption. The results of Cox regression are interpreted via the Hazard Ratio (HR). For example, in a study investigating factors affecting the survival time of horses after a specific orthopedic surgery, if the HR for age is found to be 1.15, it means that the risk of death increases by 15 percent for each one-year increase in age.

For all regression models, variable selection is a critically important step during the model building phase. Theory-based variable selection, which involves identifying variables to be included in the model based on literature knowledge and biological plausibility, is the most recommended approach (26). The use of statistical algorithms like stepwise variable selection is debatable because these methods are sensitive to sampling fluctuations and can mask true relationships. Statistics such as the Hosmer-Lemeshow test, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC) are used to evaluate model fit.

Control of Confounding Factors

One of the greatest challenges in accurately establishing cause-and-effect relationships is the presence of confounding factors (27,28). A confounder is a third variable that is related to both the exposure and the outcome. If the confounder is not controlled, the observed association may deviate from the true causal effect, potentially leading the researcher to incorrect conclusions.

Confounding is a frequent issue in veterinary epidemiology. For example, in a study that found a lower prevalence of mastitis in organic dairy farms compared to conventional ones, the relationship between farm type and mastitis needs to be evaluated. However, organic farms typically have smaller herd sizes, and herd size is also related to mastitis. In this case, herd size is a confounder, and if not controlled, it could lead to an erroneous assessment of the true effect of the organic farming system on mastitis.

Various strategies are available for the control of confounding factors (29,30). At the study design stage, randomization is the most effective method because it ensures that all confounders are equally distributed among the groups. Restriction involves including only individuals who are homogeneous with respect to the confounding factor; for instance, enrolling only animals within a specific age group. Matching involves selecting controls that are similar to each case with respect to the confounding factors and is particularly used in case-control studies.

Confounder control methods are more commonly applied at the analysis stage. Stratification involves separating the data into layers based on the levels of the confounding factor and conducting separate analyses within each stratum. The Mantel-Haenszel method is a technique used to calculate a common odds or risk ratio in stratified data (31). Multiple regression analysis allows for the control of confounders by including them as independent variables in the model, and it is the most flexible method due to its ability to simultaneously control for multiple confounders.

In recent years, propensity score methods have become popular for controlling confounding (32). The propensity score is the probability of an individual receiving a particular exposure, based on observed variables. Through propensity score matching, stratification, or weighting, balance of observed confounders between exposure groups is achieved, thus creating a form of quasi-randomization. In veterinary research, propensity score methods can be used to control for confounding factors that influence treatment choice when comparing outcomes in animals that did and did not receive a specific treatment.

Confounder control also has limitations. Unmeasured confounders always remain a problem in observational studies (33). Some confounders may not have been measured during the study, or the measurement tools may lack sufficient precision. In such cases, sensitivity analysis should be conducted to evaluate the extent to which unmeasured confounders could alter the results. Over-adjustment is also a concern. Including variables that are part of the causal pathway (mediating variables) in the model can mask the true causal effect.

Analysis of Mediating and Moderating Variables

In understanding cause-and-effect relationships, not only direct effects but also the mechanisms through which these effects occur and the conditions under which they change are important (34,35). A mediating variable is an intermediate variable that explains the mechanism by which a risk factor's effect on the outcome is realized. A moderating variable, or interaction variable, shows whether the magnitude or direction of a risk factor's effect changes across different subgroups.

The goal of mediation analysis is to partition the total effect into a direct effect and an indirect effect. For example, when investigating the effect of a high body condition score (BCS) in the peripartum period of dairy cows on the risk of metritis, part of this effect might be hypothesized to occur through a negative energy balance (NEB). That is, a causal relationship is proposed where high BCS leads to NEB, which in turn increases the risk of metritis. In this scenario, NEB is a mediating variable, and mediation analysis calculates both the direct effect of BCS on metritis and the indirect effect through NEB.

According to the Baron and Kenny approach, four conditions must be met to demonstrate a mediation effect (36). The independent variable must significantly affect the dependent variable; the independent variable must significantly affect the mediator; the mediator must significantly affect the dependent variable when controlling for the independent variable; and the effect of the independent variable must decrease or become non-significant when the mediator is added to the model. However, in modern mediation analysis, the statistical significance of the indirect effect is tested directly using methods like the Sobel test or bootstrapping.

Interaction analysis investigates whether the effect of a risk factor changes across different subgroups. For example, evaluating whether the protective effect of a specific vaccine differs between old and young animals is an analysis of a moderating effect. Statistically, interaction is tested by adding an interaction term to the model. In the veterinary literature, factors such as breed, sex, season, and geographical region are frequently evaluated as moderating variables.

One important point to consider in interaction analysis is the distinction between multiplicative interaction and additive interaction (37). While models such as logistic regression and Cox regression test interaction on a multiplicative scale, interaction on an additive scale is often more relevant from a public health perspective. Measures such as the relative risk due to interaction (RERI) can be used to assess additive interaction. Furthermore, interaction hypotheses should be pre-specified, as including multiple interaction terms in the model can lead to a loss of power and overfitting.

Advanced Statistical Techniques

Advanced statistical techniques for analyzing complex data structures and more accurately determining cause-and-effect relationships are becoming increasingly common in veterinary epidemiology (38,39). Hierarchical or multilevel data structures are frequently encountered in veterinary research. For example, individual animal-level measurements in dairy cattle may be clustered within herd-level management practices. Standard regression methods assume the independence of observations, but outcomes for animals within the same herd can be similar, and this intra-class correlation violates the independence assumption.

Multilevel models or mixed-effects models are appropriate statistical methods for analyzing hierarchical data structures. These models incorporate both fixed and random effects. Fixed effects represent general effects applicable to the entire population, while random effects account for variation at the cluster level. For instance, in a multilevel logistic regression model investigating factors affecting the risk of mastitis in dairy cattle, animal-level variables like parity and body condition score can be included as fixed effects, and herd identity can be included as a random effect.

Generalized Estimating Equations (GEE) offer an alternative approach for the analysis of clustered data. These methods, also known as marginal models, estimate population-averaged effects while accounting for correlation between observations through a working correlation matrix (40). The advantage of this method is that it produces consistent estimates even if the correlation structure is incorrectly specified. GEE are frequently used in veterinary longitudinal studies for the analysis of repeated measurements taken from the same animal at different time points.

Structural Equation Modeling (SEM) is a powerful statistical technique that allows for the simultaneous testing of multiple dependent variables and complex causal relationships. This method can model both direct and indirect effects between observed and latent variables and is ideal for multiple mediation analyses (41). In veterinary research, SEM can be used to model concepts such as animal welfare, which cannot be measured directly but can be evaluated with multiple indicators.

Machine learning methods have also become increasingly utilized in veterinary epidemiology in recent years. Algorithms such as random forests, support vector machines, and artificial neural networks are effective in discovering complex non-linear relationships and interactions (42). However, these methods have limitations for causal inference. Machine learning algorithms are generally optimized for prediction, and causal interpretation is not always straightforward. Nevertheless, they are important for identifying crucial risk factors and developing prediction models, especially in large datasets.

Bayesian statistical methods allow for the integration of prior knowledge with current data and are particularly advantageous in analyses involving small sample sizes or rare events. In the Bayesian approach, results can be interpreted probabilistically. Bayesian methods may be preferred in veterinary epidemiology when incorporating knowledge from previous studies is required or for the estimation of complex hierarchical models.

Other advanced techniques for causal inference include instrumental variable analysis and synthetic control methods. Instrumental variable analysis allows for the estimation of causal effects in the presence of unmeasured confounders (43). An instrumental variable is a variable that influences the exposure but affects the outcome only through the exposure. In veterinary genetic epidemiology, genetic variants are used as instrumental variables in the Mendelian randomization approach.

Checking Model Assumptions and Diagnostic Statistics

The validity of statistical models depends on meeting fundamental assumptions. Violation of these assumptions in regression analysis can lead to erroneous results and flawed causal inferences. The key assumptions in linear regression include the normal distribution of errors, constant variance (homoscedasticity), independence of errors, and the absence of multicollinearity among independent variables (44).

Methods such as the histogram, Q-Q plot, and the Shapiro-Wilk test can be used to check the normal distribution of errors. However, in large sample sizes, small deviations from the normality assumption do not pose a major problem due to the central limit theorem. To check the assumption of constant variance (homoscedasticity), the scatter plot of residuals versus predicted values is examined. A funnel shape indicates heteroscedasticity, which is non-constant variance. Tests such as the Breusch-Pagan test or the White test can also be used. In the presence of heteroscedasticity, robust standard errors or weighted least squares methods can be employed.

The problem of multicollinearity occurs when there is a high correlation among independent variables, leading to unreliable parameter estimates. The Variance Inflation Factor (VIF) is a measure used to assess multicollinearity. A VIF value greater than 10 indicates problematic multicollinearity (45). If a multicollinearity problem exists, methods such as removing one of the highly correlated variables from the model or combining variables may be necessary.

In logistic regression, the Hosmer-Lemeshow test, classification tables, ROC curve, and AUC values are used to evaluate model fit. The ROC curve displays sensitivity and specificity values at different cut-off points, and the AUC (Area Under the Curve) takes a value between 0.5 and 1.0. 0.5 represents random guessing, and 1.0 represents perfect discrimination. ROC analysis is widely used in veterinary diagnostic test evaluations and risk prediction models.

Outliers and influential observations can significantly affect regression results. Diagnostic statistics such as standardized residuals, studentized residuals, and Cook's distance are used to identify outliers and influential observations. A Cook's distance value greater than 1 reflects excessive influence of the observation on the model. Rather than automatically removing outliers, it should first be evaluated whether these observations stem from data entry errors or represent genuine biological variation.

Sources of Bias in Causal Inference

Recognizing and minimizing systematic errors in epidemiological studies is critical for valid causal inferences. Bias is defined as a systematic deviation from the true value and is categorized into three main types: selection bias, information bias, and confounding (46).

Selection bias arises when the study population does not adequately represent the target population. In veterinary studies, for example, a study that includes only animals brought to a veterinary clinic may have systematic differences between the clinic-referred animals and the general population. This is known as Berkson's bias. This sample, which does not reflect the characteristics of the reference population, limits generalizability. To avoid selection bias, the study population must be clearly defined, and, if possible, efforts should be concentrated on random sampling methods.

Missing observations and loss to follow-up can also lead to selection bias. In cohort studies, since the outcome status of individuals lost to follow-up is unknown, bias occurs if loss to follow-up is related to either the exposure or the outcome. For example, if animals experiencing side effects in a treatment study drop out and leave the study, the treatment effectiveness in the remaining animals might appear better than it truly is. When handling missing data, methods such as multiple imputation should be preferred over complete-case analysis (47).

Information bias is related to the erroneous measurement or classification of the exposure or outcome. There are two types: differential and non-differential. Non-differential bias occurs when the measurement error is independent of the outcome status and typically tends to attenuate (weaken) the true relationship. Differential bias is more concerning and can affect the relationship in either direction. For instance, patients in case-control studies remembering their past exposures better than controls (recall bias) can create an association that does not exist in reality.

Observer bias is the reflection of the data collector's prejudices in the measurements. In veterinary clinical studies, the person administering the treatment or assessing the outcome knowing which group received which treatment may unconsciously affect the assessments. Therefore, blinding is essential in randomized controlled trials. Ideally, both the participants and the assessors should be unaware of the exposure status; this is called a double-blind design. While blinding animals in veterinary clinical trials is often not feasible, blinding the outcome assessors is usually possible.

Publication bias is the tendency for studies with statistically significant results to be published more frequently than those with non-significant findings (48). This bias is a significant issue in systematic reviews and meta-analyses and can lead to a systematic distortion of the evidence in the literature. Practices such as trial registration and protocol publication are efforts aimed at mitigating publication bias.

Conclusion and Recommendations

The accurate establishment of cause-and-effect relationships in veterinary health research is crucial for the understanding of diseases, the development of effective interventions, and the widespread adoption of evidence-based veterinary practices. Statistical methods offer powerful tools for drawing causal inferences from observational and experimental data. However, the effective use of these tools requires a solid understanding of the foundations of causality, the strengths and weaknesses of study designs, and the assumptions and limitations of statistical techniques.

It is essential for veterinary researchers to clearly define the research question and select the most appropriate study design during the planning phase. Randomized controlled trials (RCTs) should be preferred when possible, but in situations where observational studies are necessitated by practical and ethical constraints, special attention must be paid to confounder control. When building multivariate regression models, variable selection should be grounded in biological plausibility and existing literature, and model assumptions must be carefully checked.

Advanced statistical techniques are valuable for analyzing complex data structures and elucidating causal mechanisms, but their correct application and the interpretation of results require statistical expertise.

Transparency and honesty are essential in reporting results. The limitations of studies must be clearly stated, sources of bias should be discussed, and findings should be evaluated within the context of the existing literature. It is rare for a single study to definitively prove causality. Scientific knowledge progresses through the cumulative evidence from multiple studies yielding consistent findings. Therefore, systematic reviews and meta-analyses are becoming increasingly important for evidence synthesis in the field of veterinary health.

In conclusion, determining cause-and-effect relationships in veterinary health research is a complex process that demands both methodological rigor and critical thinking. Statistical methods are indispensable tools in this process but are not sufficient on their own. As stated by Bradford Hill, arriving at a causal judgment requires the holistic assessment of statistical significance, biological plausibility, consistency, and other epidemiological criteria. By adopting this comprehensive approach and employing appropriate statistical methods, veterinary researchers can make significant contributions to animal and public health.

References

1. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. 2nd ed. Charlottetown: VER Inc; 2009.
2. Thrusfield M, Christley R. Veterinary Epidemiology. 4th ed. Oxford: Wiley-Blackwell; 2018.
3. Sargeant JM, O'Connor AM. Issues of reporting in observational studies in veterinary medicine. Preventive Veterinary Medicine. 2014;113(3):323-330.
4. Rothman KJ, Greenland S, Lash TL. Modern Epidemiology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
5. Pearl J. Causality: Models, Reasoning and Inference. 2nd ed. Cambridge: Cambridge University Press; 2009.
6. Hill AB. The environment and disease: Association or causation? Proceedings of the Royal Society of Medicine. 1965;58(5):295-300.
7. Godfroid J, Garin-Bastuji B, Saegerman C, Blasco JM. Brucellosis in terrestrial wildlife. Revue Scientifique et Technique. 2013;32(1):27-42.
8. Glass TA, Goodman SN, Hernán MA, Samet JM. Causal inference in public health. Annual Review of Public Health. 2013;34:61-75.
9. Rothman KJ. Causes. American Journal of Epidemiology. 1976;104(6):587-592.
10. Dohoo IR, Stryhn H, Sanchez J. Evaluation of underlying risk as a source of heterogeneity in meta-analyses: a simulation study of Bayesian and frequentist implementations of three models. Preventive Veterinary Medicine. 2007;80(2-3):200-212.
11. Susser M. What is a cause and how do we know one? A grammar for pragmatic epidemiology. American Journal of Epidemiology. 1991;133(7):635-648.
12. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.
13. Sibbald B, Roland M. Understanding controlled trials: Why are randomised controlled trials important? BMJ. 1998;316(7126):201.
14. Yeates JW, Main DCJ. Assessment of positive welfare: A review. The Veterinary Journal. 2008;175(3):293-300.
15. Mann CJ. Observational research methods. Research design II: Cohort, cross sectional, and case-control studies. Emergency Medicine Journal. 2003;20(1):54-60.
16. Grimes DA, Schulz KF. Cohort studies: Marching towards outcomes. The Lancet. 2002;359(9303):341-345.

17. Schulz KF, Grimes DA. Case-control studies: Research in reverse. The Lancet. 2002;359(9304):431-434.
18. Vandenbroucke JP, Pearce N. Case-control studies: Basic concepts. International Journal of Epidemiology. 2012;41(5):1480-1489.
19. Levin KA. Study design III: Cross-sectional studies. Evidence-Based Dentistry. 2006;7(1):24-25.
20. Dohoo IR, Martin W, Stryhn H. Model-building strategies. In: Veterinary Epidemiologic Research. 2nd ed. Charlottetown: VER Inc; 2009:365-394.
21. Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE. Regression Methods in Biostatistics: Linear, Logistic, Survival, and Repeated Measures Models. New York: Springer; 2004.
22. Hosmer DW, Lemeshow S, Sturdivant RX. Applied Logistic Regression. 3rd ed. Hoboken: John Wiley & Sons; 2013.
23. Gröhn YT, Erb HN, McCulloch CE, Saloniemi HS. Epidemiology of metabolic disorders in dairy cattle: Association among host characteristics, disease, and production. Journal of Dairy Science. 1989;72(7):1876-1885.
24. Cox DR. Regression models and life-tables. Journal of the Royal Statistical Society: Series B. 1972;34(2):187-220.
25. Kleinbaum DG, Klein M. Survival Analysis: A Self-Learning Text. 3rd ed. New York: Springer; 2012.
26. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code for Biology and Medicine. 2008;3:17.
27. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999;10(1):37-48.
28. VanderWeele TJ, Shpitser I. A new criterion for confounder selection. Biometrics. 2011;67(4):1406-1413.
29. Hernán MA, Robins JM. Causal Inference: What If. Boca Raton: Chapman & Hall/CRC; 2020.
30. Pouillot R, Delignette-Muller ML. Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages. International Journal of Food Microbiology. 2010;142(3):330-340.
31. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. Journal of the National Cancer Institute. 1959;22(4):719-748.
32. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. Multivariate Behavioral Research. 2011;46(3):399-424.

33. Groenwold RHH, Sterne JAC, Lawlor DA, Moons KGM, Hoes AW, Tilling K. Sensitivity analysis for the effects of multiple unmeasured confounders. Annals of Epidemiology. 2016;26(9):605-611.
34. MacKinnon DP. Introduction to Statistical Mediation Analysis. New York: Lawrence Erlbaum Associates; 2008.
35. VanderWeele TJ. Explanation in Causal Inference: Methods for Mediation and Interaction. Oxford: Oxford University Press; 2015.
36. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. Journal of Personality and Social Psychology. 1986;51(6):1173-1182.
37. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. International Journal of Epidemiology. 2012;41(2):514-520.
38. Goldstein H. Multilevel Statistical Models. 4th ed. Chichester: John Wiley & Sons; 2010.
39. Stryhn H, Sanchez J, Morley P, Booker C, Dohoo IR. Interpretation of variance parameters in multilevel Poisson regression models. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics. 2006.
40. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986;42(1):121-130.
41. Kline RB. Principles and Practice of Structural Equation Modeling. 4th ed. New York: Guilford Press; 2016.
42. Breiman L. Random forests. Machine Learning. 2001;45(1):5-32.
43. Hernán MA, Robins JM. Instruments for causal inference: An epidemiologist's dream? Epidemiology. 2006;17(4):360-372.
44. Montgomery DC, Peck EA, Vining GG. Introduction to Linear Regression Analysis. 5th ed. Hoboken: John Wiley & Sons; 2012.
45. O'Brien RM. A caution regarding rules of thumb for variance inflation factors. Quality & Quantity. 2007;41(5):673-690.
46. Delgado-Rodríguez M, Llorca J. Bias. Journal of Epidemiology and Community Health. 2004;58(8):635-641.
47. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: Potential and pitfalls. BMJ. 2009;338:b2393.
48. Rothstein HR, Sutton AJ, Borenstein M. Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments. Chichester: John Wiley & Sons; 2006.

Chapter 10

Infection Control in Dentistry: Focus on Impression Disinfection

Safa ÖZDEN[1], Hakan DEMİR[2]

The instruments and materials used during dental procedures pose a significant risk factor for the transmission of pathogenic microorganisms from patient to patient or between patients and healthcare workers. Disinfection is a process applied to eliminate or deactivate most disease-causing microorganisms on inanimate surfaces. Unlike sterilization, this process may not always eliminate bacterial spores (Pankhurst & Coulter, 2017). Proper disinfection practices play a critical role in preventing the spread of pathogens, especially bloodborne viruses like Hepatitis B and C, HIV, *Herpes simplex* virus, and *Mycobacterium tuberculosis* during dental treatments (Fulford & Stankiewicz, 2020).

Disinfection procedures in dentistry apply to situations where sterilization is not possible or necessary. The measurement materials, instruments, and surfaces used in dental practices are at risk of contamination due to direct contact with blood, saliva, and other biological fluids (Connor, 1991; Jennings & Samaranayake, 1991). Therefore, after every patient, the complete application of recommended disinfection protocols ensures protection not only for patients but also for healthcare professionals, such as dentists, auxiliary staff, and laboratory personnel, against infection risks (Pankhurst & Coulter, 2017; Fulford & Stankiewicz, 2020).

Characteristics of an Ideal Disinfectant

An ideal disinfectant should have a broad antimicrobial spectrum and be suitable for practical use. The essential characteristics that disinfectants must possess can be summarized as follows:

- **Broad Spectrum of Action:** It should be effective against many pathogens, including bacteria (including tuberculosis-causing bacteria), viruses, fungi, and ideally spores. While it is difficult for a single

[1] Department of Prosthodontics, Faculty of Dentistry, Afyonkarahisar Health Sciences University. Afyonkarahisar, Türkiye. ORCID ID: https://orcid.org/0000-0001-7485-068X,
[2] Department of Prosthodontics, Faculty of Dentistry, Sivas Cumhuriyet University. Sivas, Türkiye. ORCID ID: https://orcid.org/0000-0002-1769-1667,
Corresponding author, Hakan Demir; E-mail: hdemir@cumhuriyet.edu.tr

disinfectant to be fully effective against all microorganisms, the ideal disinfectant should provide the broadest possible range of activity.

- **Rapid Action:** It should be capable of killing microorganisms within a short period after application. In clinical practice, a disinfectant with a short contact time increases operational efficiency.
- **Unaffected by Organic Matter:** It should retain its effectiveness in environments where organic loads like blood, saliva, and tissue residues are present. An ideal disinfectant should not become inactivated by interaction with soaps or other chemicals.
- **Non-Toxic:** It must not be toxic or irritating to users or patients. It should be safe for the skin, eyes, and respiratory system, and ideally, it should be odorless.
- **Surface and Material Compatibility:** It should not cause corrosion on metal instruments, nor damage surfaces like fabric, plastic, or rubber. Disinfectant residues should not leave stains on surfaces or harm the operating mechanisms of devices.
- **Residual Effect:** It is desirable for the disinfectant to leave a short-term antimicrobial effect (residual activity) on the treated surface. This provides a period of protection against recontamination after the application.
- **Ease of Use:** Instructions should be clear, and the preparation and application should be practical. Ideally, it should come in a concentrated form that dissolves easily in water and can be diluted and applied with ease before use.
- **Cost-Effectiveness and Stability:** It should be affordable and have a long shelf life. After being prepared, it should retain its effectiveness for a certain period and not require special storage conditions.
- **Environmental Friendliness:** Post-use waste should not harm the environment. Preferably, the compound should be biodegradable or not require special waste treatment.

While it is challenging to find a disinfectant that meets all of these characteristics in practice, disinfectants used in clinical settings are selected to closely align with these criteria. In choosing a disinfectant, not only its microbial activity but also its safety in the environment where it is used and its material compatibility should be considered (Mushtaq & Khan, 2018; Hardan et al., 2022; Fulford & Stankiewicz, 2020; Dapello-Zevallos et al., 2021).

Classification of Disinfectants Based on Their Effectiveness

Disinfectants are classified into high, intermediate, and low-level activity categories based on their ability to kill microorganisms and their intended use. High-level disinfectants can inactivate all microorganisms, except for bacterial spores, and may also exhibit sporicidal activity if the appropriate contact time is provided. These disinfectants are generally used for disinfecting heat-sensitive medical instruments (Ghasemi et al., 2019). Intermediate-level disinfectants have tuberculocidal activity and effectively kill most vegetative bacteria and some viruses, but may be ineffective against bacterial spores (Hardan et al., 2022). Low-level disinfectants are primarily effective against enveloped viruses and vegetative bacteria, but they are insufficient against tuberculosis bacilli and spore-forming bacteria; therefore, they should only be used on low-risk surfaces and materials (Ganavadiya et al., 2014; Elhagali et al., 2025). These classifications provide a crucial guide to determining when and how disinfectants should be used.

In dentistry, instruments and materials are classified according to their disinfection requirements as critical, semi-critical, and non-critical. Critical and semi-critical instruments (e.g., surgical tools or equipment that come into direct contact with mucous membranes) should, if possible, be sterilized, and when sterilization is not feasible, at least high-level disinfection should be applied (Chukwu et al., 2024). For surface disinfection, intermediate-level disinfectants, especially those with tuberculocidal effects, are generally preferred (Connor, 1991). The choice of disinfection level depends on the resistance level of the target microorganism; for example, in the presence of highly resistant pathogens like Mycobacterium tuberculosis, at least an intermediate-level (tuberculocidal) disinfectant is required (Pankhurst & Coulter, 2017). This selection plays a critical role in infection control and provides important guidance for determining appropriate disinfection methods.

Classification of Disinfectants Based on Their Chemical Composition

Disinfectants are grouped into different classes based on their chemical composition, and each group exhibits distinct differences in terms of mechanism of action, antimicrobial spectrum, and intended use. The main chemical disinfectant classes and their characteristics can be summarized as follows:

Alcohols: Particularly ethyl alcohol and isopropyl alcohol (at concentrations of 60-90%) are commonly used intermediate-level disinfectants. These disinfectants provide a rapid antimicrobial effect by denaturing cell membranes and proteins. Alcohols are effective for hand antisepsis and small surface disinfection; they can effectively kill bacteria and many viruses within 30 seconds to 1 minute (Ghasemi et al., 2019). However, they are ineffective against spores, and their effectiveness can be significantly reduced in the presence of organic matter. Furthermore, due to their high evaporation rate, it is difficult to maintain sufficient contact time. In dentistry, alcohol-based solutions are often used in spray form for surface cleaning or in combination with other substances; however, they are not preferred alone for direct measurement disinfection (Dapello-Zevallos et al., 2021).

Aldehydes: These are strong high-level disinfectants, with glutaraldehyde being the most well-known example, typically used at a 2% active concentration with an alkaline activator. Glutaraldehyde exerts a strong lethal effect by cross-linking proteins in microorganisms, and with a contact time of 10-30 minutes, it can be effective against bacteria, tuberculosis bacilli, fungi, and viruses (Khatri et al., 2020). Ortho-phthalaldehyde (OPA) is an alternative aldehyde to glutaraldehyde, and 0.55% OPA solutions provide high-level disinfection within 10-12 minutes (Wezgowiec et al., 2022). The major advantage of aldehydes is their ability to effectively disinfect heat-sensitive medical devices. In dentistry, glutaraldehyde solutions are commonly used for disinfecting materials that cannot undergo thermal processing (e.g., some plastic instruments, root canal instruments, impression materials) (Al Shikh & Milosevic, 2020). However, aldehydes can be toxic and irritant, and should be avoided in contact with skin and mucous membranes. Additionally, glutaraldehyde vapors can irritate the respiratory system, and its effectiveness decreases in the presence of organic matter. Therefore, instruments and impressions must be thoroughly rinsed with water after use (Dapello-Zevallos et al., 2021).

Halogen Compounds: Halogen-based compounds are widely used effective agents in disinfection processes. Chlorine compounds, particularly sodium hypochlorite, are among the most frequently used disinfectants. Hypochlorite solutions demonstrate broad-spectrum antimicrobial activity by oxidizing proteins. Chlorine concentrations between 0.1% (1:50) and 0.5% (1:10) can kill most bacteria and viruses, including bloodborne viruses such as HIV and HBV, with 5-10 minutes of contact time (Shervani et al., 2022). Higher concentrations may also be effective against bacterial spores. The main advantages of chlorine compounds are their low cost and easy availability. However, due to their high corrosiveness, they can cause rusting on metals and bleaching of fabrics. Additionally, chlorine can produce odors and tissue irritation. Organic matter (e.g., blood and saliva) can rapidly reduce the effectiveness of chlorine, so surfaces must be cleaned before use (Masih et al., 2025; Altaf et al., 2022; Qiu et al., 2023).

Iodophors: These are disinfectants in which iodine is used in a complex form (e.g., povidone-iodine). Iodophors are intermediate-level disinfectants effective against most bacteria, viruses, and fungi, but they are not sporicidal (Chukwu et al., 2024). Iodophor solutions are commonly recommended in dentistry for both skin antisepsis and impression disinfection.

These characteristics make iodophores a useful option in disinfection applications, but it should be noted that they must be used with caution due to their high corrosiveness and irritation potential.

Phenolic compounds are disinfectants that work by damaging cell walls and proteins. Historically, phenol (carbolic acid) was widely used, but it is not preferred in its pure form today. However, bisphenols are found in some surface disinfectants. Phenolic compounds generally exhibit intermediate or low-level effectiveness (Altaf et al., 2022). In dentistry, synthetic phenolic disinfectants are particularly used for cleaning unit surfaces and floors. The disadvantage of phenolics is that they are generally not sporicidal and their effectiveness may be limited against some viruses and bacteria like *Pseudomonas* (Qiu et al., 2023). Additionally, phenolic residues can accumulate on rubber and plastic surfaces, and their odors can be unpleasant. Although phenolic sprays have been reported for disinfecting dental impressions, it has been noted that phenolic residues can negatively affect the hardening of gypsum molds on the impression surface. Therefore, phenolics are not commonly used as the first choice for dental impression disinfection (Al Mortadi et al., 2019).

Quaternary ammonium compounds (QACs), especially quaternary ammonium compounds such as benzalkonium chloride, work by disrupting cell membrane permeability, exhibiting bactericidal effects (Fulford & Stankiewicz, 2020). QACs are generally low-level disinfectants that are effective against gram-positive bacteria but may have limited activity against some gram-negative bacteria. They are also not sporicidal or tuberculocidal. Therefore, they are typically used for surface cleaning and non-critical equipment disinfection. Today, many ready-made surface disinfectants incorporate a combination of QACs and alcohol to expand the spectrum of action. Alcohol-enhanced quaternary ammonium solutions can exhibit intermediate-level activity, providing inactivation against tuberculosis bacilli and some non-enveloped viruses (Pankhurst & Coulter, 2017). However, pure QAC solutions may not be sufficient for the disinfection of dental impressions on their own, as their effectiveness may be insufficient for complete disinfection.

Oxidizing agents are strong disinfectants that destroy the cell structure of microorganisms through oxidative damage. Hydrogen peroxide and peracetic acid are the most commonly used agents in this group. Hydrogen peroxide provides disinfection from low to high levels, depending on the concentration. For example, a 3% hydrogen peroxide solution kills most bacteria and viruses but is not sporicidal, while a 7.5% solution can provide high-level disinfection and even inactivate spores (Connor, 1991). In dentistry, 1-3% hydrogen peroxide solutions are used for surface disinfection and impression disinfection. The advantage of hydrogen peroxide is that it breaks down into water and oxygen without leaving residues, making it an environmentally friendly option (Qiu et al., 2023). However, prolonged contact with metals can cause mild corrosive effects. Peracetic acid is a very strong oxidant and can show sporicidal effects even at low concentrations such as 0.2%. However, due to its corrosive properties, it is more commonly used in automatic disinfection units (e.g., endoscope washers) (Altaf et al., 2022). While peracetic acid is not commonly used in dentistry, hydrogen peroxide-based sprays or solutions have been considered as alternatives for both surface disinfection and impression disinfection (Fulford & Stankiewicz, 2020).

Biguanides are chemical compounds that play an important role in disinfection and antisepsis. Chlorhexidine, one of the most well-known antiseptics in this group, is generally considered a low-level disinfectant. Chlorhexidine is widely used in products such as mouthwashes, hand sanitizers, and for skin and mucosal antisepsis (Khatri et al., 2020). It shows strong activity against gram-positive bacteria, moderate activity against gram-negative bacteria, and is not effective against bacterial spores (Wezgowiec et al., 2022). It is usually not used alone for surface disinfection; instead, it is added to some cleaning solutions to enhance its effect (Shervani et al., 2022). In dentistry, chlorhexidine-containing solutions should not be used directly for disinfecting dental impressions, as chlorhexidine can leave residues on the impression, which can negatively affect the hardening of gypsum (Guntupalli et al., 2022). Therefore, the use of biguanides as disinfectants is mainly limited to antisepsis, and they show lower effectiveness for disinfection purposes.

Factors Affecting the Effectiveness of Disinfectants

The effectiveness of a disinfectant in laboratory conditions can vary depending on several factors in real-world usage. To increase or maintain the optimal level of disinfection effectiveness, several key factors must be taken into account.

Type of microorganism and resistance level is one of the most critical factors in the disinfection process. Not all microorganisms are equally susceptible to disinfectants. For instance, bacterial spores are the most resistant form, followed by cyst forms of parasites, mycobacteria (e.g., tuberculosis pathogens), non-enveloped viruses (e.g., poliovirus), fungi, vegetative bacteria, and finally, enveloped viruses (such as HIV, herpes virus, etc.) (Al Mortadi et al., 2019). Therefore, the target pathogen, if it is part of a highly resistant group (e.g., *Clostridium* spores), should determine the disinfectant's effectiveness. The disinfection time must also be adjusted according to the microorganism's resistance level; for example, prions are extremely resistant to chemical disinfectants and cannot be inactivated with standard disinfection methods (Jennings & Samaranayake, 1991). As a result, when selecting a disinfectant, the most resistant microorganism likely to be present in the environment should be considered (Pankhurst & Coulter, 2017).

Microbial load (number) and biofilm are other important factors that directly affect disinfection effectiveness. In the presence of a high microbial load, it is more difficult to kill all microorganisms within a specific contact time. Therefore, instruments contaminated with biological materials like blood or saliva must be cleaned under running water before applying the disinfectant (Fulford & Stankiewicz, 2020). Additionally, bacteria within a biofilm are more resistant to disinfectants than free-floating cells. Biofilm is a structure that microorganisms develop to protect themselves, and disinfectants may find it difficult to penetrate this layer. Thus, it is important to prevent biofilm formation and mechanically remove existing biofilm layers (Elhagali et al., 2025).

The presence of organic and inorganic materials can significantly affect the effectiveness of disinfectants. Organic materials, such as blood, saliva, pus, and food residue, can drastically reduce the effectiveness of disinfectants. Organic substances can react with disinfectants to form complexes that deactivate them or physically protect microorganisms from the disinfectant (Ghasemi et al., 2019). Disinfectants such as chlorine compounds and iodine can lose their effectiveness quickly when they come into contact with organic materials; for example, a hypochlorite solution loses its effectiveness when mixed with blood (Dapello-Zevallos et al., 2021). Therefore, surfaces must be properly cleaned (washed, scrubbed) before disinfection. In addition, inorganic salts (e.g., calcium and magnesium, which cause water hardness) can combine with some disinfectants and reduce their effectiveness. This highlights the necessity of using distilled water or selecting water according to the manufacturer's instructions when preparing disinfectant solutions (Khatri et al., 2020).

Concentration of the disinfectant is another critical factor that directly influences disinfection effectiveness. Generally, as the concentration of a disinfectant increases, its ability to kill microorganisms and its speed of action also increase (Wezgowiec et al., 2022). However, not every disinfectant responds the same way to changes in concentration. For example, reducing the concentration of quaternary ammonium compounds by half may result in a doubling of the exposure time, while the same change in phenolic solutions may extend the exposure time by 64 times. Therefore, to ensure the disinfectant's effectiveness, it is important to use the concentration and dilution recommended by the manufacturer. A disinfectant that is overly diluted may not show sufficient effect within the expected time. Similarly, using a concentration higher than recommended can increase the risks of toxicity and corrosion (Al Shikh & Milosevic, 2020).

Contact time is a decisive factor for the effectiveness of disinfectants. In order for a disinfectant to be effective, it must remain in contact with the microorganism for a sufficient amount of time. The minimum required contact time differs for each disinfectant and target organism. For instance, alcohol-based disinfectants should keep the surface wet for at least 1 minute, while high-level disinfection with glutaraldehyde typically requires a soaking time of 10 to 30 minutes (Shervani et al., 2022). When applying disinfectant solutions, the surface should be kept wet for the duration specified in the product instructions. In spray applications, the surface should be left undried for the required time, and in immersion methods, the material should be removed once the time is complete. If sufficient contact time is not provided, resistant microorganisms may survive, and the disinfection process may fail (Fulford & Stankiewicz, 2020).

Temperature is generally an important factor that increases the microorganism-killing speed of chemical disinfectants. Increased temperature can accelerate the reaction kinetics, thereby enhancing the effectiveness of disinfectants (Hardan et al., 2022). Hot solutions, in particular, may be more effective for many disinfectants. However, excessive heat, especially with prolonged contact, may cause some disinfectants to degrade. For example, high temperatures can cause volatile disinfectants like alcohol to evaporate quickly, which reduces the effective contact time (Khatri et al., 2020). Therefore, disinfectant solutions are typically used at room temperature. While disinfectants may slow down in cold environments, in very hot conditions, the stability of the solution can be compromised (Wezgowiec et al., 2022).

pH value is another important factor that affects the effectiveness of disinfectant solutions. The pH of the disinfectant can alter its antimicrobial activity. For example, glutaraldehyde is more effective in mildly alkaline environments, which is why many commercial glutaraldehyde solutions are activated with an alkalizer before use (most effective at pH 7.5-8). Similarly, quaternary ammonium compounds increase their effectiveness as the pH rises. In contrast, phenolic disinfectants and hypochlorite solutions are less effective in alkaline environments and are more potent in acidic conditions (Chukwu et al., 2024).

Surface characteristics and application method are other important factors that can affect the effectiveness of a disinfectant. The roughness, porosity, and shape of the surface the disinfectant contacts can influence whether the solution can reach every point of that surface. For instruments with complex shapes or hard-to-reach surfaces (e.g., hinges, channels, crevices), it can be challenging to expose every part to the disinfectant. In such cases, the immersion method may be more effective than spraying because the solution can penetrate every crack and crevice. With spray applications, areas that are shadowed or not directly exposed may remain untreated (Hardan et al., 2022). Therefore, it is recommended that instruments with indentations and protrusions be disassembled and disinfected as separate parts, and the immersion method should be preferred. In dentistry, after blood and saliva are washed off from an impression, it is common practice to cover it with a disinfectant spray and leave it in a nylon bag for a period. This ensures that the spray droplets reach every part of the impression and can act without evaporating (Khatri et al., 2020). Finally, it should be noted that the disinfected material or surface should be rinsed with sterile water or saline after the recommended time has passed (Wezgowiec et al., 2022).

Disinfection Requirements of Dental Impression Materials

Taking dental impressions is a routine practice for prosthetic restorations and orthodontic appliances. However, each impression material removed from the patient's mouth becomes heavily contaminated with saliva, blood, and oral microflora. Studies have shown the presence of serious pathogens on dental impressions, such as *Streptococcus*, *Staphylococcus*, *E. coli*, as well as HBV, HCV, HIV, and tuberculosis pathogens (Soganci et al., 2018).

When these contaminated impressions are sent to the laboratory, they can infect laboratory personnel and the environment during the production and processing of plaster models. Furthermore, microorganisms may continue to live on the surface of the plaster model obtained from a contaminated impression and could spread to other patient materials during subsequent processes. In fact, it has been experimentally shown that some microorganisms can survive on plaster models for up to 7 days (Khatri et al., 2020).

Impression disinfection is extremely important not only for the patient and the dentist but also for the safety of laboratory workers. Surveys have indicated that many dental technicians feel at risk when impressions arrive at the laboratory without disinfection (Shervani et al., 2022). In this context, continuous education and supervision regarding the necessity of impression disinfection play a major role in ensuring the safety of clinical and laboratory practices.

Contamination Risk in Dental Impression Materials

A contaminated impression material poses a high risk of cross-contamination, as it carries patient-derived pathogens and, if not properly disinfected, can transfer these pathogens to others (Fulford & Stankiewicz, 2020). The risk of contamination can vary depending on the structure of the impression material, the oral hygiene condition, and the area being imprinted; however, the risk is present in all cases. For example, an impression taken from a patient with periodontal disease and bleeding is likely to contain bloodborne pathogens. Hepatitis B virus (HBV), for instance, can remain infectious in dried blood at room temperature for at least 7 days (Pankhurst & Coulter, 2017).

Another factor that increases the risk of contamination is the time elapsed after the impression has been taken. Since the impression is initially taken from a moist and warm environment (the patient's mouth), the microorganisms on it can proliferate rapidly. While some microbes may die as the impression dries, many can remain viable within the partially preserved biomaterial. Additionally, mold and fungal spores can colonize the surface of the impression as they are deposited from the environment (Ganavadiya et al., 2014). Therefore, impressions are typically stored in a plastic bag in a moist environment until they are transported to the laboratory; however, if disinfection is not performed, this may allow microbes to survive (Elhagali et al., 2025).

While the infections that could theoretically spread from impressions to the laboratory are diverse, the most concerning ones are bloodborne pathogens. As a result, the contamination risk of dental impressions is significant and should not be overlooked. Specifically, alginate impressions, due to their porous structure and high water content, can harbor microorganisms within them. Silicone impressions, while hydrophobic on the surface and able to retain some bacteria, can generally be cleaned off with simple rinsing; however, blood and saliva proteins can adhere to the silicone surface, allowing microorganisms to persist. Regardless of the material, all impressions should be considered contaminated and subjected to a standardized disinfection procedure (Chukwu et al., 2024). If proper disinfection is not performed, it poses a health risk to laboratory personnel and can leave microorganisms on products like prostheses or orthodontic appliances. This, in turn, carries the potential to transmit infections to the patient using the product.

In summary, the contamination risk of dental impression materials can only be controlled through a strict disinfection protocol. Both clinic and laboratory staff must be aware of this and apply a standardized procedure to each impression received (Fulford & Stankiewicz, 2020).

Disinfection Methods for Dental Impression Materials

There are two main approaches for disinfecting dental impressions: immersion and spray methods. Additionally, alternative physical disinfection methods such as ozonization, UV radiation, and microwaving are being researched in recent years. The choice of method depends on the type of impression material and the properties of the disinfectant. Below, these methods and their application principles are discussed.

Cleaning the Impression (Washing): Before disinfecting, the impression should be thoroughly rinsed under running water, and if possible, any blood, saliva, and plaque should be removed using a soft brush or gauze. This step reduces organic contamination on the impression surface, increasing the effectiveness of the disinfectant and improving the surface quality of the cast model (Al Shikh & Milosevic, 2020). During cleaning, hot water should not be used, as it can cause deformation of certain impression materials, especially alginate. Therefore, lukewarm or cold water should be preferred. Furthermore, drying with pressurized air should be avoided, as this can create aerosols, leading to the spread of microorganisms (Shervani et al., 2022). After washing, excess water should be removed from the impression, and disinfection should proceed immediately without allowing the impression to dry completely. These cleaning and disinfection steps reduce the microbial load on the impressions, ensuring they safely reach the laboratory and minimize the risk of cross-contamination (Wezgowiec et al., 2022).

Immersion Method: This method involves fully immersing the impression in an appropriate disinfectant solution for a specific period. The main advantage is that it ensures complete contact between the disinfectant and all surfaces of the impression. For immersion, a plastic container with a lid is typically used, containing an appropriate amount and depth of disinfectant solution that will not damage the impression. The impression should be fully submerged without floating. The disinfectant used should be chosen according to the impression material (Khatri et al., 2020).

The immersion time is generally around 10 minutes, but this may vary depending on the manufacturer's instructions and the properties of the disinfectant used. For example, the Australian Dental Association recommends a maximum immersion time of 15 minutes (Dapello-Zevallos et al., 2021). Exceeding this time can cause distortion, especially in materials with low dimensional stability (such as alginate or polyether), while keeping the time too short may result in insufficient disinfection. Therefore, manufacturer guidelines should be followed when determining the duration (Ghasemi et al., 2019).

After immersion, the impression should be removed from the solution, rinsed thoroughly under running water, and excess water should be drained. It is then recommended to proceed with the casting process as soon as possible. When applied correctly, the immersion method is considered the most reliable disinfection method for impressions. This method is free from the potential contact shortcomings that may occur with the spray method. However, it is important to ensure that the disinfectant used does not damage the impression material, and the recommended immersion time should not be exceeded (Masih et al., 2025).

Spray Method: In this method, the impression is sprayed with a broad-spectrum disinfectant solution to ensure complete coverage of all surfaces. The impression is then placed in a closed bag or container and left to sit for the recommended contact time before the disinfectant evaporates. The biggest advantage of the spray method is that it better preserves the dimensional stability of materials like alginate and polyether, as this method prevents excessive absorption of liquid. Additionally, less disinfectant solution is used, which provides economic and practical advantages (Masih et al., 2025).

In practice, after the impression is thoroughly wetted with disinfectant, it is usually placed in a transparent plastic bag with a zipper and left for about 10 minutes. After this time, the impression is removed from the bag, rinsed under running water, and excess water is drained. It is crucial to ensure that all surfaces of the impression are covered with disinfectant during spraying. Furthermore, it is recommended that the disinfectant have tuberculocidal properties, meaning it should be moderately effective, as resistant pathogens like the tuberculosis agent need to be inactivated (Chukwu et al., 2024).

When applied correctly, the spray method provides an effective option for disinfecting impressions. However, the success of this method is directly related to ensuring full contact between the disinfectant and every surface of the impression and guaranteeing the appropriate contact time.

Disinfection of Silicone-Based Impression Materials

Silicone impression materials belong to the elastomeric group of impression materials and are typically found in two main types: condensation silicones and addition (or addition-curing) silicones, particularly polyvinylsiloxane (PVS). Today, addition silicones are the most commonly used type due to their high dimensional stability. Silicone-based impressions are highly resistant to disinfection processes due to their hydrophobic structure and chemical resistance. When the appropriate disinfection protocol is followed, clinically significant degradation or dimensional errors do not occur in silicone impressions.

Therefore, the immersion (dipping) method can be safely preferred for disinfecting silicone impressions (Mushtaq & Khan, 2018; Elhagali et al., 2025).

Recommended Disinfectants: Silicone impressions are compatible with various disinfectants. Commonly used disinfectants include 0.5% sodium hypochlorite (diluted 1:10), iodine solutions (iodophor), glutaraldehyde (2%, for 10 minutes), or phenol/alcohol-based sprays. Silicone manufacturers typically specify the type of disinfectant and the maximum contact time suitable for their products. For example, many PVS impression manufacturers consider 10-minute immersion in 0.5% hypochlorite or 2% glutaraldehyde to be safe. Iodophor solutions (1% active iodine) are popular for silicone impressions, and typically a 5-10 minute immersion time is recommended. Alcohol-based sprays may not effectively cover every surface due to the hydrophobic nature of silicone; therefore, if the spray method is used, it is essential to ensure that all areas of the impression are in contact with the disinfectant (Elhagali et al., 2025; Ganavadiya et al., 2014).

Dimensional Stability: Addition silicones are among the most dimensionally stable impression materials. Once mixed in the correct proportions and the polymerization process is complete, they are minimally affected by changes in humidity or slight chemical interactions. Studies have shown that PVS impressions do not undergo significant dimensional changes when immersed in various solutions for up to 30 minutes (Mushtaq & Khan, 2018; Elhagali et al., 2025; Ganavadiya et al., 2014). This feature allows silicone impressions to maintain dimensional stability after disinfection, enhancing the reliability of clinical outcomes.

Condensation silicones, compared to addition silicones, tend to show slightly more dimensional change. However, their resistance to disinfection processes is similar. Condensation silicones can also be immersed in hypochlorite, iodophor, or glutaraldehyde solutions for up to 5-10 minutes. However, since condensation silicones are more prone to rapid setting (ideally within 30 minutes to 1 hour), it is advisable to minimize the waiting time after disinfection (Mushtaq & Khan, 2018; Elhagali et al., 2025).

Spray and Immersion Methods for Silicone Impressions: Silicone impressions, such as PVS, may have issues with solution beading and runoff when sprayed due to their hydrophobic nature. This could prevent the disinfectant from fully contacting the surface. For this reason, the immersion method is considered a safer and more guaranteed option to ensure that all surfaces are wetted with the disinfectant. However, because silicone impressions have high dimensional stability, the spray method can also be a suitable alternative, provided that every surface is thoroughly in contact with the disinfectant. If the spray method is chosen, the double-bag technique (wet, seal, wait) can be used to improve effectiveness. Some studies have also investigated autoclaving silicone impressions; addition silicone impressions autoclaved at 121°C for 15 minutes have been shown to remain within acceptable dimensional limits. However, autoclaving may not provide the same results for all materials, and it should not be applied unless approved by the manufacturer (Connor, 1991; Guntupalli et al., 2022; Trivedi et al., 2019; Chidambaram et al., 2022).

Silicone-based impression materials are durable and easy to disinfect. The recommended protocol is to immediately rinse the impression with running water after taking the impression, then immerse it in a 0.5% sodium hypochlorite solution for 10 minutes. After this period, the impression should be thoroughly rinsed and excess water should be removed. It is advisable to prepare the plaster model as soon as possible. This method eliminates patient-derived pathogens while preserving the accuracy of the impression. Studies have shown that this procedure does not lead to dimensional changes or loss of surface detail in silicone impressions. In other words, a properly disinfected silicone impression has the potential to produce a model of the same quality as an untreated impression, but with much greater safety (Connor, 1991; Guntupalli et al., 2022; Trivedi et al., 2019; Chidambaram et al., 2022; Al Mortadi et al., 2019).

Disinfection of Irreversible Hydrocolloid (Alginate) Impression Materials: General Principles and Application Methods

Alginate impressions are commonly used materials in dentistry, and their disinfection procedures require special attention due to their water content and porous gel structure. Alginate is sensitive to environmental moisture and liquid contact; it shrinks (syneresis) when losing water and swells (imbibition) when absorbing water. These properties make it essential to select the appropriate method not only to maintain dimensional stability during disinfection but also to ensure effective microorganism killing (Trivedi et al., 2019).

Disinfection Methods and Agents: Short immersion (dipping) or spray disinfection methods are generally recommended for disinfecting alginate impressions. The classical literature suggests that alginate should not be left in disinfectant solutions for more than 10 minutes. The American Dental Association (ADA) recommends the use of 0.5% sodium hypochlorite (diluted 1:10), iodine solutions, and synthetic phenol-based sprays for alginate impressions. According to the ADA protocol, after the impression is removed from the mouth, it should be rinsed first, then completely soaked in a solution such as 0.5% hypochlorite, wrapped in a paper towel moistened with the disinfectant, and placed in a plastic bag for 10 minutes. After this period, the impression should be rinsed and excess water should be drained. This method is a practical disinfection protocol commonly applied in clinical settings (Chidambaram et al., 2022).

Comparison of Immersion and Spray Disinfection Methods: Due to the sensitivity of alginate impressions to water, the spray method is often considered a more preferred option. The spray method prevents excessive water absorption by alginate and ensures that only the surface is disinfected. This minimizes the dimensional changes in the alginate. Additionally, alginate impressions are often taken with metal impression trays, and immersing them in corrosive solutions like hypochlorite can lead to rusting of the tray. The spray method minimizes such effects. However, a careful and short immersion method can also be applied without damaging the alginate. Immersing the alginate impression in an appropriate disinfectant solution for about 3-5 minutes will be sufficient for disinfection. The critical factor here is to control the time and ensure the correct concentration. If the impression is left in hypochlorite solution for too long, it can lead to imbibition, resulting in the loss of surface detail. On the other hand, if the duration is too short, the microorganisms may not be effectively killed (Aslanimehr et al., 2021; Qiu et al., 2023).

The proper selection of the method is crucial for maintaining the dimensional stability of alginate impressions while ensuring the effective elimination of microorganisms. Short immersion and spray methods, when applied according to the correct protocol, provide effective disinfection. However, in both methods, time and concentration need to be carefully controlled. This ensures that the disinfection needs of the alginate are met while preserving its dimensional stability, resulting in precise impressions.

Alginate impressions are widely used in dentistry, but due to their water content and porous structure, they require special attention during disinfection. The sensitivity of alginate to water makes it challenging to strike a balance between killing microorganisms and maintaining dimensional stability during the disinfection process. Therefore, selecting the appropriate disinfectant and applying the correct protocol is essential.

Disinfectant Selection: Commonly recommended disinfectants for alginate impressions are chlorine and iodine-based solutions. Specifically, immersion in a 1% iodophor solution for 5-10 minutes is considered safe and effective for alginate. Although glutaraldehyde can also be used for disinfecting alginate, this disinfectant generally requires more than 10 minutes to be effective. However, since alginate carries the risk of dimensional changes and deformation if left for more than 10 minutes, glutaraldehyde is not the first choice for alginate. Short-term use of glutaraldehyde may result in incomplete disinfection. Phenolic sprays can be used on alginates, but due to concerns that phenol may affect the setting of gypsum, this method is generally not recommended. Additionally, disinfectants based on biguanides, such as chlorhexidine, may be ineffective in alginate disinfection, especially because they show limited efficacy against viruses, and are therefore not typically preferred (Aslanimehr et al., 2021). Hence, the most practical and effective agent remains the inexpensive and widely used diluted sodium hypochlorite (0.5-1.0% active chlorine) (Chidambaram et al., 2022).

Step-by-Step Sample Protocol (Using Sodium Hypochlorite):

1. After the impression is removed from the mouth, it should be thoroughly cleaned under running water on both surfaces.
2. A 1:10 diluted sodium hypochlorite solution is sprayed generously on all areas of the impression.
3. The impression is then wrapped in a clean paper towel moistened with the chlorine solution and placed in a large resealable plastic bag.
4. The bag is left at room temperature for 10 minutes.
5. At the end of the duration, the bag is opened, the impression is removed from the towel, and it is gently rinsed under tap water.
6. Excess water is drained off, and the impression should be immediately cast if possible. If immediate casting is not feasible, the impression can be wrapped in a damp tissue and stored.

Dimensional Changes and Surface Detail: Alginate impressions are among the most sensitive materials to disinfection processes. Even prolonged exposure to air can cause shrinkage, so the total disinfection time must be controlled. Excessive water loss should be avoided both before and after applying the disinfectant. Some studies have suggested that a short "dip and remove" technique, rather than fully immersing the alginate impression in disinfectant solution, helps absorb less water and achieves better disinfection (Qiu et al., 2023). However, the generally accepted view is that an immersion time of no more than 10 minutes is safe.

After disinfection of the alginate impression, it is recommended to cast the model within 30 minutes at the latest, as prolonged waiting time (even if disinfected) leads to shrinkage of the impression (Trivedi et al., 2019).

Polyether Impression Materials Disinfection

Polyether impression materials are elastomeric materials commonly used in dentistry for applications that require high precision. Polyethers have high dimensional stability, allowing for durable and highly accurate impressions. However, the disinfection of polyether impressions is an important consideration in preserving the physical properties of the material. Selecting the appropriate disinfection methods ensures effective elimination of microorganisms while also maintaining the material's physical integrity (Aslanimehr et al., 2021).

Disinfection of Polyether Impression Materials

Like other elastomeric materials, polyether impression materials require specific care during the disinfection process. Polyethers are more hydrophilic (water-attracting) compared to silicones, a characteristic that can increase the risk of water absorption and dimensional changes. Therefore, the disinfectant solutions and the duration of the process must be carefully chosen. Otherwise, the disinfection process may adversely affect the physical properties of the polyether impressions (Altaf et al., 2022).

Common disinfectants used for polyether impressions include sodium hypochlorite, iodophor solutions, and glutaraldehyde. However, each disinfectant has different effects on polyethers, and these effects are closely related to the disinfection time and solution concentration (Jennings & Samaranayake, 1991). Sodium hypochlorite solutions, typically at a concentration of 0.5-1.0%, are commonly used for polyether impressions. However, prolonged contact with sodium hypochlorite can lead to dimensional changes on the surface of polyether impressions. Therefore, the disinfection time should not be too long. Research indicates that a disinfection time of 5-10 minutes is optimal (Ganavadiya et al., 2014).

1% active iodophor solutions are considered highly effective disinfectants for polyether impressions. Iodophors are generally safer for polyethers and cause less damage to their dimensional stability. Immersing polyether impressions in iodophor solutions for 5-10 minutes is typically sufficient (Aslanimehr et al., 2021).

Glutaraldehyde is another potential disinfectant for polyether impressions. However, glutaraldehyde often requires prolonged contact time (typically 10-20 minutes), which can negatively affect the dimensional stability of polyethers. Therefore, it is recommended to use glutaraldehyde for shorter periods, such as 5-10 minutes. Prolonged exposure to glutaraldehyde can lead to undesirable changes in the dimensional properties of polyether impressions. Thus, when disinfecting polyether impressions, it is crucial to choose a disinfectant that is both effective and preserves the physical properties of the material, ensuring that it is applied for the appropriate duration (Jennings & Samaranayake, 1991; Altaf et al., 2022).

Conclusion and Recommendations

Disinfection in dentistry is one of the cornerstones of infection control. Implementing effective disinfection procedures both in clinical settings and with materials shared with the laboratory (e.g., impressions, temporary prostheses) greatly reduces the risk of cross-contamination between patients and between patients and staff. An ideal disinfectant should have a broad antimicrobial spectrum, fast and lasting action, safety of use, and compatibility with materials. In practice, the choice of disinfectant is based on the intended purpose and the resistance level of the target microorganism. The concepts of high, medium, and low-level disinfectants help determine which strength is necessary for each situation.

The disinfection of dental impression materials holds particular significance because these materials often come into direct contact with patient blood and saliva before being sent to the laboratory. If pathogens are not properly controlled, the transmission through impressions can pose a health risk to laboratory technicians and lead to contamination of prostheses. As discussed in this section, silicone-based elastomer impressions are resistant to disinfection and can be safely disinfected with various chemical agents. Hydocolloid impressions, such as alginate, are more delicate and require shorter and more careful disinfection to maintain their dimensional stability. Regardless of the material, the risks associated with not disinfecting are much greater than the minor disadvantages posed by controlled disinfection procedures.

In dental practice, the principle of treating every patient as if they are carrying an infectious disease (universal precautions) applies within the framework of standard precautions. In this context, dental impressions and all instruments should either be sterilized or properly disinfected after each patient use. It is recommended that clinics have written protocols for impression disinfection and ensure that staff members adhere to them. Clinics should communicate with laboratories to establish agreements ensuring that impressions not properly disinfected are not processed.

While new disinfection methods, such as lasers, plasma, and ozone, are being researched with the advancement of technology, the most practical and proven method today remains chemical disinfection. Using a disinfectant solution prepared with the correct concentration and within its shelf life, and adhering to the recommended contact time, is key to effective disinfection. Additionally, disinfectant solutions should be replaced at regular intervals (e.g., prepared fresh weekly) and tested if necessary, as the concentration of working solutions (especially hypochlorite) decreases over time.

In conclusion, disinfection in dentistry can be successfully carried out by knowing the ideal properties of disinfectants, making the appropriate classification and selection, and paying attention to the critical factors in application. The disinfection of dental impressions is a significant example of how these general principles are applied in practice. When performed correctly, disinfection eliminates microorganisms without compromising the quality of the impression, ensuring a safe working environment for both clinicians and the laboratory. It is essential for the entire dental team to be knowledgeable and conscientious about this issue to ensure patient safety and infection control.

Referances

Ghasemi, E., Fathi, A. H., & Parvizinia, S. (2019). Effect of three disinfectants on dimensional changes of different impression materials. Journal of Iranian Dental Association, 31(3), 169-76.

Dapello-Zevallos, G. M., Miguel-Ramírez, S., Mishell, K. N., Febre-Cuibin, K. S., Gutiérrez-Obando, D. A., & Tinedo-López, P. L. (2021). Disinfection of dental impression materials and its effects on dimensional changes: a literature review. Revista odontológica mexicana, 25(2), 154-159.

Soganci, G., Cinar, D., Caglar, A., & Yagiz, A. (2018). 3D evaluation of the effect of disinfectants on dimensional accuracy and stability of two elastomeric impression materials. Dental materials journal, 37(4), 675-684.

Khatri, M., Mantri, S. S., Deogade, S. C., Bhasin, A., Mantri, S., Khatri, N., ... & Chauhan, D. (2020). Effect of chemical disinfection on surface detail reproduction and dimensional stability of a new vinyl polyether silicone elastomeric impression material. Contemporary clinical dentistry, 11(1), 10-14.

Wezgowiec, J., Paradowska-Stolarz, A., Malysa, A., Orzeszek, S., Seweryn, P., & Wieckiewicz, M. (2022). Effects of various disinfection methods on the material properties of silicone dental impressions of different types and viscosities. International journal of molecular sciences, 23(18), 10859.

Al Shikh, A., & Milosevic, A. (2020). Effectiveness of alcohol and aldehyde spray disinfectants on dental impressions. Clinical, Cosmetic and Investigational Dentistry, 25-30.

Shervani, A. R., Saxena, D., Aggarwal, S., & Bhatnagar, P. (2022). Evaluation and comparison of the effect of autoclave and chemical sterilization on the dimensional accuracy and stability of two elastomeric impression materials-an in vitro study. Int. J. Commun. Med. Public Health, 9, 3152.

Masih, A., Gandagule, R. R., Tiwari, A., Elangovan, E., Choukse, V., Saini, N., ... & Gandagule, R. (2025). Evaluating the Influence of Spray and Immersion Disinfection on the Dimensional Accuracy of Impression Materials: A Comprehensive Review. Cureus, 17(7).

Mushtaq, M. A., & Khan, M. W. U. (2018). An Overview of Dental Impression Disinfection Techniques-A Literature Review. Journal of the Pakistan Dental Association, 27(4).

Hardan, L., Bourgi, R., Cuevas-Suárez, C. E., Lukomska-Szymanska, M., Cornejo-Ríos, E., Tosco, V., ... & Haikel, Y. (2022). Disinfection procedures and their effect on the microorganism colonization of dental impression materials: a systematic review and meta-analysis of in vitro studies. Bioengineering, 9(3), 123.

Chukwu, S., Munn, A., Wilson, J. C., Ibrahim, H., Gosling, D., Love, R. M., & Bakr, M. M. (2024). Efficacy of an impression disinfectant solution after repeated use: an in vitro study. Heliyon, 10(1).

Elhagali, A. F., Sharaf, M. Y., El-Aziz, M. E. S. A. A., Ali Bayiumy, A. S., Refaei, M. A. A., Al-Agamy, A. H., ... & Aboshama, M. (2025). The effects of different chemical disinfectants on the strength, surface, and color properties of conventional and 3D-printed fabricated denture base materials. Prosthesis, 7(2), 24.

Ganavadiya, R., Shekar, B. C., Saxena, V., Tomar, P., Gupta, R., & Khandelwal, G. (2014). Disinfecting efficacy of three chemical disinfectants on contaminated diagnostic instruments: A randomized trial. Journal of basic and clinical pharmacy, 5(4), 98.

Connor, C. (1991). Cross-contamination control in prosthodontic practice. International Journal of Prosthodontics, 4(4).

Pankhurst, C. L., & Coulter, W. A. (2017). Basic guide to infection prevention and control in dentistry. John Wiley & Sons.

Fulford, M. R., & Stankiewicz, N. R. (2020). Infection control in primary dental care (No. 180386). Springer.

Jennings, K. J., & Samaranayake, L. P. (1991). The persistence of microorganisms on impression materials following disinfection. International Journal of Prosthodontics, 4(4).

Altaf, J., Malik, M. H. A., Chaudry, S., Mushtaq, M. A., Munir, M. U., & Shah, A. A. (2022). The effect of sodium hypochlorite disinfectant on the linear dimensional stability of alginate impression material. The Professional Medical Journal, 29(09), 1310-1314.

Qiu, Y., Xu, J., Xu, Y., Shi, Z., Wang, Y., Zhang, L., & Fu, B. (2023). Disinfection efficacy of sodium hypochlorite and glutaraldehyde and their effects on the dimensional stability and surface properties of dental impressions: a systematic review. PeerJ, 11, e14868.

Aslanimehr, M., Gholami, F., Torbati, S., & Aalaei, S. (2021). Effect of different Disinfecting Agents on Dental Impressions contaminated with Candida albicans. Dental Hypotheses, 12(3), 139-143.

Guntupalli, Y., Ganesh, S. B., & Jayalakshmi, S. (2022). Effect of disinfectants on the tear strength of addition silicone impression material–An in vitro study. Journal of Advanced Pharmaceutical Technology & Research, 13(Suppl 2), S458-S461.

Trivedi, R., Sangur, R., Bathala, L. R., Srivastava, S., Madhav, S., & Chaubey, P. (2019). Evaluation of efficacy of aloe vera as a disinfectant by immersion and spray methods on irreversible hydrocolloid impression

material and its effect on the dimensional stability of resultant gypsum cast-an in vitro study. Journal of Medicine and Life, 12(4), 395.
Chidambaram, S. R., George, A. M., Muralidharan, N. P., Arvind, T. P., Subramanian, A., & Rahaman, F. (2022). Current overview for chemical disinfection of dental impressions and models based on its criteria of usage: A microbiological study. Indian Journal of Dental Research, 33(1), 30-36.
Al Mortadi, N., Al-Khatib, A., Alzoubi, K. H., & Khabour, O. F. (2019). Disinfection of dental impressions: knowledge and practice among dental technicians. Clinical, cosmetic and investigational dentistry, 103-108.
Chidambaranathan, A. S., Rajendiran, S., & Kumar, B. M. (2025). Disinfection of Dental Impression: Knowledge, Attitude, and Practice Among Dental Specialists, Post-Graduates, and Dental Technicians. Annals of Dental Specialty, 13(2-2025), 40-44.

Chapter 11

Important Points on Antiepileptic Drugs

Hasan YAŞAR[1]

Introduction

Epilepsy is a disease characterized by recurrent and spontaneous seizures that occur as a result of increased synchronized electrical discharges in neurons in the brain. Epilepsy can be diagnosed when at least one seizure occurs spontaneously and has a recurrence risk of more than 60%. It is most common before the age of 1 and after the age of 50. History, neurological examination, imaging methods such as EEG and MRI are used for diagnosis. Differential diagnosis should be made of syncope, migraine, and psychogenic seizures that may be confused with seizures. Epileptic seizures are classified as focal or generalized seizures. Focal seizures begin in a specific area of the brain, and consciousness may be preserved or impaired. Generalized seizures affect both hemispheres of the brain simultaneously and have subtypes such as absence, tonic-clonic, and myoclonic. Up to 70% seizure control can be achieved with medications. Medications are classified as broad- and narrow-spectrum, each acting on different types of seizures. When starting medications, factors such as drug interactions, side effects and teratogenic properties should be taken into consideration(1). If seizures persist despite two appropriate antiepileptic treatments, the patient is considered drug resistant. In other words, the continuation of seizures for 12 months can also be considered as drug resistance(2). Estrogen can increase seizures, while progesterone can decrease them. The menstrual cycle may have an impact on epilepsy(3).

Antiepileptics and important points

Clonazepam

The benefits of clonazepam in cortical and subcortical myoclonus are well known. It has been shown to provide significant benefit in post-hypoxic myoclonus and post-encephalitic myoclonus(4).

[1] Assoc. Prof. Dr. Trabzon Kanuni Training and Research Hospital

Topiramate

Topiramate is excreted predominantly by the kidneys without being metabolized. In severe liver disease, dose reductions of up to 30% may be necessary. It may cause hyperammonemia when used in combination with valproic acid, but hepatotoxicity is very rare when used as monotherapy(5). In the treatment of epilepsy, it is recommended at doses of 200–400 mg/day. Side effects may include paresthesia, weight loss, dizziness, fatigue, and impairments in memory, attention, and language skills. Glaucoma, metabolic acidosis, kidney stones, and teratogenic effects are seen as serious side effects(6-8). The effectiveness of topiramate is reduced when administered with caffeine(9).

Lacosamide

It is a functional amino acid. It is available in both oral and intravenous forms. Side effects such as dizziness, headache, nausea, diplopia, hyponatremia may occur; cardiac side effects (PR interval prolongation) may be dose-related but are usually not clinically significant. It is considered an effective option in the treatment of focal-onset seizures(10, 11). Almost half of the drug is excreted unchanged in the urine, while some is metabolized in the liver(5). It has low interaction with other drugs. The starting dose is usually 50-100 mg per day, increased in weekly increments up to 200-400 mg per day. It is effective both as monotherapy and as adjunctive therapy. It should not be used in patients with severe hepatic impairment. It is an important option for patients with refractory epilepsy(12). The maximum dose is 600 mg/day(13).

Zonisamide

It can cause weight loss, cognitive slowdown, metabolic acidosis, kidney stones, and hyperammonemia(8, 14). As serious side effects, it may cause Stevens-Johnson syndrome, oligohidrosis, and teratogenicity(15). It is effective in many types of seizures, including focal, generalized tonic-clonic, absence, myoclonic, and atonic seizures. Due to its long half-life (50–60 hours), it can be used as a once-daily dose. It is used as an adjunctive treatment in resistant epilepsies. The effective dose range is 300–500 mg/day(16).

Vigabatrin

It causes irreversible visual field loss.

Phenobarbital

There is a risk of sedation, cognitive slowing, depression and long-term addiction(8).

Ethosuximide

It does not cause liver toxicity. Caution is required when used with valproic acid. It is a first-line antiepileptic drug. It is used only for absence seizures and is the first choice. It may cause gastrointestinal side effects, insomnia and psychotic attacks(5, 17).

Felbamate

This drug was widely used in the post-marketing period until reports of aplastic anemia and liver failure emerged. These side effects restricted its use.

Phenytoin

Phenytoin is metabolized in the liver by cytochrome P450. Blood levels must be monitored frequently to avoid toxicity(5). It has an antiarrhythmic effect on the heart. Common side effects include dizziness, unsteadiness, fatigue, nausea, and gum enlargement. Cerebellar atrophy may occur with chronic use. Overdose may cause neurological symptoms such as coma, ataxia, and nystagmus. Serious side effects include systemic allergic reactions, liver damage, bone marrow suppression, and Stevens-Johnson syndrome. Hemodialysis does not remove phenytoin from the body because it is 90% bound to albumin. There is no specific antidote to phenytoin toxicity(18) It is primarily used in the treatment of tonic-clonic and partial seizures. If warfarin and oral contraceptives are used together with phenytoin, their effectiveness decreases(8).

Carbamazepine

Carbamazepine may exacerbate myoclonic seizures. Non-epileptic multifocal myoclonus has also been reported in patients with epilepsy(4). The risk of Stevens-Johnson syndrome (especially in those who are HLA-B*1502 positive) may occur(17). Dizziness, diplopia, nausea, rarely, agranulocytosis, aplastic anemia, and hyponatremia may occur. Concomitant use of carbamazepine and valproic acide accelerates the metabolism of carbamazepine and may cause toxicity by increasing active metabolites in the blood(8, 19). Macrolide antibiotics may increase its level, leading to toxicity(20).

Oxbarbazepine

It can cause hyponatremia, dizziness, fatigue and has fewer hematological side effects than Carbamazepine(8). Oxcarbazepine is effective against focal seizures. It may exacerbate absence and myoclonic seizures and should be avoided in patients with generalized epilepsy. The highest dose used in clinical trials was 1200 mg twice daily. When the carbamazepine dose is 800 mg or less, conversion from carbamazepine can be achieved by using 300 mg of oxcarbazepine for every 200 mg of carbamazepine(21).

Valproic acid

It has been shown to provide significant benefit in post-hypoxic myoclonus and postencephalitic myoclonus(4). It is one of the first choices in generalized seizures and myoclonic seizures(17). It has side effects such as weight gain, tremor, hepatotoxicity, pancreatitis, polycystic ovary syndrome, high teratogenicity and the risk of cognitive impairment in babies of mothers who use it during pregnancy.

Levetiracetam

It may cause neuropsychiatric side effects such as irritability, depression, and aggression. It is generally well tolerated(8). It is the most effective antiepileptic against myoclonic seizures (22). It has been shown to provide significant benefit in post-hypoxic myoclonus and postencephalitic myoclonus(4).

Lamotrigine

It is the first choice for focal seizures. It carries a risk of Stevens-Johnson syndrome, especially in those who are HLA-B*1502 positive, when the dose is increased rapidly and when used with valproic acid. It is generally well tolerated. Drug levels may decrease with birth control pills, and dose adjustment may be necessary(17).

Epilepsy treatment in pregnant women

Most women with epilepsy have normal pregnancies and childbirth, but special monitoring is necessary. Monotherapy is preferred. Valproic acid should not be used during pregnancy due to its teratogenic effects. Seizure frequency generally remains unchanged during pregnancy, but drug levels may decrease, so regular monitoring of drug blood levels is recommended. Topiramate is pregnancy category D and should not be preferred(6). Lamotrigine and levetiracetam are preferred in pregnancy(1). Folate supplementation should be started at a dose of $\geq$0.4 mg/day(2). The rate of cesarean section is higher in women with epilepsy, but epilepsy alone is not an indication for cesarean section. The decision to perform a Caesarean section in women with epilepsy is made for reasons such as those who have had a previous Caesarean section, those with a history of seizures during birth, or the physician's preference due to an epilepsy diagnosis. Vaginal delivery is possible in patients with good seizure control and a stable pregnancy. If a seizure occurs during labor, intervention with fast-acting benzodiazepines should be considered(17). Seizures can lead to complications such as premature birth and fetal hypoxia during pregnancy(23). While levetracetam and lamotrigine are safe when used alone during pregnancy, the risk of teratogenicity increases when used together. Therefore, they are not preferred in combination. If a combination is necessary, safer pairs such as carbamazepine and gabapentin or oxcarbazepine and levetracetam should be preferred(23).

Antiepileptic drugs and breastfeeding

Breastfeeding is generally safe for newborns of women with epilepsy and is beneficial for neurodevelopment. Antiepileptics pass into breast milk to varying degrees. However, these levels do not cause significant side effects. It should be recommended for at least 6 months and preferably 12 months(23). Newer-generation medications such as lamotrigine and levetiracetam are less likely to pass into breast milk and are generally considered safe. Breastfed infants demonstrate better cognitive development despite exposure to antiepileptic drugs. Therefore, breastfeeding is beneficial and often recommended. If side effects such as excessive sleepiness, difficulty feeding, or rash are observed in the baby, the doctor should be informed(24). Sleep deprivation and medication dose changes in mothers can increase the risk of seizures(17).

Conclusion

Antiepileptics prevent seizures but do not cure epilepsy(25). The goal of treatment is to maximize efficacy and minimize toxicity(5). In clinical practice, interactions with oral contraceptives, anticoagulants, and immunosuppressants are particularly important. Levetiracetam, Gabapentin, and Pregabalin have no drug interactions because they are not metabolized by the liver(8). When a medication fails, there are two options: switching to another medication or combining it. In patients who have achieved seizure control and are experiencing long-term remission, discontinuation of the medication may be considered. In patients who have been seizure-free for two years, discontinuation of medication may be considered, but this decision should be based on individual risk factors. Therefore, seizure recurrence after discontinuation suggests that the underlying epileptogenic process persists(2). Lamotrigine, carbamazepine, and levetiracetam are suitable first-line options for focal seizures. Valproic acid is the best option for generalized seizures(26). Combinations of antiepileptic drugs with different mechanisms of action may have a higher probability of success(22).

References

1. Milligan TA. Epilepsy: a clinical overview. The American Journal of Medicine. 2021;134(7):840-7.
2. Schmidt D, Schachter SC. Drug treatment of epilepsy in adults. Bmj. 2014;348.
3. Stephen LJ, Harden C, Tomson T, Brodie MJ. Management of epilepsy in women. The Lancet Neurology. 2019;18(5):481-91.
4. Sáenz-Farret M, Tijssen MA, Eliashiv D, Fisher RS, Sethi K, Fasano A. Antiseizure drugs and movement disorders. CNS drugs. 2022;36(8):859-76.
5. Vidaurre J, Gedela S, Yarosz S. Antiepileptic drugs and liver disease. Pediatric Neurology. 2017;77:23-36.
6. Spritzer SD, Bravo TP, Drazkowski JF. Topiramate for treatment in patients with migraine and epilepsy. Headache: The Journal of Head and Face Pain. 2016;56(6):1081-5.
7. Pearl NZ, Babin CP, Catalano NT, Blake JC, Ahmadzadeh S, Shekoohi S, et al. Narrative review of topiramate: clinical uses and pharmacological considerations. Advances in Therapy. 2023;40(9):3626-38.
8. Hakami T. Neuropharmacology of antiseizure drugs. Neuropsychopharmacology reports. 2021;41(3):336-51.
9. Supuran CT. An update on drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors. Expert Opinion on Drug Metabolism & Toxicology. 2020;16(4):297-307.
10. Hoy SM. Lacosamide: a review in focal-onset seizures in patients with epilepsy. CNS drugs. 2018;32(5):473-84.
11. Li J, Sun M, Wang X. The adverse-effect profile of lacosamide. Expert Opinion on Drug Safety. 2020;19(2):131-8.
12. Scott LJ. Lacosamide: a review in focal seizures in patients with epilepsy. Drugs. 2015;75(18):2143-54.
13. Paquette V, Culley C, Greanya ED, Ensom MH. Lacosamide as adjunctive therapy in refractory epilepsy in adults: a systematic review. Seizure. 2015;25:1-17.
14. Ozsoy HZ. Anticonvulsant effects of carbonic anhydrase inhibitors: the enigmatic link between carbonic anhydrases and electrical activity of the brain. Neurochemical Research. 2021;46(11):2783-99.
15. Gidal BE, Resnick T, Smith MC, Wheless JW. Zonisamide: a comprehensive, updated review for the clinician. Neurology: Clinical Practice. 2024;14(1):e200210.

16.Brigo F, Lattanzi S, Igwe SC, Behzadifar M, Bragazzi NL. Zonisamide add-on therapy for focal epilepsy. Cochrane Database of Systematic Reviews. 2018(10).
17.Li Y, Meador KJ. Epilepsy and pregnancy. CONTINUUM: Lifelong learning in neurology. 2022;28(1):34-54.
18.Patocka J, Wu Q, Nepovimova E, Kuca K. Phenytoin–An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology. Food and Chemical Toxicology. 2020;142:111393.
19.Lu X, Wang X. Hyponatremia induced by antiepileptic drugs in patients with epilepsy. Expert opinion on drug safety. 2017;16(1):77-87.
20.Albani F, Riva R, Baruzzi A. Carbamazepine clinical pharmacology: a review. Pharmacopsychiatry. 1995;28(06):235-44.
21.Abou-Khalil BW. Update on antiepileptic drugs 2019. CONTINUUM: Lifelong Learning in Neurology. 2019;25(2):508-36.
22.Abou-Khalil BW. Bassel W. Abou-Khalil, MD, FAAN. Continuum (Minneap Minn). 2016;22(1):132-56.
23.Błaszczyk B, Miziak B, Pluta R, Czuczwar SJ. Epilepsy in pregnancy—management principles and focus on valproate. International journal of molecular sciences. 2022;23(3):1369.
24.Dupont S, Vercueil L. Epilepsy and pregnancy: What should the neurologists do? Revue Neurologique. 2021;177(3):168-79.
25.Sills GJ, Rogawski MA. Mechanisms of action of currently used antiseizure drugs. Neuropharmacology. 2020;168:107966.
26.Nevitt SJ, Sudell M, Cividini S, Marson AG, Smith CT. Antiepileptic drug monotherapy for epilepsy: a network meta-analysis of individual participant data. Cochrane database of systematic reviews. 2022(4).

Chapter 12

Leading Secondary Metabolites with Potential for Use in Cancer Treatment

Süphan ÖZÇAĞDAVUL[1], Cansu BEYTUR[2], Ramazan DEMİREL[3], Özkan ÖZDEN[4]

1. INTRODUCTION

1.1. Secondary Metabolites

Plants contain various compounds that differ in function; some directly contribute to growth, while others assist in the plant's adaptation to the environment without directly supporting growth. These compounds, classified as primary and secondary metabolites, respectively (Nwodo et al., 2016). Secondary metabolites are complex molecules synthesized by plants and are quite small molecules (Delgoda & Murray, 2017). Secondary metabolites have allelopathic effects against various diseases, adverse environmental conditions, and weeds (Ramakrishna & Ravishankar, 2011). These compounds are soluble in both water and oil and are synthesized in various parts of plants through complex pathways by biosynthetic enzymes. They can be stored in the region where they are synthesized or transported to distant locations. Secondary metabolites are used in many areas, including biotechnology, industrial applications, and, most importantly, healthcare. Secondary metabolites are found in trace amounts and are synthesized during specific developmental stages and under special conditions, such as stress (Thakur et al., 2019; Alvarez, 2014). Secondary metabolites are divided into three classes: terpenes, alkaloids, and phenolic compounds.

[1] (Ph.D. student) Department of Biotechnology, Institute of Natural and Applied Sciences, Kafkas University, Kars – Türkiye
Email:subhanozcagdavul@gmail.com, ORCID:0000-0022-5338-6731
[2] (Ph.D. student) Department of Molecular Biology, Institute of Natural and Applied Sciences, Kafkas University, Kars – Türkiye
Email:cnsbytrc@gmail.com, ORCID:0000-0002-4361-5035
[3] (M.Sc.) Department of Biotechnology, Institute of Natural and Applied Sciences, Kafkas University, TR-36300 Kars - Türkiye
Email:demirelramazan938@gmail.com, ORCID: 0000-0001-8654-5359
[4] (Prof. Dr. Ph.D.) Department of Bioengineering, Faculty of Engineering and Architecture, Kafkas University, Kars – Türkiye
Email:ozzkan1@gmail.com, ORCID:0000-0002-9467-3761

1.1.1. Terpenes

This class of secondary compounds comprises a wide range of hydrocarbons. Terpenes are classified according to the number of carbon atoms they contain. They play various roles in plants, including acting as photosynthetic pigments (carotenoids) and being involved in membrane structure. Terpenes have important functions in plants, such as oxidative stress, pollinator attraction, UV protection, and allelopathic interaction (Bohlmann & Keeling, 2008). These compounds are commonly used in food, perfume and cosmetics as essential oils, and are notable for their antioxidant, antimicrobial, anti-inflammatory and anticancer properties (Masyita et al., 2022).

1.1.2. Alkaloids

Alkaloids are another group of secondary compounds that contain nitrogen. Alkaloids, produced by plants, act as a defense mechanism by exerting toxic effects. The special structures with amino acid properties in their content can protect plants against possible external factors (Pagare et al., 2015). Although these structures are mostly seen in plants, some types of alkaloids are also produced by organisms, such as fungi or bacteria. Important compounds such as morphine, caffeine, nicotine, strychnine, and quinine belong to the alkaloid class (Wink & Roberts, 1998).

1.1.3. Phenolic Compounds

Phenolic compounds, found in abundance in nature and of which around 8,000 have been identified, play a crucial role in various aspects of plant life, including growth, defense and fertilization (Zamora-Ros et al., 2012). Previous studies have shown that these compounds have various effects on human health, including anti-inflammatory, anticarcinogenic, antidiabetic, anti-obesity and anti-allergic properties (Magdy et al., 2020). Phenolic compounds are classified according to their ring structure. Secondary compounds are frequently preferred in the health sector due to their active biological properties and are also among the most noteworthy metabolites in cancer research.

1.2. Cancer

Cancer is a group of diseases that directly affects public health and has more than 100 different types (Pavlopoulou et al., 2015). Particular genes are involved in the development of cancer and the most important of them are proto-oncogenes, tumor suppressor genes, and genes involved in the DNA repair mechanism, and any change in them can cause a cell to become cancerous (Blackadar, 2016). Cancer cells share several characteristics. They possess their own growth signals and evade growth-inhibiting factors. They also escape programmed cell death (apoptosis) and have the potential for continuous division. They form new blood vessels (angiogenesis) and evade immune system cells and inflammation that supports cancer formation. They also exhibit mutations (errors in gene structure) and genetic instability. They invade and metastasise (Hanahan, 2014). In addition to all these characteristics, cancer cells perform anaerobic respiration even in the presence of oxygen, which is referred to in the literature as the Warburg effect (Warburg et al., 1927). This effect, proposed by the scientist of the same name, describes the effect created by cancer cells to obtain more energy.

1.3. Secondary Metabolites Used in Cancer Treatment

Cancer treatment is one of the most frequently researched topics by scientists. In this regard, scientists are turning to various alternatives for cancer research. Plants have been used for treatment purposes throughout human history. As a result of scientific advances, plant contents have been identified, and the concept of secondary metabolites has been introduced.

1.3.1. Vincristine

This secondary metabolite is an alkaloid obtained from the *Catharanthus roseus* L. plant. Vincristine is generally used in pediatric oncology, but it is also used as a chemotherapeutic agent in adults (Evans et al., 1963). Another important feature of this compound is that it is one of the first plant-derived compounds approved by the Food and Drug Administration (FDA) (Newman & Cragg, 2016). Survival rates with vincristine use are approximately 80%, which increases the compound's preference in treatment (Moore & Pinkerton, 2009). Studies have shown that vincristine affects cell division in a dose-dependent manner. Its effect on cell division is based on its interaction with tubulin, the main component of mitotic spindle fibers, directly inhibiting cell division. Thus, the compound kills cells in the process of division (Wang et al., 2015). Another study has shown that differences in the dimeric structure of this compound also show variability in its anticancer effect (Sertel et al., 2011). Vincristine generally produces better results when combined with other antitumor agents. In addition to its effectiveness in combination therapy, its role here may be to reduce toxicity, but it may also affect drug resistance (Yoshihara et al., 2017). There are also clinical leukemia studies conducted with this compound (Ollila et al., 2022). Clinical studies with different chemotherapeutic agents have also determined that the efficacy of the compound increases as a result of its different formulations (Shah et al., 2025).

1.3.2. Paclitaxel

Paclitaxel is a secondary metabolite isolated from the bark of *Taxus brevifolia*. This compound is a tricyclic diterpenoid containing a 6,6,8-tri-fused skeleton called the taxane ring system, sometimes defined as a pseudoalkaloid. Paclitaxel is a non-ionic molecule with low solubility in aqueous media (Bernabeu et al., 2016). In recent years, experimental and clinical studies conducted in line with the increased use of this compound have yielded successful results in breast and ovarian cancer, as well as lung cancer (Bernabeu et al., 2017). The antitumor activity of this compound has a wide range of effects. Its effects on DNA repair mechanisms and cell proliferation have been clearly demonstrated. The main reason for paclitaxel's antitumor activity is the specific positioning of the groups in its structure and, accordingly, the targeting of beta-tubulin during cell division (Weaver, 2014). Researchers and pharmacologists have conducted various studies to develop a version of this secondary metabolite with the least toxic effects, resulting in the development of docetaxel and cabazitaxel. The therapeutic efficacy of the paclitaxel compound is also reduced due to multidrug resistance. While the cause of multidrug resistance is being investigated, scientists believe that it is caused by the overexpression of a protein called multidrug resistance protein 7 and mutations occurring in tubulin binding sites (Barbuti & Chen, 2015; Wang et al., 2013). Various versions of paclitaxel have been developed by scientists and continue to be developed. The paclitaxel derivatives developed are actively used in cancer treatment. Paclitaxel is actively used not only in cancer treatment but also in the treatment of diseases, such as psoriasis and botulinum neurotoxin (Ehrlich et al., 2004; Dadgar et al., 2013). A clinical study published in 2024 reported that the prognosis of pancreatic cancer was better when nab-paclitaxel was used in combination (Bodeker et al., 2024). In another clinical study published in 2025, the use of nab-paclitaxel in platinum-resistant ovarian cancer resulted in a significant improvement in prognosis (Olawaiye et al., 2025).

1.3.3. Homoharringtonine

The homoharringtonine compound is found in the trees Cephalotaxus harringtonii and Cephalotaxus fortunei, and was first used in traditional Chinese medicine to treat cancer. This alkaloid contains cephalotaxine in its core (Kantarjian et al., 2013). Cephalotaxine, which is found in high amounts in the leaves of Cephalotaxus species, is isolated and converted to homoharringtonine after extraction (Butler et al., 2014). The compound, which has an important place in Chinese medicine, attracted the attention of Western medicine after the development of a semi-synthetic procedure showed its effectiveness against leukemia (Lü & Wang, 2014). The working principle of this compound is as follows: it exerts its effect by inhibiting protein translation. This inhibition occurs by binding to the A site of the large subunit of proteins, thereby blocking tRNA access. In this way, it directs cells towards apoptosis by inhibiting antiapoptotic proteins and short-lived oncogenes (Gandhi et al., 2014). Recent studies have shown that this compound can also affect various signaling pathways. The compound, administered to chemotherapy-resistant patients, promoted apoptosis and also demonstrated antitumor activity in a myeloma xenograft model (Meng et al., 2008). A study on the MCF-7 breast cancer cell line revealed that the homoharringtonine compound induces apoptosis and reduces cell survival in vitro, including in cells resistant to imatinib treatment, by acting through the Bcl-6 and p53 pathways (Wang et al., 2021; Wang et al., 2017).

1.3.4. Curcumin

Curcumin is a polyphenolic compound obtained from turmeric. The plant is commonly used in traditional East Asian medicine and is primarily employed for its anti-inflammatory, antioxidant, chemopreventive and anticancer properties (Kocaadam & Şanlier, 2017). This compound has a low solubility in water and is more likely to degrade in a basic environment (Wang et al., 1997). The reason scientists are researching this compound is the low incidence of gastrointestinal cancer in Asia and its association with turmeric consumption (Sinha et al., 2003). Various *in vivo* and *in vitro* studies conducted with curcumin have shown that this compound exhibits anticancer effects on various types of cancer (Perrone et al., 2015). Studies conducted with curcumin reveal that this compound has therapeutic value, as it has a toxic effect on cancer cells but does not show a toxic effect on normal cells (Sordillo & Helson, 2015). Curcumin affects cancer cells through different pathways. The MAPK and Akt signaling pathways increase the level of the transcription factor Nrf2, leading to modulation of the CYP enzyme and ultimately resulting in the death of cells resistant to apoptosis (Schwertheim et al., 2017). Another study found that this secondary compound affects cancer by interfering with the control points of the cell cycle through the inhibition of cyclin-dependent kinases (Dasiram et al., 2017). Studies by various scientists have shown that curcumin, when administered alongside chemotherapy drugs, acts as a chemosensitizer and also exhibits a synergistic effect with naturally sourced compounds such as resveratrol and honokiol, thereby reducing the proliferation of cancer cells (Di Martino et al., 2017; Klippstein et al., 2016; Pimentel-Gutiérrez et al., 2016). In one recent study, curcumin doses of 5, 15, and 30 μM blocked the cell cycle in the G2/M phase in the 22Rv1 pancreatic cell line while cell death was observed at concentrations of 15 and 30 μM (Tossetta et al., 2025).

1.3.5. Betulinic Acid

It is a secondary compound that is found in the *Gratiola officinalis* L. plant, as well as other plant species, and has been given different names. Detailed research by scientists revealed that they all have the same content and that the common name is betulinic acid (Ali-Seyed et al., 2016). Researchers at the University of Illinois first identified the anti-cancer properties of betulinic acid in 1995, when they found that it killed melanoma cells in mice at very low doses (Pisha et al., 1995). Research on betulinic acid is also supported by the National Cancer Institute's RAID program (Ali-Seyed et al., 2016). This compound affects cancer in various ways, but its most notable mechanism is through apoptosis. The activation of the apoptotic pathway occurs through changes in the regulation of the Bcl-2 family, as well as the release of pre-apoptotic factors such as cytochrome c (Zhang et al., 2015). There is also evidence that betulinic acid can induce apoptosis via the p53 pathway, as well as via NF-κB-mediated signaling (Shankar et al., 2017). This compound has also been reported to have an antimetastatic effect (Gheorgheosu et al., 2014). Application of betulinic acid in the MDA-MB-231 breast cancer cell line increased intracellular reactive oxygen species (ROS) levels and induced apoptosis (Lestari et al., 2024). Similarly, a study on the anticancer activity of betulinic acid in U937 human myeloid leukemia cells revealed that it induces apoptosis through ROS (Park et al., 2021).

1.3.6. Gallic acid

Gallic acid (3,4,5-trihydroxybenzoic acid) is a polyphenolic secondary metabolite. This compound is soluble in compounds, such as water, ether, and alcohol. Gallic acid is found in many plants, such as walnuts, sumac, oak bark, and tea leaves. The rapid absorption of this compound also increases its medical value. In addition to its antidiabetic, antioxidant, anti-obesity, and anti-inflammatory properties, the compound also exhibits noteworthy neuroprotective effects (Liu et al., 2020). Researchers have discovered that reactive oxygen species (ROS) contribute to increased brain damage, while gallic acid exerts a protective effect (Schimites et al., 2020). A 2002 study found that gallic acid influences apoptosis by increasing ROS which in turn cause mitochondrial membrane changes and activate caspases via cytochrome c release (Wilcox, 2002). In a study conducted on gastric cancer cells, gallic acid application resulted in decreased cell viability at different doses and incubation times (Tsai et al., 2018). In a study conducted on OVCAR-3 and A2780/CP70 ovarian cancer cell lines and healthy ovarian cell IOSE-364, it was reported that gallic acid selectively inhibited cell growth in cancer cells (He et al., 2016). In HepG2 and

SMMC-7721 human liver cancer cells, gallic acid treatment was reported to have a toxic effect depending on the dose and incubation time (Sun et al., 2016).

1.3.7. Resveratrol

Resveratrol is a polyphenolic compound consisting of two linked phenolic rings. This natural compound is found in the skin of red grapes and is also present in approximately 70 different plants. Resveratrol is a compound that exhibits active effects against pathogens found in many bacteria and fungi. In addition to its antipathogenic properties, this compound's notable feature is its high antioxidant potential. Studies have also revealed its effects on cardiovascular diseases (Izzo et al., 2021). The effects of this compound on lipid metabolism are also known. Some studies have shown that resveratrol reduces the fat content and body weight of animals with high fat levels (Szkudelska and Szkudelski, 2010). Resveratrol has many analogues in various plants. One such analogue is pterostilbene. Cancer studies conducted with this compound have revealed that it induces apoptosis and metastasis, thereby affecting cancer cells (McCormack & McFadden, 2012). Studies have also shown that pterostilbene affects the cell cycle (Bai et al., 2010). Researchers have also determined that this compound is effective against drug resistance (Gupta et al., 2011). Various in vivo and in vitro studies have been conducted with resveratrol. Researchers have found that it induces apoptosis in the MCF-7 human breast cancer cell line but does not show the same effect in the MDA-MB-231 cell line, another estrogen-insensitive breast cancer cell line (Pozo-Guisado et al., 2005). A study conducted in 2023 revealed that resveratrol even affects prostate cancer cell lines resistant to docetaxel and exhibits anticancer activity. In addition to this effect, it was also found that resveratrol combined with docetaxel treatment exhibits a synergistic effect in cells (Badawi, 2023). Another study reported that 50 mg of resveratrol administered to approximately 40 women at risk of breast cancer for three months reduced RASSF-1a methylation and PGE2 expression in these women (Choudhari et al., 2020). Another study showed that resveratrol inhibited cell proliferation in different breast cancer cell lines (Nakagawa et al., 2001).

1.3.8. Honokiol

Honokiol is a phenolic compound found in *Magnolia obovata* and many other plants (Fujita et al., 1973). Researchers have demonstrated the anticancer properties of this compound in various studies. It has been determined that honokiol exerts antitumor activity by inhibiting angiogenesis. In a study of patients with chronic lymphocytic leukaemia, researchers demonstrated this compound's antitumour activity. This study found that the honokiol compound triggers apoptosis via caspases. The effect of the compound on Bax regulation was also demonstrated in this study (Battle et al., 2005). Experimental studies conducted by different researchers have shown that the honokiol compound affects different types of cancer. In a study conducted in 2004, scientists demonstrated that this compound induces apoptosis in the RKO colon cancer cell line (Wang et al., 2004). The study found that increased permeability of the mitochondrial outer membrane led to elevated ROS levels and necrosis, and that honokiol, when administered to mice with cancer via various routes, directly extended their lifespan (Li et al., 2007). Furthermore, it was determined that this compound reverses the mechanism of drug resistance — an adverse effect of cancer treatments — via the ABCC transporter gene (Garcia et al., 2008).

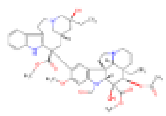
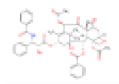
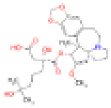
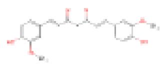
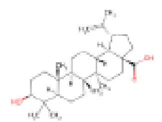
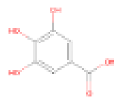
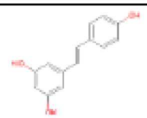
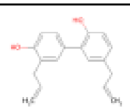
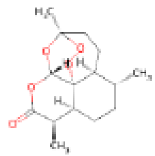
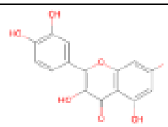
1.3.9. Artemisinin

This compound, isolated from the *Artemisia annua* L. plant, was discovered through malaria research (Krishna et al., 2008). Since its discovery, this compound has attracted attention for its effects against malaria and other diseases. Researchers have been interested in whether the compound's effective treatment against malaria involves antiviral, antibacterial, anti-inflammatory, and anticancer effects (Ho et al., 2014). The compound has various mechanisms that are effective against cancer. In one study, researchers found that applying artemisinin to prostate cancer cells altered the levels of CDK2 and CDK4 proteins, determining that the compound effectively inhibits cell proliferation (Willoughby et al., 2009). A study investigating the effects of artemisinin on ovarian cancer used A2780 and OVCAR-3 cell lines. Xenograft models were also created and concluded that the compound increases apoptosis (Chen et al., 2009). Following the application of artemisinin, selective toxicity was observed in the MCF-7, MDA-MB-231 (breast cancer) and MCF10A (epithelial breast) cell lines. It has been reported that artemisinin may exhibit anti-cancer effects (Kumari et al., 2017). The combination of artemisinin with the chemotherapeutic agent 5-FU has also been reported to increase 5-FU sensitivity in colon cancer cells (Wang et al., 2020).

1.3.10. Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a phenolic secondary compound. This compound is naturally found in apples, cherries, seeds, tea, red wine, and coffee (Samova et al., 2018). Quercetin is known to have many properties, including antioxidant, antiapoptotic, antitumor, and antiallergenic effects (Alizadeh and Ebrahimzadeh, 2022). The mechanism of action of this compound in cells is to inhibit apoptosis by affecting the production of ROS and blocking the caspase-3 pathway by increasing the mitochondrial membrane potential (Hu et al., 2019). This compound's antioxidant activity is due to the catechol-type B ring and hydroxyl arrangement in its structure (Bartekova et al., 2016). Studies have shown that quercetin exhibits a synergistic effect when combined with agents such as cisplatin by enhancing the expression of p53 and BAX genes and influencing epigenetic mechanisms (Rauf et al., 2018). Quercetin causes cell apoptosis by increasing Foxo3a activation and altering the transcription of genes involved in activating the Foxo3a pathway and apoptosis (Nguyen et al., 2017). A study reported that this compound reduced cell viability in A549 and H1299 cell lines in a concentration-dependent manner, induced mitochondria-dependent apoptosis, and increased SIRT1 protein levels in a dose-dependent manner (Guo et al., 2021). In a study investigating the effects of quercetin on stomach cancer, it was determined that the compound's mechanism of action on cancer cells was through lipid peroxidation (Ding et al., 2024). The chemical structures of the 10 common secondary metabolites mentioned in this study and the types of cancer they affect are summarized in Table 1.

Table 1: Chemical structures of the common secondary metabolites and the types of cancer they affect.

Secondary Metabolites	Secondary Metabolite Class	Chemical Structure	Cancer Types	References
Vincristine	Alkaloid		Leukemia	(Ollila et al., 2022)
Paclitaxel	Terpenoid		Breast, Ovarian and lung cancer	(Bernabeu et al., 2017)
Homoharringtonine	Alkaloid		Breast cancer	(Wang et al., 2021)
Curcumin	Phenolic compound		Lymphoblastic leukemia and thyroid cancer	(Wang et al. 2012), (Zhang et al. 2016)
Betulinic Acid	Terpenoid		Breast cancer and myeloid leukemia	(Lestari et al., 2024), (Park et al., 2021).
Gallic acid	Phenolic compound		Gastric, ovarian and liver cancer	(Tsai et al., 2018), (He et al., 2016), (Sun et al., 2016)
Resveratrol	Phenolic compound		Breast and prostate cancer	(Pozo-Guisado et al., 2005), (Badawi, 2023)
Honokiol	Phenolic compound		Colon cancer	(Wang et al., 2004)
Artemisinin	Terpenoid		Prostate, ovarian, breast and colon cancer	(Willoughby et al., 2009), (Chen et al., 2009), (Kumari et al., 2017), (Y. Wang et al., 2020).
Quercetin	Phenolic compound		Oral and lung cancer	(Saadh et al. 2025), (Guo et al. 2021)

2. CONCLUSION

Secondary metabolites are compounds found in plants that play a crucial role in regulating many of their functions, as well as in communication between plants and other ecological systems. Beyond their importance for plants, secondary metabolites are also important for human health. These compounds directly affect many diseases, from obesity to diabetes, neurodegenerative diseases to cancer. These compounds are preferred because they are naturally sourced, have low toxicity, are soluble and have positive properties, such as being able to be transmitted in the body. The complex nature of cancer has led researchers to explore various avenues for its treatment. The preference for naturally sourced compounds in the treatment of this disease is not only due to economic factors and accessibility, but also because they are less toxic than synthetic compounds. Research conducted on secondary metabolites has revealed that these compounds affect cancer through multiple pathways. Our literature review indicates that secondary compounds are frequently favored in the development of anticancer drugs and are poised to become a major focus of future research.

References

Ali-Seyed, Mohamed, Ibrahim Jantan, Kavitha Vijayaraghavan, and Syed Nasir Abbas Bukhari. 2016. "Betulinic Acid: Recent Advances in Chemical Modifications, Effective Delivery, and Molecular Mechanisms of a Promising Anticancer Therapy." *Chemical Biology & Drug Design* 87(4):517–36. doi:10.1111/cbdd.12682.

Alizadeh, Seyedeh Roya, and Mohammad Ali Ebrahimzadeh. 2022. "O-Glycoside Quercetin Derivatives: Biological Activities, Mechanisms of Action, and Structure-Activity Relationship for Drug Design, a Review." *Phytotherapy Research: PTR* 36(2):778–807. doi:10.1002/ptr.7352.

Alvarez, María Alejandra. 2014. "Plants for Health: From Secondary Metabolites to Molecular FarmingChapter 1." Pp. 1–2 in *Plant Biotechnology for Health: From Secondary Metabolites to Molecular Farming*, edited by Maria Alejandra Alvarez. Cham: Springer International Publishing.

Badawi, Jasmin Katrin. 2023. "Resveratrol Used as Nanotherapeutic: A Promising Additional Therapeutic Tool against Hormone-Sensitive, Hormone-Insensitive and Resistant Prostate Cancer." *American Journal of Clinical and Experimental Urology* 11(1):1–11.

Bai, Yu, Qi-Qi Mao, Jie Qin, Xiang-Yi Zheng, Yun-Bin Wang, Kai Yang, Hua-Feng Shen, and Li-Ping Xie. 2010. "Resveratrol Induces Apoptosis and Cell Cycle Arrest of Human T24 Bladder Cancer Cells in Vitro and Inhibits Tumor Growth in Vivo." *Cancer Science* 101(2):488–93. doi:10.1111/j.1349-7006.2009.01415.x.

Barbuti, Anna Maria, and Zhe-Sheng Chen. 2015. "Paclitaxel Through the Ages of Anticancer Therapy: Exploring Its Role in Chemoresistance and Radiation Therapy." *Cancers* 7(4):2360–71. doi:10.3390/cancers7040897.

Bartekova, M., J. Radosinska, D. Pancza, M. Barancik, and T. Ravingerova. 2016. "Cardioprotective Effects of Quercetin against Ischemia-Reperfusion Injury Are Age-Dependent." *Physiological Research* 65 Suppl 1:S101-107. doi:10.33549/physiolres.933390.

Battle, Traci E., Jack Arbiser, and David A. Frank. 2005. "The Natural Product Honokiol Induces Caspase-Dependent Apoptosis in B-Cell Chronic Lymphocytic Leukemia (B-CLL) Cells." *Blood* 106(2):690–97. doi:10.1182/blood-2004-11-4273.

Bernabeu, Ezequiel, Maximiliano Cagel, Eduardo Lagomarsino, Marcela Moretton, and Diego A. Chiappetta. 2017. "Paclitaxel: What Has Been Done and the Challenges Remain Ahead." *International Journal of Pharmaceutics* 526(1–2):474–95. doi:10.1016/j.ijpharm.2017.05.016.

Bernabeu, Ezequiel, Lorena Gonzalez, Maximiliano Cagel, Esteban P. Gergic, Marcela A. Moretton, and Diego A. Chiappetta. 2016. “Novel Soluplus(®)-TPGS Mixed Micelles for Encapsulation of Paclitaxel with Enhanced in Vitro Cytotoxicity on Breast and Ovarian Cancer Cell Lines.” *Colloids and Surfaces. B, Biointerfaces* 140:403–11. doi:10.1016/j.colsurfb.2016.01.003.

Blackadar, Clarke Brian. 2016. “Historical Review of the Causes of Cancer.” *World Journal of Clinical Oncology* 7(1):54. doi:10.5306/wjco.v7.i1.54.

Bodeker, Kellie L., Brian J. Smith, Daniel J. Berg, Chandrikha Chandrasekharan, Saima Sharif, Naomi Fei, Sandy Vollstedt, Heather Brown, Meghan Chandler, Amanda Lorack, Stacy McMichael, Jared Wulfekuhle, Brett A. Wagner, Garry R. Buettner, Bryan G. Allen, Joseph M. Caster, Barbara Dion, Mandana Kamgar, John M. Buatti, and Joseph J. Cullen. 2024. “A Randomized Trial of Pharmacological Ascorbate, Gemcitabine, and Nab-Paclitaxel for Metastatic Pancreatic Cancer.” *Redox Biology* 77:103375. doi:10.1016/j.redox.2024.103375.

Bohlmann, Jörg, and Christopher I. Keeling. 2008. “Terpenoid Biomaterials.” *The Plant Journal* 54(4):656–69. doi:10.1111/j.1365-313X.2008.03449.x.

Butler, Mark S., Avril A. B. Robertson, and Matthew A. Cooper. 2014. “Natural Product and Natural Product Derived Drugs in Clinical Trials.” *Natural Product Reports* 31(11):1612–61. doi:10.1039/c4np00064a.

Chen, Tao, Mian Li, Ruiwen Zhang, and Hui Wang. 2009. “Dihydroartemisinin Induces Apoptosis and Sensitizes Human Ovarian Cancer Cells to Carboplatin Therapy.” *Journal of Cellular and Molecular Medicine* 13(7):1358–70. doi:10.1111/j.1582-4934.2008.00360.x.

Choudhari, Amit S., Pallavi C. Mandave, Manasi Deshpande, Prabhakar Ranjekar, and Om Prakash. 2020. “Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice.” *Frontiers in Pharmacology* 10. https://www.frontiersin.org/articles/10.3389/fphar.2019.01614.

Dadgar, Saedeh, Zack Ramjan, and Wely B. Floriano. 2013. “Paclitaxel Is an Inhibitor and Its Boron Dipyrromethene Derivative Is a Fluorescent Recognition Agent for Botulinum Neurotoxin Subtype A.” *Journal of Medicinal Chemistry* 56(7):2791–2803. doi:10.1021/jm301829h.

Dasiram, Jade Dhananjay, Ramamoorthi Ganesan, Janani Kannan, Venkatesan Kotteeswaran, and Nageswaran Sivalingam. 2017. “Curcumin Inhibits Growth Potential by G1 Cell Cycle Arrest and Induces Apoptosis in P53-Mutated COLO 320DM Human Colon Adenocarcinoma Cells.” *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 86:373–80. doi:10.1016/j.biopha.2016.12.034.

Delgoda, Rupika, and Jeann Murray. 2017. "Evolutionary Perspectives on the Role of Plant Secondary Metabolites." Pp. 93–100 in *Pharmacognosy: Fundamentals, Applications and Strategy*.

Di Martino, Rita Maria Concetta, Barbara Luppi, Alessandra Bisi, Silvia Gobbi, Angela Rampa, Angela Abruzzo, and Federica Belluti. 2017. "Recent Progress on Curcumin-Based Therapeutics: A Patent Review (2012-2016). Part I: Curcumin." *Expert Opinion on Therapeutic Patents* 27(5):579–90. doi:10.1080/13543776.2017.1276566.

Ding, Lixian, Shuwei Dang, Mingjun Sun, Dazhi Zhou, Yanyan Sun, Encheng Li, Shuqi Peng, Jinxing Li, and Guodong Li. 2024. "Quercetin Induces Ferroptosis in Gastric Cancer Cells by Targeting SLC1A5 and Regulating the P-Camk2/p-DRP1 and NRF2/GPX4 Axes." *Free Radical Biology and Medicine* 213:150–63. doi:10.1016/j.freeradbiomed.2024.01.002.

Ehrlich, Alison, Susan Booher, Yvonne Becerra, Debra L. Borris, W. Douglas Figg, Maria L. Turner, and Andrew Blauvelt. 2004. "Micellar Paclitaxel Improves Severe Psoriasis in a Prospective Phase II Pilot Study." *Journal of the American Academy of Dermatology* 50(4):533–40. doi:10.1016/j.jaad.2003.09.018.

Evans, A. E., S. Farber, S. Brunet, and P. J. Mariano. 1963. "VINCRISTINE IN THE TREATMENT OF ACUTE LEUKEMIA IN CHILDREN." *Cancer* 16:1302–6. doi:10.1002/1097-0142(196310)16:10<1302::aid-cncr2820161011>3.0.co;2-a.

Fujita, M., H. Itokawa, and Y. Sashida. 1973. "[Studies on the components of Magnolia obovata Thunb. 3. Occurrence of magnolol and hõnokiol in M. obovata and other allied plants]." *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan* 93(4):429–34. doi:10.1248/yakushi1947.93.4_429.

Gandhi, Varsha, William Plunkett, and Jorge E. Cortes. 2014. "Omacetaxine: A Protein Translation Inhibitor for Treatment of Chronic Myelogenous Leukemia." *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 20(7):1735–40. doi:10.1158/1078-0432.CCR-13-1283.

Garcia, Avalon, Yang Zheng, Chen Zhao, Alfredo Toschi, Judy Fan, Natalie Shraibman, H. Alex Brown, Dafna Bar-Sagi, David A. Foster, and Jack L. Arbiser. 2008. "Honokiol Suppresses Survival Signals Mediated by Ras-Dependent Phospholipase D Activity in Human Cancer Cells." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* 14(13):4267–74. doi:10.1158/1078-0432.CCR-08-0102.

Gheorgheosu, Dorina, Oana Duicu, Cristina Dehelean, Codruta Soica, and Danina Muntean. 2014. “Betulinic Acid as a Potent and Complex Antitumor Phytochemical: A Minireview.” *Anti-Cancer Agents in Medicinal Chemistry* 14(7):936–45. doi:10.2174/1871520614666140223192148.

Guo, Hengjuan, Hui Ding, Xin Tang, Maoli Liang, Shuo Li, Jing Zhang, and Jie Cao. 2021. “Quercetin Induces Pro-Apoptotic Autophagy via SIRT1/AMPK Signaling Pathway in Human Lung Cancer Cell Lines A549 and H1299 in Vitro.” *Thoracic Cancer* 12(9):1415–22. doi:10.1111/1759-7714.13925.

Gupta, Subash C., Ramaswamy Kannappan, Simone Reuter, Ji Hye Kim, and Bharat B. Aggarwal. 2011. “Chemosensitization of Tumors by Resveratrol.” *Annals of the New York Academy of Sciences* 1215:150–60. doi:10.1111/j.1749-6632.2010.05852.x.

Hanahan, Douglas. 2014. “Rethinking the War on Cancer.” *The Lancet* 383(9916):558–63. doi:10.1016/S0140-6736(13)62226-6.

He, Zhiping, Allen Y. Chen, Yon Rojanasakul, Gary O. Rankin, and Yi Charlie Chen. 2016. “Gallic Acid, a Phenolic Compound, Exerts Anti-Angiogenic Effects via the PTEN/AKT/HIF-1α/VEGF Signaling Pathway in Ovarian Cancer Cells.” *Oncology Reports* 35(1):291–97. doi:10.3892/or.2015.4354.

Ho, Wanxing Eugene, Hong Yong Peh, Tze Khee Chan, and W. S. Fred Wong. 2014. “Artemisinins: Pharmacological Actions beyond Anti-Malarial.” *Pharmacology & Therapeutics* 142(1):126–39. doi:10.1016/j.pharmthera.2013.12.001.

Hu, Yue, Zhipeng Gui, Yuning Zhou, Lunguo Xia, Kaili Lin, and Yuanjin Xu. 2019. “Quercetin Alleviates Rat Osteoarthritis by Inhibiting Inflammation and Apoptosis of Chondrocytes, Modulating Synovial Macrophages Polarization to M2 Macrophages.” *Free Radical Biology & Medicine* 145:146–60. doi:10.1016/j.freeradbiomed.2019.09.024.

Izzo, Carmine, Monica Annunziata, Giuseppe Melara, Roberta Sciorio, Marcello Dallio, Mario Masarone, Alessandro Federico, and Marcello Persico. 2021. “The Role of Resveratrol in Liver Disease: A Comprehensive Review from In Vitro to Clinical Trials.” *Nutrients* 13(3):933. doi:10.3390/nu13030933.

Kantarjian, Hagop M., Susan O’Brien, and Jorge Cortes. 2013. “Homoharringtonine/Omacetaxine Mepesuccinate: The Long and Winding Road to Food and Drug Administration Approval.” *Clinical Lymphoma, Myeloma & Leukemia* 13(5):530–33. doi:10.1016/j.clml.2013.03.017.

Klippstein, Rebecca, Sukhvinder S. Bansal, and Khuloud T. Al-Jamal. 2016. "Doxorubicin Enhances Curcumin's Cytotoxicity in Human Prostate Cancer Cells in Vitro by Enhancing Its Cellular Uptake." *International Journal of Pharmaceutics* 514(1):169–75. doi:10.1016/j.ijpharm.2016.08.003.

Kocaadam, Betül, and Nevin Şanlier. 2017. "Curcumin, an Active Component of Turmeric (Curcuma Longa), and Its Effects on Health." *Critical Reviews in Food Science and Nutrition* 57(13):2889–95. doi:10.1080/10408398.2015.1077195.

Krishna, Sanjeev, Leyla Bustamante, Richard K. Haynes, and Henry M. Staines. 2008. "Artemisinins: Their Growing Importance in Medicine." *Trends in Pharmacological Sciences* 29(10):520–27. doi:10.1016/j.tips.2008.07.004.

Kumari, Kanchan, Sunita Keshari, Debomita Sengupta, Surendra C. Sabat, and Sandip K. Mishra. 2017. "Transcriptome Analysis of Genes Associated with Breast Cancer Cell Motility in Response to Artemisinin Treatment." *BMC Cancer* 17:858. doi:10.1186/s12885-017-3863-7.

Lestari, Dian Yuliartha, Gondo Mastutik, and Indri Safitri Mukono. 2024. "Betulinic Acid and Oleanolic Acid Modulate CD81 Expression and Induce Apoptosis in Triple-Negative Breast Cancer Cells through ROS Generation." *Medical Oncology (Northwood, London, England)* 42(1):24. doi:10.1007/s12032-024-02574-4.

Li, Ling, Weidong Han, Ying Gu, Shuang Qiu, Qinghua Lu, Jie Jin, Jianhong Luo, and Xun Hu. 2007. "Honokiol Induces a Necrotic Cell Death through the Mitochondrial Permeability Transition Pore." *Cancer Research* 67(10):4894–4903. doi:10.1158/0008-5472.CAN-06-3818.

Liu, Y. L., C. C. Hsu, H. J. Huang, C. J. Chang, S. H. Sun, and A. M. Y. Lin. 2020. "Gallic Acid Attenuated LPS-Induced Neuroinflammation: Protein Aggregation and Necroptosis." *Molecular Neurobiology* 57(1):96–104. doi:10.1007/s12035-019-01759-7.

Lü, Shuqing, and Jianmin Wang. 2014. "Homoharringtonine and Omacetaxine for Myeloid Hematological Malignancies." *Journal of Hematology & Oncology* 7:2. doi:10.1186/1756-8722-7-2.

Magdy, Ahmed, Emad Sadaka, Nemany Hanafy, Mohammed A. El-Magd, Nasr Allahloubi, and Maged El Kemary. 2020. "Green Tea Ameliorates the Side Effects of the Silver Nanoparticles Treatment of Ehrlich Ascites Tumor in Mice." *Molecular & Cellular Toxicology* 16(3):271–82. doi:10.1007/s13273-020-00078-6.

Masyita, Ayu, Reka Mustika Sari, Ayun Dwi Astuti, Budiman Yasir, Nur Rahma Rumata, Talha Bin Emran, Firzan Nainu, and Jesus Simal-Gandara. 2022. “Terpenes and Terpenoids as Main Bioactive Compounds of Essential Oils, Their Roles in Human Health and Potential Application as Natural Food Preservatives.” *Food Chemistry: X* 13:100217. doi:10.1016/j.fochx.2022.100217.

McCormack, Denise, and David McFadden. 2012. “Pterostilbene and Cancer: Current Review.” *The Journal of Surgical Research* 173(2):e53-61. doi:10.1016/j.jss.2011.09.054.

Meng, Haitao, Chunmei Yang, Jie Jin, Yuhong Zhou, and Wenbin Qian. 2008. “Homoharringtonine Inhibits the AKT Pathway and Induces in Vitro and in Vivo Cytotoxicity in Human Multiple Myeloma Cells.” *Leukemia & Lymphoma* 49(10):1954–62. doi:10.1080/10428190802320368.

Moore, Andrew, and Ross Pinkerton. 2009. “Vincristine: Can Its Therapeutic Index Be Enhanced?” *Pediatric Blood & Cancer* 53(7):1180–87. doi:10.1002/pbc.22161.

Nakagawa, H., Y. Kiyozuka, Y. Uemura, H. Senzaki, N. Shikata, K. Hioki, and A. Tsubura. 2001. “Resveratrol Inhibits Human Breast Cancer Cell Growth and May Mitigate the Effect of Linoleic Acid, a Potent Breast Cancer Cell Stimulator.” *Journal of Cancer Research and Clinical Oncology* 127(4):258–64. doi:10.1007/s004320000190.

Newman, David J., and Gordon M. Cragg. 2016. “Natural Products as Sources of New Drugs from 1981 to 2014.” *Journal of Natural Products* 79(3):629–61. doi:10.1021/acs.jnatprod.5b01055.

Nguyen, Lich Thi, Yeon-Hee Lee, Ashish Ranjan Sharma, Jong-Bong Park, Supriya Jagga, Garima Sharma, Sang-Soo Lee, and Ju-Suk Nam. 2017. “Quercetin Induces Apoptosis and Cell Cycle Arrest in Triple-Negative Breast Cancer Cells through Modulation of Foxo3a Activity.” *The Korean Journal of Physiology & Pharmacology : Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology* 21(2):205–13. doi:10.4196/kjpp.2017.21.2.205.

Nwodo, Justina N., Akachukwu Ibezim, Conrad V. Simoben, and Fidele Ntie-Kang. 2016. “Exploring Cancer Therapeutics with Natural Products from African Medicinal Plants, Part II: Alkaloids, Terpenoids and Flavonoids.” *Anti-Cancer Agents in Medicinal Chemistry* 16(1):108–27. doi:10.2174/1871520615666150520143827.

Olawaiye, Alexander B., Laurence Gladieff, David M. O’Malley, Jae-Weon Kim, Gabriel Garbaos, Vanda Salutari, Lucy Gilbert, Linda Mileshkin, Alix Devaux, Elizabeth Hopp, Yong Jae Lee, Ana Oaknin, Mariana Scaranti, Byoung-Gie Kim, Nicoletta Colombo, Michael E. McCollum, Connie Diakos, Andrew Clamp, Aliza L. Leiser, Boglárka Balázs, Bradley J. Monk, Giuseppa Scandurra, Emily McClung, Emilie Kaczmarek, Brian Slomovitz, Helena De La Cueva, Aknar Freire de Carvalho Calabrich, Chiara Cassani, Benoit You, Toon Van Gorp, Cristina Churruca, Giuseppe Caruso, Shibani Nicum, Andrea Bagaméri, Grazia Artioli, Lubomir Bodnar, Sokbom Kang, Ignace Vergote, Amanda Kesner-Hays, Hristina I. Pashova, Sachin G. Pai, Iulia Cristina Tudor, Adrian M. Jubb, and Domenica Lorusso. 2025. "Relacorilant and Nab-Paclitaxel in Patients with Platinum-Resistant Ovarian Cancer (ROSELLA): An Open-Label, Randomised, Controlled, Phase 3 Trial." *Lancet (London, England)* 405(10496):2205–16. doi:10.1016/S0140-6736(25)01040-2.

Ollila, Thomas, James Butera, Pamela Egan, John Reagan, Anthony Thomas, Inna Yakirevich, Kelsey MacKinnon, Jeannine Margolis, Jessica McMahon, Valerie Rosati, and Adam J. Olszewski. 2022. "Vincristine Sulfate Liposome Injection with Bendamustine and Rituximab as First-Line Therapy for B-Cell Lymphomas: A Phase I Study." *The Oncologist* 27(7):532-e542. doi:10.1093/oncolo/oyab079.

Pagare, Saurabh, Manila Bhatia, Niraj Tripathi, and Y. K. Bansal. 2015. "Secondary Metabolites of Plants and Their Role: Overview." *Current Trends in Biotechnology and Pharmacy* 9:293–304.

Park, Cheol, Jin-Woo Jeong, Min Ho Han, Hyesook Lee, Gi-Young Kim, Soojung Jin, Jung-Ha Park, Hyun Ju Kwon, Byung Woo Kim, and Yung Hyun Choi. n.d. "The Anti-Cancer Effect of Betulinic Acid in U937 Human Leukemia Cells Is Mediated through ROS-Dependent Cell Cycle Arrest and Apoptosis." *Animal Cells and Systems* 25(2):119–27. doi:10.1080/19768354.2021.1915380.

Pavlopoulou, Athanasia, Demetrios A. Spandidos, and Ioannis Michalopoulos. 2015. "Human Cancer Databases (Review)." *Oncology Reports* 33(1):3–18. doi:10.3892/or.2014.3579.

Perrone, Donatella, Fatima Ardito, Giovanni Giannatempo, Mario Dioguardi, Giuseppe Troiano, Lucio Lo Russo, Alfredo DE Lillo, Luigi Laino, and Lorenzo Lo Muzio. 2015. "Biological and Therapeutic Activities, and Anticancer Properties of Curcumin." *Experimental and Therapeutic Medicine* 10(5):1615–23. doi:10.3892/etm.2015.2749.

Pimentel-Gutiérrez, Helia Judith, Lucina Bobadilla-Morales, César Cenobio Barba-Barba, Citlalli Ortega-De-La-Torre, Fernando Antonio Sánchez-Zubieta, Jorge Román Corona-Rivera, Betsy Annel González-Quezada, Juan S. Armendáriz-Borunda, Rocío Silva-Cruz, and Alfredo Corona-

Rivera. 2016. “Curcumin Potentiates the Effect of Chemotherapy against Acute Lymphoblastic Leukemia Cells via Downregulation of NF-κB.” *Oncology Letters* 12(5):4117–24. doi:10.3892/ol.2016.5217.

Pisha, Emily, Heebyung Chai, Ik-Soo Lee, Tangai E. Chagwedera, Norman R. Farnsworth, Geoffrey A. Cordell, Christopher W. W. Beecher, Harry H. S. Fong, A. Douglas Kinghorn, Daniel M. Brown, Mansukh C. Wani, Monroe E. Wall, Tina J. Hieken, Tapas K. Das Gupta, and John M. Pezzuto. 1995. “Discovery of Betulinic Acid as a Selective Inhibitor of Human Melanoma That Functions by Induction of Apoptosis.” *Nature Medicine* 1(10):1046–51. doi:10.1038/nm1095-1046.

Pozo-Guisado, Eulalia, Jaime M. Merino, Sonia Mulero-Navarro, M. Jesús Lorenzo-Benayas, Francisco Centeno, Alberto Alvarez-Barrientos, and Pedro M. Fernandez Salguero. 2005. “Resveratrol-Induced Apoptosis in MCF-7 Human Breast Cancer Cells Involves a Caspase-Independent Mechanism with Downregulation of Bcl-2 and NF-κB.” *International Journal of Cancer* 115(1):74–84. doi:10.1002/ijc.20856.

Ramakrishna, Akula, and Gokare Aswathanarayana Ravishankar. 2011. “Influence of Abiotic Stress Signals on Secondary Metabolites in Plants.” *Plant Signaling & Behavior* 6(11):1720–31. doi:10.4161/psb.6.11.17613.

Rauf, Abdur, Muhammad Imran, Imtiaz Ali Khan, Mujeeb- Ur-Rehman, Syed Amir Gilani, Zaffar Mehmood, and Mohammad S. Mubarak. 2018. “Anticancer Potential of Quercetin: A Comprehensive Review.” *Phytotherapy Research: PTR* 32(11):2109–30. doi:10.1002/ptr.6155.

Saadh, Mohamed J., Hanan Hassan Ahmed, Muktesh Chandra, Ali Fawzi Al-Hussainy, Junainah Abd Hamid, Anurag Mishra, Waam Mohammed Taher, Mariem Alwan, Mahmood Jasem Jawad, Ali M. Ali Al-Nuaimi, Fahad Alsaikhan, Bagher Farhood, and Reza Akhavan-Sigari. 2025. “Therapeutic Effects of Quercetin in Oral Cancer Therapy: A Systematic Review of Preclinical Evidence Focused on Oxidative Damage, Apoptosis and Anti-Metastasis.” *Cancer Cell International* 25(1):66. doi:10.1186/s12935-025-03694-1.

Samova, Sanman, Chirag N. Patel, Hetal Doctor, Himanshu A. Pandya, and R. J. Verma. 2018. “The Effect of Bisphenol A on Testicular Steroidogenesis and Its Amelioration by Quercetin: An in Vivo and in Silico Approach.” *Toxicology Research* 7(1):22–31. doi:10.1039/c7tx00161d

Schimites, P. I., H. J. Segat, L. G. Teixeira, L. R. Martins, L. T. Mangini, P. S. Baccin, H. Z. Rosa, L. H. Milanesi, M. E. Burger, and A. V. Soares. 2020. “Gallic Acid Prevents Ketamine-Induced Oxidative Damages in Brain

Regions and Liver of Rats." *Neuroscience Letters* 714:134560. doi:10.1016/j.neulet.2019.134560.

Schwertheim, Suzan, Frederik Wein, Klaus Lennartz, Karl Worm, Kurt Werner Schmid, and Sien-Yi Sheu-Grabellus. 2017. "Curcumin Induces G2/M Arrest, Apoptosis, NF-κB Inhibition, and Expression of Differentiation Genes in Thyroid Carcinoma Cells." *Journal of Cancer Research and Clinical Oncology* 143(7):1143–54. doi:10.1007/s00432-017-2380-z.

Sertel, Serkan, Yujie Fu, Yuangang Zu, Blanka Rebacz, Badireenath Konkimalla, Peter K. Plinkert, Alwin Krämer, Jürg Gertsch, and Thomas Efferth. 2011. "Molecular Docking and Pharmacogenomics of Vinca Alkaloids and Their Monomeric Precursors, Vindoline and Catharanthine." *Biochemical Pharmacology* 81(6):723–35. doi:10.1016/j.bcp.2010.12.026.

Shah, Nirali N., Eric S. Schafer, Yueh-Yun Chi, Jemily Malvar, Kenneth M. Heym, Andrew E. Place, Melissa Burns, Bill H. Chang, Tamra Slone, Anupam Verma, Nathan Gossai, Peter H. Shaw, Michael J. Burke, Michelle Hermiston, Reuven J. Schore, Todd Cooper, Melinda Pauly, Teresa Rushing, Paul Jarosinski, Ellynore Florendo, Bonnie Yates, Brigitte C. Widemann, Cody J. Peer, William D. Figg, Lewis B. Silverman, Deepa Bhojwani, and Alan S. Wayne. 2025. "Vincristine Sulfate Liposome Injection With Combination Chemotherapy for Children, Adolescents, and Young Adults With Relapsed Acute Lymphoblastic Leukemia: A Therapeutic Advances in Childhood Leukemia and Lymphoma Consortium Trial." *Pediatric Blood & Cancer* 72(5):e31584. doi:10.1002/pbc.31584.

Shankar, Eswar, Ailin Zhang, Daniel Franco, and Sanjay Gupta. 2017. "Betulinic Acid-Mediated Apoptosis in Human Prostate Cancer Cells Involves P53 and Nuclear Factor-Kappa B (NF-κB) Pathways." *Molecules (Basel, Switzerland)* 22(2):264. doi:10.3390/molecules22020264.

Sinha, R., D. E. Anderson, S. S. McDonald, and P. Greenwald. 2003. "Cancer Risk and Diet in India." *Journal of Postgraduate Medicine* 49(3):222–28.

Sordillo, Peter P., and Lawrence Helson. 2015. "Curcumin and Cancer Stem Cells: Curcumin Has Asymmetrical Effects on Cancer and Normal Stem Cells." *Anticancer Research* 35(2):599–614.

Sun, Guojun, Shuqin Zhang, Yanru Xie, Ziyu Zhang, and Wenjing Zhao. 2016. "Gallic Acid as a Selective Anticancer Agent That Induces Apoptosis in SMMC-7721 Human Hepatocellular Carcinoma Cells." *Oncology Letters* 11(1):150–58. doi:10.3892/ol.2015.3845.

Szkudelska, Katarzyna, and Tomasz Szkudelski. 2010. "Resveratrol, Obesity and Diabetes." *European Journal of Pharmacology* 635(1–3):1–8. doi:10.1016/j.ejphar.2010.02.054.

Thakur, Menaka, Sujata Bhattacharya, Prem Kumar Khosla, and Sunil Puri. 2019. "Improving Production of Plant Secondary Metabolites through Biotic and Abiotic Elicitation." *Journal of Applied Research on Medicinal and Aromatic Plants* 12:1–12. doi:10.1016/j.jarmap.2018.11.004.

Tossetta, Giovanni, Sonia Fantone, Elena Marinelli Busilacchi, Daniela Marzioni, and Roberta Mazzucchelli. 2025. "Dose-Dependent Effects of Curcumin on 22Rv1 Prostate Cancer Cell Line." *Molecular Biology Reports* 52(1):339. doi:10.1007/s11033-025-10448-9.

Tsai, Chung-Lin, Ying-Ming Chiu, Tin-Yun Ho, Chin-Tung Hsieh, Dong-Chen Shieh, Yi-Ju Lee, Gregory J. Tsay, and Yi-Ying Wu. 2018. "Gallic Acid Induces Apoptosis in Human Gastric Adenocarcinoma Cells." *Anticancer Research* 38(4):2057–67. https://ar.iiarjournals.org/content/38/4/2057.

Wang, Hua, Qi-Rong Geng, Liang Wang, and Yue Lu. 2012. "Curcumin Potentiates Antitumor Activity of L-Asparaginase via Inhibition of the AKT Signaling Pathway in Acute Lymphoblastic Leukemia." *Leukemia & Lymphoma* 53(7):1376–82. doi:10.3109/10428194.2011.649478.

Wang, Li-Bin, Dan-Ni Wang, Li-Gang Wu, Jia Cao, Jin-Hai Tian, Rong Liu, Rong Ma, Jing-Jing Yu, Jia Wang, Qi Huang, Wen-Yong Xiong, and Xu Zhang. 2021. "Homoharringtonine Inhibited Breast Cancer Cells Growth via miR-18a-3p/AKT/mTOR Signaling Pathway." *International Journal of Biological Sciences* 17(4):995–1009. doi:10.7150/ijbs.44907.

Wang, Ning-Ning, Li-Jun Zhao, Li-Nan Wu, Ming-Feng He, Jun-Wei Qu, Yi-Bing Zhao, Wan-Zhou Zhao, Jie-Shou Li, and Jin-Hua Wang. 2013. "Mechanistic Analysis of Taxol-Induced Multidrug Resistance in an Ovarian Cancer Cell Line." *Asian Pacific Journal of Cancer Prevention: APJCP* 14(9):4983–88. doi:10.7314/apjcp.2013.14.9.4983.

Wang, Qian, Wei Ding, Yihan Ding, Jingjing Ma, Zhaoye Qian, Jingxian Shao, and Yufeng Li. 2017. "Homoharringtonine Suppresses Imatinib Resistance via the Bcl-6/P53 Pathway in Chronic Myeloid Leukemia Cell Lines." *Oncotarget* 8(23):37594–604. doi:10.18632/oncotarget.16731.

Wang, Tao, Fei Chen, Zhe Chen, Yi-Feng Wu, Xiao-Li Xu, Shu Zheng, and Xun Hu. 2004. "Honokiol Induces Apoptosis through P53-Independent Pathway in Human Colorectal Cell Line RKO." *World Journal of Gastroenterology* 10(15):2205–8. doi:10.3748/wjg.v10.i15.2205.

Wang, Xuling, Yanzhi Song, Yuqing Su, Qingjing Tian, Boqun Li, Jingjing Quan, and Yihui Deng. 2015. "Are PEGylated Liposomes Better than

Conventional Liposomes? A Special Case for Vincristine.” *Drug Delivery* 29:1–9. doi:10.3109/10717544.2015.1027015.

Wang, Ying-Jan, Min-Hsiung Pan, Ann-Lii Cheng, Liang-In Lin, Yuan-Soon Ho, Chang-Yao Hsieh, and Jen-Kun Lin. 1997. “Stability of Curcumin in Buffer Solutions and Characterization of Its Degradation Products.” *Journal of Pharmaceutical and Biomedical Analysis* 15(12):1867–76. doi:10.1016/S0731-7085(96)02024-9.

Wang, Yongqi, Yong Chen, Lijie Liu, Donglei He, and He Li. 2020. “Effects of Artemisinin Combined with 5-Fluorouracil on Colon Cancer Cell Proliferation, Migration, and Drug Sensitivity via PI3K/AKT Signaling.” *Journal of Biomaterials and Tissue Engineering* 10(11):1600–1604. doi:10.1166/jbt.2020.2468.

Warburg, O., F. Wind, and E. Negelein. 1927. “The Metabolism of Tumors in The Body.” *The Journal of General Physiology* 8(6):519–30. doi:10.1085/jgp.8.6.519.

Weaver, Beth A. 2014. “How Taxol/Paclitaxel Kills Cancer Cells.” *Molecular Biology of the Cell* 25(18):2677–81. doi:10.1091/mbc.E14-04-0916.

Wilcox, Christopher S. 2002. “Reactive Oxygen Species: Roles in Blood Pressure and Kidney Function.” *Current Hypertension Reports* 4(2):160–66. doi:10.1007/s11906-002-0041-2.

Willoughby, Jamin A., Shyam N. Sundar, Mark Cheung, Antony S. Tin, Jaime Modiano, and Gary L. Firestone. 2009. “Artemisinin Blocks Prostate Cancer Growth and Cell Cycle Progression by Disrupting Sp1 Interactions with the Cyclin-Dependent Kinase-4 (CDK4) Promoter and Inhibiting CDK4 Gene Expression*.” *Journal of Biological Chemistry* 284(4):2203–13. doi:10.1074/jbc.M804491200.

Wink, Michael, and Margaret F. Roberts. 1998. “Compartmentation of Alkaloid Synthesis, Transport, and Storage.” Pp. 239–62 in *Alkaloids: Biochemistry, Ecology, and Medicinal Applications*, edited by M. F. Roberts and M. Wink. Boston, MA: Springer US.

Yoshihara, Hiroki, Yuri Yoshimoto, Yosuke Hosoya, Daisuke Hasegawa, Takafumi Kawano, Akiko Sakoda, Hajime Okita, and Atsushi Manabe. 2017. “Infantile Fibrosarcoma Treated with Postoperative Vincristine and Dactinomycin.” *Pediatrics International: Official Journal of the Japan Pediatric Society* 59(3):371–74. doi:10.1111/ped.13229.

Zamora-Ros, Raul, Montserrat Rabassa, Rafael Llorach, Carlos A. González, and Cristina Andres-Lacueva. 2012. “Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future.” *Journal of*

Agricultural and Food Chemistry 60(27):6648–57. doi:10.1021/jf204742e.

Zhang, Dong-Mei, Hong-Gui Xu, Lei Wang, Ying-Jie Li, Ping-Hua Sun, Xiao-Ming Wu, Guang-Ji Wang, Wei-Min Chen, and Wen-Cai Ye. 2015. "Betulinic Acid and Its Derivatives as Potential Antitumor Agents." *Medicinal Research Reviews* 35(6):1127–55. doi:10.1002/med.21353.

Zhang, Junjia, Jichun Yu, Rong Xie, Wanzhi Chen, and Yunxia Lv. 2016. "Combinatorial Anticancer Effects of Curcumin and Sorafenib towards Thyroid Cancer Cells via PI3K/Akt and ERK Pathways." *Natural Product Research* 30(16):1858–61. doi:10.1080/14786419.2015.1074229.

Chapter 13

From Food To Fertility: Bioaccumulation Of Micro/Nanoplastics (MNPs) And Female Reproductive Health Risks

Seda KESKIN [1*], Neşe ÇÖLÇİMEN[2]

Abstract

The global increase in plastic production has made environmental micro- and nanoplastics (MNPs) pollution a critical public health issue. MNPs are released into the environment through physical fragmentation, UV light exposure, and biological degradation, and can enter the human body through various routes, including ingestion, inhalation, and dermal absorption. These particles can exhibit endocrine-disrupting, genotoxic, and immunomodulatory effects through the additives they carry and the toxic agents adsorbed on their surfaces. The female reproductive system is susceptible to the impact of such chemicals due to its hormone-dependent functioning. Exposure to environmental toxins can disrupt physiological processes, including ovulation, embryo implantation, and pregnancy. Experimental studies conducted in recent years have shown that MNPs can accumulate in target tissues, including the ovary, endometrium, and placenta, thereby affecting the reproductive process in multiple ways. This indicates that MNPs pose a risk to female fertility not only systemically but also locally. In this context, the effects of MNP exposure on female fertility should be addressed holistically within the framework of environmental health, toxicology, and reproductive biology.

Keywords: Micro-Nanoplastics (MNPs), Female Fertility, Endocrine Disruptors, Environmental Exposure,

[1] Department of Medical Histology and Embryology, Faculty of Medicine, Van Yuzuncu Yil University, ORCID: 0000-0002-4726-982X, Van, Turkey

* Correspondence: sedakeskin@yyu.edu.tr, ORCID: 0000-0002-4726-982X

[2] Department of Medical Histology and Embryology, Faculty of Medicine, Van Yuzuncu Yil University, ORCID: 0000-0002-7695-3049, Van, Turkey

1. Introduction

The global increase in plastic production and use has led to significant pollution of microplastics and nanoplastics (MNP) in environmental and biological systems, posing a substantial health issue. Microplastics generally refer to plastic particles smaller than 5 mm, while nanoplastics refer to plastic particles smaller than 1000 nanometres. These particles are released into the environment through the physical breakdown of plastics, exposure to UV light, and biological degradation in marine and terrestrial ecosystems. They can enter the human body through various direct/indirect pathways. MNPs may exhibit potential endocrine-disrupting, genotoxic, and immunomodulatory effects, both through the additives they carry and through other toxic agents they adsorb in the environment (Ashokkumar et al., 2025; Dar et al., 2025).

Female reproductive health is a complex, dynamic biological system susceptible to environmental pollutants. In particular, physiological events such as ovulation, menstruation, fertilisation, embryo implantation, and pregnancy are regulated by the delicate balance of the endocrine system. The regular functioning of these processes is highly fragile, both in internal hormonal cycles and in response to external chemical disruptors (Inam, 2025). In this context, it has been scientifically established that environmentally sourced endocrine-disrupting chemicals, particularly molecules exhibiting oestrogenic or anti-oestrogenic properties, can have serious and irreversible effects on female reproductive physiology.

Recent studies have demonstrated that MNPs may exhibit endocrine-disrupting effects (Anbuchelvan et al., 2025; Lee et al., 2025; Pathak, 2025). These particles can influence hormonal regulation directly or indirectly, both through their chemical structure and through toxic compounds (such as heavy metals, pesticides, phthalates, and bisphenol A) that can be carried on their surfaces. The potential for MNPs to enter systemic circulation and accumulate in reproductive organs, such as the ovaries, endometrium, and placenta, underscores their potential effects on reproductive health. Findings from both animal models and cell culture studies increasingly suggest that reproductive processes such as folliculogenesis, ovulation, luteinisation, the endometrial cycle, fertilisation, and embryonic development may be disrupted by MNP exposure (Inam, 2025; Land et al., 2025). This situation highlights the risk that MNP exposure may cause not only systemic toxicity but also localised disruptions in tissue and cellular microarchitecture. Therefore, the potential effects of MNPs on fertility are emerging as a priority research area that must be addressed not only from an environmental health perspective but also at the molecular level, at the intersection of reproductive biology, endocrinology, and toxicology disciplines.

Evidence is growing that MNPs can affect these processes at both systemic and local levels; therefore, the effects of MNP exposure on female fertility are a priority topic that warrants investigation at the intersection of environmental health and reproductive biology.

2. Sources and Routes of Human Exposure to Micro- and Nanoplastics

2.1. Dietary Intake: Contaminated Food and Water

The most common route of entry of MNP particles into the human body is oral exposure. Seafood, in particular, is among the primary sources for the biological accumulation of these particles. Marine organisms, such as mussels, oysters, and small pelagic fish, can accumulate high levels of microplastics due to their filter-feeding habits and tendency to accumulate at higher trophic levels. However, it is not only marine organisms; sea-sourced table salt, packaged drinking water, beverages served in plastic containers, and some industrially processed food products are also potential sources of MNP exposure for human health.

Recent studies have revealed that the average individual consumes approximately 5 grams of microplastics per week (Mohamed Nor et al., 2021). This amount equates to a plastic load equivalent to the size of a credit card, indicating a serious public health problem across society. However, the long-term physiopathological effects of this exposure have not yet been fully established. Current findings are associated with various adverse effects, including chronic local inflammation in the gastrointestinal tract, increased intestinal mucosal permeability (leaky gut), dysbiosis of the microbiota, and impairments in mucosal immune responses. This situation indicates that it may have indirect effects not only on gut health but also on systemic inflammation, immune responses, and even neuroendocrine functions (Thin et al., 2025). When explicitly evaluated in terms of women's health, the potential for MNPs absorbed through the gastrointestinal system to reach the reproductive system via the circulatory system raises the possibility of direct effects on reproductive functions. In this context, hypotheses suggesting that oral exposure may affect not only digestive system diseases but also processes such as hormonal balance, follicle development, embryo implantation, and placental barrier function require further support from comprehensive experimental data.

2.2. Inhalation and Dermal Contact

Microplastics are increasingly recognised as being absorbed into the human body not only orally, but also through inhalation and dermal contact (Keskin et al., 2024). Particularly in enclosed living spaces, microplastic particles released into the environment from sources such as carpeting, synthetic textile products, furniture coverings, and plastic-based household items can enter the respiratory system through inhalation (Lombardi et al., 2022). In this context, a 2022 study that directly detected microplastic particles in human lung tissue at the histological level clearly demonstrated that environmental particles can enter the body through respiration and reach systemic circulation (Amato-Lourenço et al., 2022). It is believed that these particles, ingested through the respiratory tract, reach the alveoli via the bronchial epithelium, then enter the circulatory system, where they may accumulate in target organs. This situation suggests that micro/nanoplastics can exhibit distribution levels in biological systems that can lead to systemic effects, not just local toxicity.

On the other hand, although exposure through the skin is considered less extensive than through the respiratory and oral routes, it is significant for nanoscale particles. The current literature suggests that nanoparticles can penetrate epidermal barriers, such as the stratum corneum, and reach the dermis, potentially entering systemic circulation via transdermal absorption (Keskin et al., 2024). It is suggested that skin penetration may be facilitated and MNPs may be absorbed into the body, particularly in areas of skin where the epidermal integrity is compromised, such as open wounds or in conditions involving barrier dysfunction, such as atopic dermatitis. Therefore, MNP exposure is not solely food-related, and the combined assessment of multiple exposure routes (oral, inhalation, dermal) is of great importance for realistic risk estimation (Aristizabal et al., 2024).

2.3. Translocation and Systemic Distribution Mechanisms

When ingested through the digestive system, MNPs can bypass the intestinal epithelial barrier via paracellular transport mechanisms, primarily through M cells, and enter the systemic circulation. Nanoplastics, in particular, can pass through cell membranes more effectively due to their small size and surface properties. After being taken up into the cell via endocytosis, MNPs can reach the cytoplasm or various organelles directly, avoiding lysosomal degradation. This property increases the potential for nanoplastics to affect intracellular biological processes directly. Following the entry of MNPs into the systemic circulation, their accumulation in metabolically active organs with high blood flow, such as the liver, spleen, lungs, and kidneys, has been demonstrated in both animal models and human autopsy studies (Hoang et al., 2025; Nouri et al., 2025; Thin et al., 2025).

Furthermore, various experimental and clinical observations support the notion that these particles are not limited to adult tissues but can also cross the placental barrier and reach foetal tissues (Anifowoshe et al., 2025). This situation raises critical concerns regarding the potential teratogenic, epigenetic, and immunological effects of maternal MNP exposure on the developing foetus, particularly during pregnancy. Therefore, the ability of MNPs to cross biological barriers and accumulate in both maternal and foetal tissues necessitates further toxicological investigations, not only in terms of women's reproductive health but also in terms of intergenerational health effects. This raises the issue of the potential harmful effects of exposure during pregnancy on foetal development (Anifowoshe et al., 2025; C. Wang et al., 2025).

3. Physicochemical Properties and Bioaccumulation Dynamics

3.1. Particle Size, Surface Chemistry, and Toxicological Relevance

The toxic effects of MNPs in biological systems depend primarily on their physicochemical properties. Particles of nanoplastic size (≤1000 nm), in particular, are more advantageous in terms of crossing biological barriers and reaching intracellular structures due to their high surface-to-diameter ratios and increased reactivity. These properties significantly increase their potential to disrupt the functions of cellular organelles, such as mitochondria, lysosomes, and even the nucleus, by directly interacting with them (Molenaar et al., 2021). During intracellular nanoplastic distribution, pathophysiological processes such as the induction of oxidative stress responses, disruption of mitochondrial membrane integrity, and leakage of lysosomal enzymes have been observed (Banerjee et al., 2021). However, the predominantly hydrophobic surface properties of MNPs facilitate their interaction with other toxic compounds present in the environmental matrix. Persistent organic pollutants (e.g., polychlorinated biphenyls [PCBs], dichlorodiphenyltrichloroethane [DDT]), heavy metals (e.g., lead, cadmium), and antibiotics, in particular, can be transported by adsorbing onto the surface of these particles (Zhang & Xu, 2022). This situation highlights a mechanism defined in the literature as the 'Trojan horse effect,' which results in a combined toxic effect by increasing both the toxicity of the carrier particle itself and the bioavailability of the substances it carries (Mahendran & Ramaswamy, 2024). Therefore, MNPs should be evaluated not only for their toxicity based on their physical structure, but also for their synergistic or potentiated toxic effects resulting from interactions with other environmental pollutants. Understanding such complex exposure scenarios is crucial for comprehensively assessing the ecological and biological risks associated with MNPs.

3.2. Interaction with Biological Membranes and Endocrine Systems

The biological effects of MNP particles at the cellular level primarily begin with their uptake via endocytosis. Following endocytic uptake, these particles destabilise lysosomal membranes, negatively affecting intracellular redox balance and significantly increasing the production of reactive oxygen species (ROS). Increased oxidative stress can lead to impaired cell membrane integrity, increased cell damage via lipid peroxidation, DNA breakage, and mitochondrial dysfunction (Jiang et al., 2019; Tyc et al., 2025). These molecular disruptions negatively affect tissue physiological functions by triggering processes such as cellular ageing, apoptosis, and genetic instability (Mahendran & Ramaswamy, 2024; Zhang & Xu, 2022). Furthermore, certain additives frequently used in the production of polymer structures, particularly phthalate esters and bisphenol A (BPA), are classified as endocrine-disrupting chemicals. These substances can mimic or block natural hormone signalling pathways by binding to oestrogen receptors. By disrupting estrogenic activity, they irregularize feedback mechanisms in the hypothalamic-pituitary-gonadal axis and destabilise hormonal balance. As a result, fundamental steps in female reproductive physiology, such as folliculogenesis, ovulation, luteinisation, and corpus luteum function, may be directly affected (Jahedi et al., 2025; Nowak-Jary & Machnicka, 2024). Consequently, the interaction of MNPs with cells is not limited to a physical particle effect but also leads to multi-layered disruptions at the biochemical and endocrinological levels, posing serious risks to female fertility. These effects can directly impact numerous reproductive processes, from follicle development to corpus luteum function.

3.3. Accumulation in Reproductive Organs: Evidence from *In Vivo* and *In Vitro* Studies

Various *in vivo* studies using animal models have clearly demonstrated that MNP particles can accumulate in specific organs of the female reproductive system, particularly in ovarian, uterine, and placental tissues. The bioaccumulative nature of these particles suggests they may cause serious biological effects on reproductive health, especially with repeated, low-dose exposure (Balali et al., 2024; Geng et al., 2023; Shahsavari et al., 2025). Zeng et al. (2023) investigated the ovarian effects of polystyrene nanoplastics (PS-NPs) on human ovarian granulosa cells and female mice. They suggested that these nanoplastics may have potentially harmful effects on female reproductive health, notably that PS-NPs may cause cell death in ovarian cells and that oxidative stress may be a pathway through which PS-NPs exert their effects. The study also addressed folliculogenesis, the process of follicle development, and reported that PS-NPs had harmful effects on ovarian function and fertility. These findings suggest that exposure to PS-NPs has observable effects on ovarian cells and processes in both cellular and animal models, through mechanisms involving apoptosis and oxidative stress, and that it impacts women's reproductive health. Similarly, *in vitro* findings indicate that MNPs can cause direct cellular toxicity in granulosa cells. These studies showed increased apoptosis-related molecular markers, particularly caspase-3 activity, along with significant downregulation of key steroidogenic genes (StAR, CYP19A1). This suggests that MNPs can disrupt not only hormonal signalling but also fundamental reproductive events such as follicle development, ovulation, and oocyte maturation at the molecular level (Zeng et al., 2023).

In a similar study, Xue et al. showed that PS-NP exposure suppresses the PI3K-AKT signalling pathway in granulosa cells, thereby increasing autophagy and apoptosis associated with this pathway, disrupting the balance of steroidogenesis, and ultimately leading to decreased oocyte quality. RNA sequencing analysis confirmed that the PI3K-AKT pathway is the primary signalling pathway in response to PS-NPs. PS-NPs cannot directly enter oocytes, but they can indirectly affect oocyte function via granulosa cells. Pre-incubation of granulosa cells with PS-NPs resulted in a significant decrease in oocyte quality; this effect could be partially reversed by 17β-estradiol. In conclusion, it was emphasised that PS-NPs may reduce female fertility by disrupting ovarian functions, and that developing strategies to prevent these effects is essential (Xue et al., 2024). In addition to the direct impacts on the ovaries, studies by Balali et al. have documented that these materials contribute to significant reproductive health challenges, including decreased ovarian reserve capacity (Balali et al., 2024). These findings collectively underscore the multifaceted adverse impact of nanoplastics on female reproductive health. In another study, PS-MPs characterised by FTIR, TEM, and fluorescence microscopy, measuring 1 μm, were administered to female mice at a dose of 30 mg/kg for 35 days.

The results revealed that PS-MPs accumulated in ovarian tissue, leading to follicular atresia and increased granulosa cell apoptosis. TEM analyses detected mitochondrial morphological deterioration and ultrastructural damage in granulosa cells. Post-superovulation analyses revealed a decrease in the number of ovulations, disruption of spindle fibres and chromosome integrity in metaphase II (MII) oocytes, abnormalities in mitochondrial distribution patterns, and increased ROS levels. These effects indicate that PS-MPs exposure reduces oocyte quality via oxidative stress. A significant decrease in the number of offspring born to females exposed to PS-MPs was determined. Immunohistochemical analyses revealed increased Cleaved Caspase-3 levels and decreased Bcl-2 expression, indicating activation of apoptosis in granulosa cells. At the molecular level, it was found that PS-MPs induce cell death by activating the JNK and ERK signalling pathways, while suppressing the AKT pathway, thereby weakening cell survival and ovarian function. In addition, the *in vitro* follicle culture model demonstrated that PS-MPs exposure negatively affects female fertility, reduces oocyte quality, and may lead to loss of ovarian function by impairing granulosa cell survival (Jeong et al., 2025). The results obtained both *in vivo* and *in vitro* strongly support the notion that MNPs may cause target organ toxicity in the female reproductive system and have direct adverse effects on cellular function, hormone production, and fertility. In this context, conducting further research into the mechanical basis of MNP-related reproductive toxicity is crucial for understanding the effects of environmental pollutants on reproductive health.

4. Mechanisms of MNPs-Induced Female Reproductive Toxicity

Recent experimental and epidemiological studies have shown that MNPs can enter the systemic circulation, reach ovarian tissue, accumulate there, and affect female reproductive function in multiple ways. These particles not only cause direct cellular toxicity but also threaten reproductive health through indirect mechanisms. Particularly after their intracellular uptake, they disrupt cellular homeostasis by triggering processes such as increased ROS production, mitochondrial dysfunction, lipid peroxidation, and DNA damage. This leads to the suppression of antioxidant defence systems (SOD, GSH-Px, CAT) in granulosa cells and the activation of early cellular ageing (senescence) and apoptosis. It has also been reported that MNPs enhance inflammatory responses by increasing the expression of proinflammatory cytokines (IL-1β, IL-6, TNF-α) in ovarian tissue. This increase in cytokines negatively affects the proliferative capacity and steroid hormone synthesis of cells involved in follicle development, leading to suppression of folliculogenesis and reduced fertility potential. In parallel, MNPs have been shown to disrupt the balance of estrogen and progesterone by targeting the endocrine system. Chemical additives, especially bisphenol A (BPA) and phthalates, carried by or contained in these particles, compete with natural hormones by binding to estrogen and progesterone receptors with high affinity, disrupting hormonal signaling in the hypothalamus-pituitary-ovarian (HPO) axis. This antagonistic interaction starting at the receptor level disrupts mechanisms regulating gonadotropin secretion (GnRH, LH, FSH), suppressing the steroidogenesis process and ovarian functions. Consequently, this can lead to clinical conditions such as estrogen/progesterone imbalance, follicle maturation disorders, anovulation, luteal phase defects, and menstrual irregularities. Moreover, these hormonal imbalances might adversely impact endometrial receptivity and embryo implantation, ultimately leading to early pregnancy losses.

Oocyte development is a complex biological process that involves the precise coordination of hormonal signals, intraovarian factors, and cellular interactions, and is highly sensitive to environmental conditions. In this process, the microenvironment of the ovaries, particularly supportive cell populations such as theca and granulosa cells, play critical roles in oocyte maturation, metabolic stability, and genomic integrity. Recent experimental studies have shown that MNPs can interact with cells in this microenvironment, thereby negatively affecting folliculogenesis Specifically, polystyrene-based nanoparticles have been reported to significantly reduce oocyte quality, disrupt the microtubule network, leading to spindle disorganisation, and trigger chromosomal aneuploidy (Haddadi et al., 2022; Raghuvir et al., 2025). The structural integrity of the microtubule-spindle complex is vital for proper chromosome separation and healthy embryo development. Therefore, the relationship between MNP exposure and disruption of spindle integrity highlights the vulnerability of fertility to environmental toxins. Furthermore, evidence is mounting that MNPs disrupt cell skeleton dynamics by disrupting the cell skeleton (particularly actin filaments and microtubules) during mitotic and meiotic divisions, thereby reducing the maturation capacity of oocytes and their post-fertilisation development potential (Cui et al., 2025; Tang, 2025; Zhao et al., 2025). This can lead to segmentation errors, blastomere dysfunction, and implantation failure in early embryo development. Therefore, it is thought that the effects of MNPs on ovarian function are not limited to disrupting hormonal balance but also constitute a multi-layered risk factor for female fertility by directly interfering with cytoskeletal structure. These findings provide an essential foundation for guiding future research into understanding the effects of environmental pollutants on reproductive biology. When all these results are evaluated together, it becomes clear that micro/nanoplastics create a multi-layered toxicity model by simultaneously targeting oxidative stress, the inflammatory response, and endocrine disruption mechanisms in the female reproductive system. These multifaceted effects not only impair ovarian functions and oocyte quality in the short term but also become a serious environmental risk factor threatening fertility potential in the long term.

5. Impacts on Uterine Environment, Endometrial Receptivity and Fetal Exposure

Maintaining optimal female fertility is not limited solely to the presence of a morphologically and genetically healthy oocyte; it also requires that the endometrium be hormonally synchronised and prepared, and that an immunological and cellular microenvironment conducive to successful embryo implantation be provided (Strowitzki et al., 2006). All of these processes require a delicate hormonal balance and complex physiological coordination operating at the tissue level (Ahmad et al., 2025). Recent studies have shown that MNPs can enter the systemic circulation within the maternal organism and accumulate not only in ovarian structures but also in target organs directly related to pregnancy, such as the uterus and placenta (Aghaei et al., 2022; He & Zhang, 2025; Xu et al., 2024). It has been suggested that this accumulation may adversely affect endometrial tissue structure, vascularisation, and embryo receptivity; it may also directly threaten the foetal development environment by disrupting the integrity of the placental barrier (Haddadi et al., 2025; P. Wang et al., 2025; Xu et al., 2025). For this reason, the effects of MNP exposure on female fertility constitute a multifaceted risk profile that extends beyond ovulation and folliculogenesis processes to encompass numerous critical physiological stages from the onset of pregnancy to foetal implantation.

The successful implantation of the embryo depends on the endometrial tissue reaching a physiologically receptive state, i.e., the 'receptive phase.' This receptive state requires the timely and coordinated occurrence of cellular proliferation, stromal differentiation (decidualisation), the development of immune tolerance, and appropriate cytokine-matrix interactions (Qin et al., 2024). Reported experimental studies have shown that after reaching the uterine tissue, MNPs trigger cellular stress responses, particularly in endometrial epithelial cells, and weaken the integrity of the mucosal barrier. This disruption increases epithelial permeability and facilitates the easier penetration of pathogenic or environmental toxins, thereby triggering a local inflammatory response (Hu et al., 2021). The disruption of cytokine balance resulting from MNP exposure, . It particularly disrupts the critical balance between anti-inflammatory and pro-inflammatory cytokines, prevents the formation of an embryo-supportive immune microenvironment, and adversely affects implantation (N. Kim et al., 2025).

Additionally, owing to their endocrine-disturbing capabilities, MNPs have been documented to inhibit the actions of oestrogen and progesterone on target cells, hence disrupting the continuity of proliferative and secretory alterations in the endometrium. This situation may lead to the interruption of molecular-level interactions between the embryo and maternal tissue and, consequently, to implantation failure (Qiao et al., 2025; Z. Wang et al., 2025). Therefore, MNP exposure involves multiple mechanisms that may compromise female fertility, not only through cellular stress and inflammation but also through hormonal signalling inhibition.

Recent advanced studies have demonstrated that MNPs can reach fetal tissues by crossing the placental barrier, not just maternal tissues. This situation has made it even more urgent to assess the effects of plastic-derived environmental pollutants on pregnancy physiology. Indeed, various *in vitro* models using human placental tissue have demonstrated that nanoplastic particles can be internalised by cytotrophoblast and syncytiotrophoblast cells via endocytosis, leading to disruptions in cytoskeletal organisation and reduced cell viability. The transplacental passage of these particles not only causes toxic accumulation in fetal tissues but is also associated with multidimensional effects, including developmental toxicity, organogenesis anomalies, and epigenetic reprogramming (Andonotopo et al., 2025; J. Kim et al., 2025; Ragusa et al., 2022; Zhang et al., 2025).

Oxidative stress induced by MNPs in placental tissue leads to increased ROS and inadequate antioxidant defence mechanisms, which can result in pathophysiological consequences, including placental inflammation, inhibition of trophoblast invasion, and disorders of spiral artery remodelling. These processes are directly related to an increased risk of severe obstetric complications frequently encountered during pregnancy, such as intrauterine growth restriction (IUGR), pre-eclampsia, placental insufficiency, and spontaneous miscarriage. Moreover, it has been suggested that such environmental exposures during the developmental period of fetal organ systems may have long-term effects at the epigenetic level (e.g., DNA methylation, histone modifications), and that these effects may be associated with childhood disease risks and the development of chronic diseases later in life (Inam, 2025; N. Kim et al., 2025; Qiao et al., 2025; Z. Wang et al., 2025). Scientific evidence supports the notion that MNP exposure poses a threat not only to the maternal system but also to the fetal development environment at the cellular and molecular levels. Therefore, it is crucial to develop risk assessment protocols specific to pregnancy and to encourage multidisciplinary research focusing on placenta-fetal interactions.

6. Conclusion

The effects of MNPs on women's reproductive health remain an emerging field of research that has not yet been sufficiently elucidated, despite growing scientific interest. It has been reported that these particles, which enter the body through food, water, and environmental sources, may accumulate in biological systems, leading to cellular stress, hormonal imbalances, and genetic disruptions. Evidence is mounting that sensitive processes such as ovarian function, endocrine regulation, and embryo implantation may be adversely affected by this exposure.

The failure of current regulatory systems to adequately address the toxicological risks specific to MNPs creates a gap that threatens not only individual fertility but also public health and the reproductive potential of future generations. The inadequacy of current regulatory frameworks for these new generation contaminants paves the way for a public health problem that could jeopardise both individual health and the reproductive capacity of future generations. Therefore, an interdisciplinary approach is required, involving collaboration between toxicology, environmental science, women's health and policymakers, to conduct scientific research and implement evidence-based regulations. Consequently, rapidly increasing comprehensive studies evaluating the effects of MNP on the female reproductive system will pave the way for both social awareness and science-based policy-making. In this process, preventive strategies developed on the basis of scientific data will play a fundamental role in protecting reproductive health.

References

Aghaei, Z., Mercer, G. V., Schneider, C. M., Sled, J. G., Macgowan, C. K., Baschat, A. A., Kingdom, J. C., Helm, P. A., Simpson, A. J., Simpson, M. J., Jobst, K. J., & Cahill, L. S. (2022). Maternal exposure to polystyrene microplastics alters placental metabolism in mice. *Metabolomics*, *19*(1), 1. https://doi.org/10.1007/s11306-022-01967-8

Ahmad, F., Ahmed, S. H., Choucair, F., Chouliaras, S., Awwad, J., & Terranegra, A. (2025). A disturbed communication between hypothalamic-pituitary-ovary axis and gut microbiota in female infertility: is diet to blame? *Journal of Translational Medicine*, *23*(1), 92. https://doi.org/10.1186/s12967-025-06117-x

Anbuchelvan, S. K., Udayakumaran, H., Xin, A. Y., Loshni, Y., & Rashid, F. (2025). Microplastics and endocrine disruption: Emerging risks for human fertility. *Journal of Biomedical Sciences*, *12*(1), 11–14.

Andonotopo, W., Bachnas, M. A., Dewantiningrum, J., Pramono, M. B. A., Sanjaya, I. N. H., Stanojevic, M., & Kurjak, A. (2025). Microplastics in the Perinatal Period: Emerging Evidence on Maternal Exposure, Placental Transfer, and Fetal Health Outcomes. *Sarvodaya International Journal of Medicine*, *1*(3).

Anifowoshe, A. T., Akhtar, M. N., Majeed, A., Singh, A. S., Ismail, T. F., & Nongthomba, U. (2025). Microplastics: A threat to Fetoplacental unit and Reproductive systems. *Toxicology Reports*, 101938.

Aristizabal, M., Jiménez-Orrego, K. V., Caicedo-León, M. D., Páez-Cárdenas, L. S., Castellanos-García, I., Villalba-Moreno, D. L., Ramírez-Zuluaga, L. V., Hsu, J. T., Jaller, J., & Gold, M. (2024). Microplastics in dermatology: Potential effects on skin homeostasis. *Journal of Cosmetic Dermatology*, *23*(3), 766–772.

Ashokkumar, V., Chandramughi, V. P., Mohanty, K., & Gummadi, S. N. (2025). Microplastic pollution: Critical analysis of global hotspots and their impact on health and ecosystems. *Journal of Environmental Management*, *381*, 124995. https://doi.org/https://doi.org/10.1016/j.jenvman.2025.124995

Balali, H., Morabbi, A., & Karimian, M. (2024). Concerning influences of micro/nano plastics on female reproductive health: focusing on cellular and molecular pathways from animal models to human studies. *Reproductive Biology and Endocrinology*, *22*(1), 141.

Banerjee, A., Billey, L. O., & Shelver, W. L. (2021). Uptake and toxicity of polystyrene micro/nanoplastics in gastric cells: Effects of particle size and surface functionalization. *PloS One*, *16*(12), e0260803.

Cui, Z., Zhang, J., Zhang, J., Zhong, J., Wu, J., Miao, Y., & Wang, H. (2025). Microplastics (MPs) exposure impairs porcine oocyte quality by triggering oxidative stress-directed DNA damage and apoptosis with metabolomic alterations. *Ecotoxicology and Environmental Safety*, *300*, 118461.

Dar, M. A., Palsania, P., Satya, S., Dashora, M., Bhat, O. A., Parveen, S., Patidar, S. K., & Kaushik, G. (2025). Microplastic pollution: A global perspective in surface waters, microbial degradation, and corresponding mechanism. *Marine Pollution Bulletin*, *210*, 117344. https://doi.org/https://doi.org/10.1016/j.marpolbul.2024.117344

Geng, Y., Liu, Z., Hu, R., Huang, Y., Li, F., Ma, W., Wu, X., Dong, H., Song, K., & Xu, X. (2023). Toxicity of microplastics and nanoplastics: invisible killers of female fertility and offspring health. *Frontiers in Physiology*, *14*, 1254886.

Haddadi, A., Kessabi, K., Boughammoura, S., Rhouma, M. B., Mlouka, R., Banni, M., & Messaoudi, I. (2022). Exposure to microplastics leads to a defective ovarian function and change in cytoskeleton protein expression in rat. *Environmental Science and Pollution Research*, *29*(23), 34594–34606.

Haddadi, A., Venditti, M., Kessabi, K., & Messaoudi, I. (2025). Adverse effects of a realistic concentration of human exposure to microplastics on markers of placental barrier permeability in pregnant rats. *Environmental Science and Pollution Research*, *32*(24), 14782–14795. https://doi.org/10.1007/s11356-025-36474-5

He, S., & Zhang, Y. (2025). Detection and quantification of microplastics in endometrial polyps and their role in polyp formation. *Reproductive Toxicology*, *132*, 108757. https://doi.org/https://doi.org/10.1016/j.reprotox.2024.108757

Hoang, H. G., Nguyen, N. S. H., Zhang, T., Tran, H.-T., Mukherjee, S., & Naidu, R. (2025). A review of microplastic pollution and human health risk assessment: current knowledge and future outlook. *Frontiers in Environmental Science*, *13*, 1606332.

Hu, J., Qin, X., Zhang, J., Zhu, Y., Zeng, W., Lin, Y., & Liu, X. (2021). Polystyrene microplastics disturb maternal-fetal immune balance and cause reproductive toxicity in pregnant mice. *Reproductive Toxicology*, *106*, 42–50. https://doi.org/https://doi.org/10.1016/j.reprotox.2021.10.002

Inam, Ö. (2025). Impact of microplastics on female reproductive health: insights from animal and human experimental studies: a systematic review.

Archives of Gynecology and Obstetrics, *312*(1), 77–92. https://doi.org/10.1007/s00404-024-07929-w

Jahedi, F., Frad, N. J. H., Khaksar, M. A., Rashidi, P., Safdari, F., & Mansouri, Z. (2025). Nano and microplastics: unveiling their profound impact on endocrine health. *Toxicology Mechanisms and Methods*, *35*(8), 865–893.

Jeong, J., Mai, N. T. Q., Moon, B.-S., & Choi, J. K. (2025). Impact of polystyrene microplastics (PS-MPs) on the entire female mouse reproductive cycle: Assessing reproductive toxicity of microplastics through in vitro follicle culture. *Ecotoxicology and Environmental Safety*, *297*, 118228.

Jiang, Z., Shan, K., Song, J., Liu, J., Rajendran, S., Pugazhendhi, A., Jacob, J. A., & Chen, B. (2019). Toxic effects of magnetic nanoparticles on normal cells and organs. *Life Sciences*, *220*, 156–161.

Keskin, S., Çakır, M., Açıkgöz, E., & Çölçimen, N., (2024). Can Micro(Nano)Plastics Exacerbate Inflammation In Psoriasis?. *The Broad Spectrum of Biological Sciences* (pp.343-362), Gaziantep: Gaziantep University Press.

Kim, J., Chen, M., & White, R. S. (2025). Microplastics and the Placenta: A Call to Action for Perinatal Research. *American Journal of Perinatology*(EFirst). https://doi.org/10.1055/a-2657-6249

Kim, N., Lee, J. H., Lee, I., Park, J. H., Jung, G. S., Lee, M. J., Im, W., Cho, S., & Choi, Y. S. (2025). Investigation of potential toxic effects of nano- and microplastics on human endometrial stromal cells. *Reproductive Toxicology*, *132*, 108848. https://doi.org/https://doi.org/10.1016/j.reprotox.2025.108848

Land, K. L., Ghuneim, S. M., Williams, B. A., & Hannon, P. R. (2025). IMPACT OF REAL-LIFE ENVIRONMENTAL EXPOSURES ON REPRODUCTION: Phthalates disrupt female reproductive health: a call for enhanced investigation into mixtures. *Reproduction*, *169*(2), e240117. https://doi.org/10.1530/rep-24-0117

Lee, S.-W., Kim, H.-Y., Jang, S., Kim, W., Kim, C., Lee, J., Ryoo, Z. Y., Han, J. E., Baek, S.-M., & Park, J.-K. (2025). Exploratory investigation of the correlation between microplastics and breast cancer: Polystyrene-derived microplastics promote cell proliferation via estrogenic endocrine disruption. *Journal of Environmental Chemical Engineering*, *13*(2), 115473.

Lombardi, G., Di Russo, M., Zjalic, D., Lanza, T., Simmons, M., Moscato, U., Ricciardi, W., & Chiara, C. (2022). Microplastics inhalation and their effects on human health: A systematic review. *European Journal of Public Health*, *32*(Supplement_3), ckac131. 152.

Mahendran, R., & Ramaswamy, S. (2024). Nanoplastics as Trojan horses: deciphering complex connections and environmental ramifications: a review. *Chemistry Africa*, *7*(5), 2265–2282.

Mohamed Nor, N. H., Kooi, M., Diepens, N. J., & Koelmans, A. A. (2021). Lifetime accumulation of microplastic in children and adults. *Environmental Science & Technology*, *55*(8), 5084–5096.

Molenaar, R., Chatterjee, S., Kamphuis, B., Segers-Nolten, I. M., Claessens, M. M., & Blum, C. (2021). Nanoplastic sizes and numbers: quantification by single particle tracking. *Environmental Science: Nano*, *8*(3), 723–730.

Nouri, M., Massahi, T., & Hossini, H. (2025). Microplastics in human body: a narrative on routes of exposure to contamination and potential health effects. *Environmental Pollutants and Bioavailability*, *37*(1), 2538535.

Nowak-Jary, J., & Machnicka, B. (2024). Comprehensive analysis of the potential toxicity of magnetic iron oxide nanoparticles for medical applications: cellular mechanisms and systemic effects. *International Journal of Molecular Sciences*, *25*(22), 12013.

Pathak, D. (2025). Enemies of the hormones: microplastics and endocrine disruptors impacting public health. In *Health and Climate Change* (pp. 119–150). Elsevier.

Qiao, Q.-F., Wang, L.-Q., Xu, Q.-J., Wu, X.-M., Chen, Q.-D., Sheng, T.-Y., Cui, M.-X., Li, J.-A., Pang, X.-Q., & Zhou, Y.-J. (2025). Exposure to Polystyrene Nanoplastics Compromise Ovarian Reserve Function and Endometrial Decidualization in Early Pregnant Mice. *Journal of Applied Toxicology*, *45*(7), 1230–1242. https://doi.org/https://doi.org/10.1002/jat.4765

Qin, X., Cao, M., Peng, T., Shan, H., Lian, W., Yu, Y., Shui, G., & Li, R. (2024). Features, Potential Invasion Pathways, and Reproductive Health Risks of Microplastics Detected in Human Uterus. *Environmental Science & Technology*, *58*(24), 10482–10493. https://doi.org/10.1021/acs.est.4c01541

Raghuvir, S., Alex, S., & Clegg, D. (2025). A review of the impact of micro-and nanoplastics on female reproduction: What we know and gaps in knowledge. *International Journal of Gynecology & Obstetrics*.

Ragusa, A., Matta, M., Cristiano, L., Matassa, R., Battaglione, E., Svelato, A., De Luca, C., D'Avino, S., Gulotta, A., Rongioletti, M. C. A., Catalano, P., Santacroce, C., Notarstefano, V., Carnevali, O., Giorgini, E., Vizza, E., Familiari, G., & Nottola, S. A. (2022). Deeply in Plasticenta: Presence of Microplastics in the Intracellular Compartment of Human Placentas.

International Journal of Environmental Research and Public Health, *19*(18), 11593.

Shahsavari, S., Akbari-Adergani, B., Shafaroodi, H., Akbari, N., Basaran, B., Sadighara, M., & Sadighara, P. (2025). A systematic review on the effect of microplastics on the hypothalamus-pituitary-ovary axis based on animal studies. *Toxicology Letters*, 111745.

Strowitzki, T., Germeyer, A., Popovici, R., & von Wolff, M. (2006). The human endometrium as a fertility-determining factor. *Human Reproduction Update*, *12*(5), 617–630. https://doi.org/10.1093/humupd/dml033

Tang, K. H. D. (2025). Genotoxicity of microplastics on living organisms: effects on chromosomes, DNA and gene expression. *Environments*, *12*(1), 10.

Thin, Z. S., Chew, J., Ong, T. Y. Y., Raja Ali, R. A., & Gew, L. T. (2025). Impact of microplastics on the human gut microbiome: a systematic review of microbial composition, diversity, and metabolic disruptions. *BMC Gastroenterology*, *25*(1), 583.

Tyc, H. J., Kłodnicka, K., Teresińska, B., Karpiński, R., Flieger, J., & Baj, J. (2025). Micro-and Nanoplastics as Disruptors of the Endocrine System—A Review of the Threats and Consequences Associated with Plastic Exposure. *International Journal of Molecular Sciences*, *26*(13), 6156.

Wang, C., Chang, H., Wang, H., Li, H., Ding, S., & Ren, F. (2025). Exposure to microplastics during pregnancy and fetal liver function. *Ecotoxicology and Environmental Safety*, *294*, 118099.

Wang, P., Li, G., Shou, H., Huang, X., Xu, H., Zhang, S., & Zhu, H. (2025). Microplastic abundance in placental chorionic villi detected by pyrolysis-gas chromatography/mass spectrometry in cases of spontaneous miscarriage during early pregnancy. *EBioMedicine*, *120*. https://doi.org/10.1016/j.ebiom.2025.105918

Wang, Z., Shi, R., Wang, R., Ma, Z., Jiang, S., Zhang, F., & Wu, W. (2025). Gestational exposure to polystyrene microplastics incurred placental damage in mice: Insights into metabolic and gene expression disorders. *Ecotoxicology and Environmental Safety*, *294*, 118056. https://doi.org/https://doi.org/10.1016/j.ecoenv.2025.118056

Xu, H., Dong, C., Yu, Z., Hu, Z., Yu, J., Ma, D., Yao, W., Qi, X., Ozaki, Y., & Xie, Y. (2024). First identification of microplastics in human uterine fibroids and myometrium. *Environmental Pollution*, *360*, 124632. https://doi.org/https://doi.org/10.1016/j.envpol.2024.124632

Xu, H., Dong, C., Zhang, B., Jin, Y., Cheng, J., Yu, Z., Ozaki, Y., Yu, J., Ma, D., & Xie, Y. (2025). The role of microplastics in elevated risk and tissues metabolic alterations in uterine fibroid of female patients. *Environmental Pollution*, *374*, 126251. https://doi.org/https://doi.org/10.1016/j.envpol.2025.126251

Xue, Y., Cheng, X., Ma, Z.-Q., Wang, H.-P., Zhou, C., Li, J., Zhang, D.-L., Hu, L.-L., Cui, Y.-F., & Huang, J. (2024). Polystyrene nanoplastics induce apoptosis, autophagy, and steroidogenesis disruption in granulosa cells to reduce oocyte quality and fertility by inhibiting the PI3K/AKT pathway in female mice. *Journal of Nanobiotechnology*, *22*(1), 460.

Zeng, L., Zhou, C., Xu, W., Huang, Y., Wang, W., Ma, Z., Huang, J., Li, J., Hu, L., & Xue, Y. (2023). The ovarian-related effects of polystyrene nanoplastics on human ovarian granulosa cells and female mice. *Ecotoxicology and Environmental Safety*, *257*, 114941.

Zhang, M., & Xu, L. (2022). Transport of micro-and nanoplastics in the environment: Trojan-Horse effect for organic contaminants. *Critical Reviews in Environmental Science and Technology*, *52*(5), 810–846.

Zhang, S., Chen, H., Li, L., Li, Z., & Wang, D. (2025). Exposure to placental microplastic and placental and umbilical cord blood telomere length. *Ecotoxicology and Environmental Safety*, *302*, 118536. https://doi.org/https://doi.org/10.1016/j.ecoenv.2025.118536

Zhao, Q., Zong, M., Song, E., Linghu, H., Li, X., Wang, B., Xiao, S., Guan, W., Su, J., & Zhang, J. (2025). Low-dose exposure to microplastics retards meiotic maturation via HDAC3 insufficiency. *Science China Life Sciences*, *68*(5), 1410–1422.

Chapter 14

Ethical Climate in Health Care

Selda YILDIZ[1]

1. Introduction

Human behavior is shaped not only by cognitive and physiological processes but also by the sociocultural environment in which individuals function. Within organizational settings, both organizational climate and organizational culture exert substantial influence on employee attitudes and behaviors. Consequently, the concept of work climate has become a central focus in the domains of organizational behavior, healthcare management, sociology, and applied psychology (Peterson, 2002; VanSandt, Shepard, & Zappe, 2006; Martin & Cullen, 2006).

The ethical climate, as an integral dimension of organizational culture, is of critical significance because it provides normative and procedural guidance for ethical conduct within the organization (Martin & Cullen, 2006). In healthcare delivery systems, ethical climate defines the moral framework through which professionals interpret and respond to ethical dilemmas encountered in daily practice.

Healthcare organizations operate within complex sociotechnical environments that demand not only advanced clinical competencies and technical proficiency but also a robust foundation of ethical principles and professional integrity. Increasing patient expectations, chronic staff shortages, hierarchical structures, and the inherently multidisciplinary nature of healthcare delivery present persistent challenges for professionals (Faraco et al., 2022). These organizational and systemic pressures often contribute to moral distress, diminish ethical resilience, and adversely influence ethical decision-making processes (Faraco et al., 2022; Mutonyi, Slåtten, Lien, & González-Piñero, 2022).

Despite its significance, empirical research on ethical climate and its antecedents in healthcare settings remains limited. In high-stakes environments where decisions directly affect human life, the quality of ethical climate has profound implications not only for employee behavior and interprofessional collaboration but also for patient safety, quality of care, and organizational

[1] Lecturer PhD. Eskişehir Osmangazi University, School of Health Services, Department of Health Care Services
ORCID: 0000-0002-3650-0539

effectiveness. Therefore, the ethical climate is increasingly regarded as a pivotal determinant of both individual moral conduct and institutional performance within healthcare systems (Essex et al., 2023).

2. The Concept of Ethical Climate and Its Theoretical Foundations

The ethical climate is recognized as a distinct form of organizational climate (Martin & Cullen, 2006) and simultaneously regarded as one of the fundamental pillars of organizational culture (Şahin & Dündar, 2011). The ethical climate, which determines both how ethical behaviors are manifested within organizations and how employees perceive such behaviors, plays a critical role in shaping not only institutional ethical values but also individual decision-making processes.

2.1. Concepts of Organizational Climate and Organizational Culture

Organizational climate represents an abstract construct that embodies a cognitive structure emerging from the holistic interpretation of multiple organizational experiences and observations. In other words, the concept of climate refers to a framework of perception through which organizational members interpret and assign meaning to their work environment (Ehrhart, Schneider, & Macey, 2013).

Schneider and Reichers (1983, 1987) examined organizational climate through the Attraction–Selection–Attrition (ASA) process, which explains the dynamics that determine the continuity of individuals' relationship with the organization. According to this framework, individuals who achieve alignment with the organization's values and norms tend to maintain their membership, whereas those who fail to adapt are more likely to withdraw. Hence, the development of organizational climate is shaped by employees' organizational entry processes, socialization patterns, and their interaction with institutional values (Denison, 1996).

On the other hand, organizational climate can also be conceptualized as a socially constructed phenomenon, rather than being limited solely to the individual–organization fit process. Scholars such as Ashforth (1985), Poole (1985), and Poole and McPhee (1983) considered climate to be a reflection of an organization's core value system, thereby emphasizing its close interrelationship with organizational culture (Denison, 1996). Indeed, the concepts of organizational climate and organizational culture have coexisted for decades, and numerous studies have demonstrated that their interrelationship plays a critical role in enhancing organizational effectiveness. However, the literature frequently highlights that a complete theoretical integration between these two constructs has not yet been achieved (Ehrhart et al., 2013).

In this context, organizational climate reflects employees' shared perceptions of organizational policies, practices, and procedures, whereas organizational culture represents the deeper-level values, beliefs, and assumptions that underlie these perceptions (Denison, 1996). Culture can be defined as a set of shared assumptions developed by an organization to address the challenges of external adaptation and internal integration, which, once proven effective, are accepted as valid and transmitted to new members. These shared assumptions guide members' thinking, feeling, and behavioral patterns when solving organizational problems. While climate mirrors employees' perceptions based on their lived experiences, culture provides an interpretive framework that explains the origins and meanings of those experiences (Mutonyi et al., 2022).

Organizational culture shapes the deep structural composition of an organization within the framework of values and beliefs embraced by its members, while meaning is continuously constructed through social interaction and organizational socialization. Culture creates a symbolic world that ensures relative stability within organizations but also retains an inherent fragility due to its dependence on individual perceptions and behaviors. Conversely, organizational climate encompasses the consciously perceived dimensions of social environments that are defined by the organizational value system (Denison, 1996). Therefore, climate is considered temporary, context-dependent, and administratively controllable, limited to the environmental factors that are consciously recognized by organizational members.

The interaction between organizational culture and organizational climate is of great importance for enhancing organizational effectiveness and establishing a sustainable organizational structure. Recent studies in the healthcare sector have demonstrated that particularly leadership climate and organizational culture exert direct effects on employees' innovative behaviors, organizational commitment, and job satisfaction (Mutonyi et al., 2022). This finding indicates that sustainable organizational transformation cannot be achieved solely through structural modifications but requires profound cognitive and perceptual changes within employees' value systems (Schneider, Brief, & Guzzo, 1996). Therefore, achieving organizational sustainability and effectiveness necessitates a holistic management approach that integrates the complementary dynamics of culture and climate.

2.2. The Concept of Ethical Climate

The ethical climate is defined as a *normative dimension of organizational climate* that embodies the moral implications of organizational policies, procedures, and practices (Martin & Cullen, 2006). Within this conceptual framework, the ethical climate serves as a comprehensive reflection of the organization's prevailing ethical perceptions, institutionalized practices, and governance mechanisms (Barnett, Bass, & Brown, 2000; Treviño, Butterfield, & McCabe, 1998). In essence, it encompasses the shared perceptions among employees regarding both the formal and informal operations of the organization and the patterns of behavior that are rewarded, supported, or expected (Ehrhart et al., 2013; West, Topakas, & Dawson, 2014).

The ethical climate emerges as a result of organizational governance structures, managerial practices, and leadership approaches, directly influencing members' ethical decision-making, moral reasoning, and workplace conduct (Newman, Round, Bhattacharya, & Roy, 2017). An ethical climate is established when organizational members collectively perceive a particular form of ethical reasoning or behavior as normatively acceptable within the institution. Consequently, the ethical climate fosters a shared understanding of *"what constitutes right behavior"* in the workplace and functions as a psychological regulatory mechanism for addressing ethical challenges. This mechanism not only shapes employees' decision-making processes but also guides their behavioral responses when confronted with moral dilemmas.

Moreover, the ethical climate determines not only which issues employees deem ethically salient but also the moral criteria they employ to evaluate, interpret, and resolve these issues. At this juncture, organizational values are translated into ethical actions, exerting both direct and indirect effects on a wide range of organizational outcomes (Martin & Cullen, 2006). In this regard, the ethical climate embodies the normative systems that govern organizational decision-making, collective responses to ethical dilemmas, and the translation of institutional values into individual conduct (Victor & Cullen, 1988).

The construct of ethical climate also seeks to elucidate how the social context of organizations fosters collective moral reasoning and, in turn, influences the ethical behavior of employees (Essex et al., 2023). Thus, ethical climate refers to the set of ethical norms, values, and principles that define behavioral expectations within an organization. The degree to which employees comply with these expectations reflects the extent of internalization and adoption of ethical standards within the institution (Şahin & Dündar, 2011).

The scope of ethical climate encompasses all organizational values associated with notions of "right" and "wrong." Employees' shared perceptions regarding what constitutes appropriate conduct (*content*) and how ethical challenges should be managed (*process*) form the foundational basis of the ethical climate (Cullen, Victor, & Bronson, 1989). Ethical climate not only identifies the parameters of appropriate behavior but also provides guidance on moral action in ethically ambiguous situations (Peterson, 2002). This enables organizations to encourage ethical conduct while predicting and mitigating behaviors that deviate from moral standards.

In this regard, the ethical climate assumes a critical governance function in reducing unethical behavior and maintaining organizational integrity. As Peterson (2002) emphasizes, ethical climate mediates the internalization of ethical norms, ensuring that employee behaviors remain aligned with institutional values and professional standards. Hence, ethical climate should be viewed not merely as a contextual factor influencing individual moral choices, but as a core determinant of organizational integrity, trustworthiness, and long-term sustainability.

3. Ethical Climate Theory

Groundbreaking work on the concept of ethical climate was conducted by Bart Victor and John Cullen (1987, 1988), who are widely recognized as the principal scholars responsible for its conceptualization and operationalization. Victor and Cullen (1987, p. 51) defined ethical climate as *"the shared perception of what constitutes appropriate behavior and how ethical situations should be managed within an organization."*

Within this framework, the Ethical Climate Theory (ECT) was formulated to establish a shared conceptual framework for addressing ethical issues and defining what constitutes ethically appropriate behavior in organizations. ECT has provided a theoretical foundation for numerous empirical and conceptual studies, focusing on identifying employees' perceptions of acceptable and unacceptable behaviors within the workplace, as well as their expectations regarding ethically appropriate conduct (Victor & Cullen, 1988; Malloy & Agarwall, 2001; Wimbush, Shepard, & Markham, 1997; Fritzsche, 2000).

Victor and Cullen integrated sociological and philosophical paradigms to construct a systematic approach for assessing ethical climates in organizational settings (Victor & Cullen, 1988). Accordingly, ECT draws upon typologies inspired by ethical philosophy, developmental and cognitive moral psychology, and sociological theories of reference groups. Thus, the theory offers a comprehensive framework encapsulating ethical criteria that encompass the majority of philosophical approaches to ethics (Martin & Cullen, 2006).

The intellectual origins of Ethical Climate Theory are grounded in Schneider's (1983) conceptualization of organizational climate and Lawrence Kohlberg's (1984) theory of moral development (Victor & Cullen, 1988).

3.1. Schneider's Influence on Ethical Climate Theory

Schneider (1983), along with his subsequent works (Schneider, Brief, & Guzzo, 1996), advanced the concept of *organizational climate* as a central construct in understanding organizational behavior, effectiveness, and change processes. According to Schneider, for transformations implemented within organizations to be both enduring and effective, structural or administrative modifications alone are insufficient; it is also necessary to transform the psychological climate—that is, the "collective affect" or "organizational feel" shared by employees.

Schneider defined organizational climate as a collective psychological state emerging from individuals' shared perceptions and experiences, emphasizing that this shared cognition functions as a driving force behind employees' motivation, effort, and behavioral orientation. Consequently, in processes of organizational transformation, sustainable change necessitates the restructuring of employees' perceptual and value-based frameworks, in conjunction with technical or structural interventions (Schneider et al., 1996).

Victor and Cullen's conceptualization of ECT was deeply influenced by Schneider's perspective. They posited that ethical perceptions and norms within organizations could similarly be viewed as a form of collective organizational cognition guiding employee conduct. This interpretation underscores that ethical climate is shaped not merely by individual moral characteristics but by the social, cultural, and structural context of the organization. Accordingly, ethical climate is conceptualized as a collective organizational phenomenon extending beyond individual moral development, encompassing institutional psychology, normative expectations, and the influence of reference groups (Victor & Cullen, 1988).

3.2. Lawrence Kohlberg and the Moral Development Perspective

A second theoretical foundation of Ethical Climate Theory derives from Lawrence Kohlberg's theory of moral development, itself rooted in Jean Piaget's (1932) framework of moral judgment in children. Piaget delineated moral development in two primary stages: the heteronomous (externally dependent) and the autonomous stages.

- Heteronomous stage (ages 0–10): Events and behaviors are evaluated primarily based on their tangible consequences; moral realism becomes increasingly dominant toward the end of this stage.
- Autonomous stage (age 11 and above): Moral reasoning incorporates not only the physical outcomes of actions but also underlying intentions; empathy and moral perspective-taking begin to emerge (Çam et al., 2012; Yıldız, 2017).

Building on Piaget's cognitive-developmental model, Kohlberg (1981) conceptualized moral development as progressing through three hierarchical levels, each comprising two distinct stages (Warren & Smith-Crowe, 2008; Gürses & Kılavuz, 2016):

1. Pre-conventional level
   a. Punishment–obedience orientation
   b. Instrumental–relativist orientation
2. Conventional level
   a. Interpersonal concordance orientation
   b. Law-and-order orientation
3. Post-conventional level
   a. Social contract orientation
   b. Universal ethical principles orientation

According to Kohlberg, these six stages of moral reasoning are organized around principles of justice, with each stage building upon its predecessor and forming the foundation for subsequent moral development. However, individuals may display reasoning patterns corresponding to different stages depending on situational and contextual factors. The progression of moral development may thus be accelerated, impeded, or stagnated depending on the broader sociocultural environment (Waterman, 1988; Gürses & Kılavuz, 2016).

Although Kohlberg initially conceptualized moral development as an individual cognitive process, he later acknowledged the formative role of social context in shaping moral reasoning. Consequently, he introduced the notions of the "moral atmosphere" and the "just community." The concept of moral atmosphere parallels the notion of ethical climate, in that it reflects group-level normative orientations rather than individual moral competence. Nonetheless, despite his emphasis on collectivist norms and moral education, Kohlberg's framework remained largely disconnected from the organizational context, both methodologically and theoretically (Victor & Cullen, 1988).

By integrating L. Kohlberg's individual-centered moral development framework with Schneider's organizational climate paradigm, the Ethical Climate Theory (ECT) demonstrates that ethical behavior is influenced not only by individual-level moral reasoning but also by institutional norms, reference groups, and collectively shared perceptions. Accordingly, Victor and Cullen conceptualized *ethical climate* as a construct that bridges micro-level moral cognition and macro-level organizational psychology (Martin & Cullen, 2006; Fritzsche, 2000).

Within this framework, the ethical climate as theorized by Victor and Cullen is distinguished from other moral constructs such as *moral identity* and *moral awareness*. The ethical climate focuses on the influence of the organizational social environment in shaping employees' ethical conduct through collective moral reasoning. In contrast, *moral identity* pertains to the extent to which morality is central to an individual's self-concept, whereas *moral awareness* refers to "an individual's recognition that a given situation possesses moral implications and can justifiably be evaluated from an ethical standpoint" (Newman et al., 2017).

3.3. Types of Ethical Climates

Victor and Cullen (1988) demonstrated that organizations embody multidimensional and heterogeneous ethical climates that guide ethical decision-making and behavior (Malloy & Agarwall, 2001; Upchurch & Ruhland, 1996). They also emphasized that significant intra-organizational variation can exist across subunits with respect to perceived ethical climates.

In explaining the emergence of ethical climate, Victor and Cullen identified two principal dimensions:

Ethical criteria: egoistic (i.e., maximizing individual or organizational self-interest), benevolent (i.e., promoting collective welfare), and deontological or principled (i.e., adherence to moral rules and duties) (Victor & Cullen, 1988; Cullen et al., 1993).

Locus of analysis: this dimension concerns the referential focus of moral judgment. The three loci are: *individual* (i.e., personal), *local* (i.e., organizational or subunit level), and *cosmopolitan* (i.e., external to the organization) (Newman et al., 2017).

The intersection of these two dimensions produces a 3x3 typology of nine distinct ethical climate types: *self-interest*, *company profit*, *efficiency*, *friendship*, *team interest*, *social responsibility*, *personal morality*, *company rules/procedures*, and *laws/professional codes* (Mayer, 2014, p. 416).

Table 2. Focus of Analysis in Theoretical Ethical Climate Typology

Ethical Criterion	**Individual**	**Local**	**Cosmopolitan**
Egoism	Self-interest	Company Profit	Efficiency
Benevolence	Friendship	Team Interest	Social Responsibility
Principle-based (Deontology)	Personal Morality	Company Rules and Procedures	Laws and Professional Codes

Egoistic Climate: An egoistic climate refers to an environment in which ethical decision-making is primarily driven by individual or organizational self-interest (Barnett & Vaicy, 2000). Within such contexts, decisions are made with minimal consideration of others' welfare, and consequently, organizational commitment tends to remain weak (Cullen et al., 2003).

In egoistically oriented ethical climates, the dominant evaluative criterion is the extent to which actions advance personal gain or institutional advantage. Thus, in organizations characterized by an egoistic ethical orientation, normative systems reinforce behaviors that prioritize self-interest. Employees in such climates tend to elevate personal or departmental benefit over collective or ethical considerations, resulting in diminished organizational loyalty and an increased incidence of unethical or opportunistic behaviors (Victor & Cullen, 1988; Peterson, 2002).

Benevolent Climate: A benevolent ethical climate is one in which moral reasoning extends beyond individual self-interest and prioritizes the welfare of others and the collective good. In such climates, ethical dilemmas are addressed from the standpoint of mutual benefit and communal well-being. Decision-makers engage in deliberative evaluations of alternatives, taking into account the needs, rights, and potential outcomes for all relevant stakeholders. Consequently, decision-making processes are guided by an orientation toward interpersonal harmony and social responsibility, emphasizing how organizational actions impact others (Weber & Seger, 2002).

Principled (Deontological) Climate: A principled ethical climate refers to an environment in which decision-making is guided by established rules, statutory regulations, and professional ethical codes. Within this climate, ethical issues are resolved based on objective and universal moral standards, although individual interests are not entirely disregarded (Victor & Cullen, 1988). This form of climate embodies a deontological orientation, wherein ethical judgment derives its legitimacy from adherence to duties, principles, and formalized norms rather than from the outcomes of actions.

Given that ethical climate represents a collective organizational phenomenon, its typologies are classified at the group or organizational level, rather than at the individual level of moral cognition. Consequently, these typologies do not correspond directly to the hierarchical stages of moral development outlined by individual moral development theories. As Kohlberg (1984) asserted, individuals may operate within groups whose normative frameworks differ from their own level of moral reasoning, resulting in behavioral conformity and adaptive responses to the prevailing ethical norms.

According to Kohlberg (1984), Gilligan (1982), Haan, Aerts, and Cooper (1985), and other scholars examining moral cognition and ethical reasoning, types of ethical reasoning may exhibit relative incompatibility across individuals and contexts. The literature suggests that benevolent-oriented individuals may display less emphasis on formalized rules and legal constraints, whereas principled-oriented individuals demonstrate greater resistance to external pressures that could compromise their ethical integrity and normative commitments (Victor & Cullen, 1988). Consequently, various organizations—or even subgroups within the same institution—may cultivate distinct prototypical ethical climates, such as benevolent, principled, or egoistic orientations.

Although a single ethical climate may dominate a particular organization, multiple climate types often coexist, creating a complex moral ecosystem that influences how ethical dilemmas are recognized, negotiated, and resolved. This plurality determines which ethical conflicts become salient, the modes through which they are addressed, and the procedural attributes of ethical resolution processes. Kohlberg's (1984) concept of the "sociomoral atmosphere" further illustrates that the ethical climate of an organization serves as a critical determinant in shaping individuals' moral decision-making processes and ethical conduct.

Within this framework:

- Teleological reasoning, emphasizing the welfare and interests of others, predominates in benevolent climates;
- Interpretive reasoning, centered on the application and contextual understanding of rules and laws, characterizes principled climates; and
- Instrumental reasoning, focused on the pursuit of self-interest and personal advantage, is dominant within egoistic climates (Victor & Cullen, 1988).

The determinative role of ethical climate in shaping organizational architecture and influencing employee behavior extends beyond theoretical discourse and has been empirically validated across diverse organizational settings. In their comprehensive meta-analysis of 42 studies, Martin and Cullen (2006) examined the evolution of Victor and Cullen's ethical climate typology within the organizational behavior and business ethics literature. Their findings demonstrated that ethical climate exerts a significant impact not only on ethical decision-making processes but also on key organizational outcomes, including organizational commitment, job satisfaction, psychological well-being, and the prevalence of deviant or counterproductive behaviors (Martin & Cullen, 2006).

4. Ethical Climate in Healthcare

The ethical climate is recognized as one of the most influential organizational constructs shaping the ethical attitudes and behaviors of individuals within healthcare institutions. Conceptually positioned as a sub-dimension of the broader organizational climate, ethical climate is defined as the "shared perceptions regarding what constitutes ethically appropriate conduct and how ethical dilemmas should be addressed" (Aly et al., 2020). Functioning as a behavioral reference framework, ethical climate is shaped by an organization's underlying culture, regulatory structures, managerial policies, and institutional ethical principles.

Healthcare represents one of the most ethically complex sectors, characterized by continuous moral challenges that require multidimensional judgment and accountability. Issues such as patient safety, equitable allocation of scarce resources, end-of-life decision-making, and malpractice liability underscore the centrality of ethical climate as a determinant of institutional integrity and care quality (Olson, 1998; Dalmolin et al., 2022). Within this framework, ethical climate encapsulates healthcare professionals' shared perceptions concerning how ethical issues are recognized and managed across clinical care, diagnostic processes, treatment decisions, and professional interactions. A positive ethical climate promotes a supportive organizational structure that reinforces professional autonomy, facilitates participatory decision-making, and strengthens interprofessional collaboration (Lanes et al., 2021).

Conversely, a negative ethical climate fosters moral distress, emotional exhaustion, and empathy erosion, ultimately contributing to malpractice anxiety, defensive medical practices, and adverse patient outcomes (Hauser et al., 1991; Dzeng & Curtis, 2018; Labarge et al., 2018). Accordingly, cultivating an ethical climate that ensures a psychologically and clinically safe care environment is fundamental to sustaining both healthcare professional well-being and patient safety outcomes.

The concept of ethical climate has been explored through diverse theoretical and philosophical paradigms, yet the healthcare environment presents unique organizational dynamics that distinguish it from traditional business or industrial contexts where ethical climate theories were originally developed. The healthcare setting is inherently multidisciplinary, emotionally intensive, and value-laden, necessitating context-specific frameworks to understand how healthcare professionals perceive ethical conduct within their clinical, managerial, and interpersonal practices. Consequently, there is a growing need for empirically grounded studies that delineate the distinct dimensions of ethical climate within healthcare organizations. In this regard, the development and implementation of institutional policies emphasizing ethical care, supported by structured guidelines that promote a morally responsible and safe environment for both healthcare providers and patients, are crucial (Dalmolin et al., 2022).

Examples of institutional mechanisms that reflect the ethical climate in healthcare may include:

- Policies emphasizing accountability, such as the consistent enforcement of disciplinary measures for ethical violations;
- Practices like performance evaluations and professional development reviews that incorporate ethical competence as a criterion; and
- Procedures designed to encourage transparent error and incident reporting systems that foster learning rather than punishment.

Moreover, behaviors that exemplify respect, empathy, and courtesy toward patients constitute visible manifestations of a positive ethical climate (West et al., 2014).

The concept of ethical climate in healthcare was first introduced by Olson (1995), who defined it as the collective perception of ethically appropriate decision-making processes related to patient care and the conditions that enable ethical reflection (Liu, Zhang, & Jin, 2022). Subsequently, Olson developed the Hospital Ethical Climate Survey (HECS) to empirically measure perceptions of ethical climate within healthcare institutions.

This instrument evaluates healthcare professionals' perceptions across five key dimensions:

1. Colleagues – the degree of ethical interaction, collegial solidarity, and mutual support among employees;
2. Patients – the extent to which patient rights, dignity, and autonomy are respected and upheld;
3. Managers – the leadership's commitment to ethical principles and its role in modeling moral conduct;
4. Hospital – the alignment of institutional policies, protocols, and governance structures with ethical standards;
5. Physicians – the extent to which clinical decisions are guided by ethical reasoning and professional codes of conduct (Olson, 1998).

Empirical research examining the organizational consequences of ethical climate has predominantly focused on four core outcome domains:

1. Work attitudes,
2. Turnover intentions and behaviors,
3. Ethical and unethical conduct, and

4. Miscellaneous organizational and psychological outcomes.

Findings consistently demonstrate that a positive ethical climate perception is positively associated with favorable job attitudes—such as job satisfaction and organizational commitment—and negatively associated with moral distress, turnover intention, and unethical conduct (Martin & Cullen, 2006; Ulrich et al., 2007; Neubert et al., 2009; Rosenblatt, Shapira-Lishchinsky, & Shirom, 2010; Shapira-Lishchinsky & Even-Zohar, 2011; Stewart, Volpone, Avery, & McKay, 2011; Lanes, Magnago, Schutz, Carneiro, Morais, & Dalmolin, 2021).

Among healthcare professionals, nurses represent the group most profoundly influenced by the ethical climate, as they occupy the frontline of patient care and serve as the primary mediators between patients, physicians, and management. The literature reveals that nurses' ethical climate perceptions are directly correlated with job satisfaction, organizational commitment, professional burnout, intention to leave the organization, and the likelihood of clinical error (Joseph & Deshpande, 1997; Hwang & Park, 2014).

A study investigating the relationship between nurses' perceptions of the ethical climate, their experiences of medical errors, and turnover intentions found that nurses perceiving a more positive ethical climate were less prone to making medical errors. Furthermore, favorable perceptions across the "patient," "manager," "hospital," and "physician" dimensions of the ethical climate were negatively associated with turnover intention. The findings emphasize that enhancing the ethical climate in healthcare settings can reduce medical errors and improve workforce retention in public hospitals (Hwang & Park, 2014).

Nurses perceiving a positive ethical climate have been found to:

- Commit fewer medical errors (Hwang & Park, 2014),
- Exhibit higher job satisfaction and stronger organizational commitment (Mulki et al., 2006),
- Experience lower levels of moral distress (Dzeng & Curtis, 2018), and
- Contribute to higher standards of patient safety (Ulrich et al., 2007).

Conversely, nurses perceiving a negative ethical climate tend to experience:

- Decreased professional motivation,
- Increased emotional exhaustion and decreased empathy,
- Reduced teamwork and collaboration,
- Diminished trust in leadership,
- Increased turnover intentions (Karagözoğlu et al., 2014; Abou Hashish, 2017),
- Declining interest in clinical and ethical matters, and

• Compromised quality of care delivery (Hwang & Park, 2014; Essex et al., 2023).

These adverse effects are particularly pronounced in high-risk clinical settings such as intensive care, oncology, and emergency departments, where ethical conflicts and emotional labor are more prevalent.

Furthermore, the detrimental effects of a negative ethical climate extend beyond staff outcomes to clinical safety and patient care quality, being associated with:

• Heightened fear of malpractice (Hauser et al., 1991),
• The adoption of defensive medical practices (Aly et al., 2020),
• Burnout and diminished empathy among clinicians (Dzeng & Curtis, 2018), and
• Adverse patient outcomes (Labarge et al., 2018).

Conversely, a constructive ethical climate fosters a learning-oriented organizational culture where healthcare professionals feel empowered to report errors voluntarily, engage in reflective practice, and collaborate to improve patient safety (West et al., 2014).

Although the existing body of literature has primarily concentrated on nurses, empirical evidence on physicians' perceptions of ethical climate remains relatively limited (Essex et al., 2023). Nonetheless, physicians are directly influenced by the ethical climate in their clinical decision-making processes. A positive ethical climate facilitates evidence-based and patient-centered medical decisions consistent with ethical and professional standards, whereas a negative ethical climate may contribute to unethical conduct, moral disengagement, and defensive medical practices driven by malpractice anxiety and institutional pressures (Dzeng & Curtis, 2018; Aly et al., 2020).

A positive ethical climate facilitates physicians' clinical decision-making in alignment with evidence-based practice and ethical principles, thereby promoting actions that prioritize the best interests of patients. Conversely, a negative ethical climate can predispose physicians to unethical conduct as a result of malpractice anxiety, defensive medical behaviors, and institutional pressures (Dzeng & Curtis, 2018; Aly et al., 2020).

Among the individual-level outcomes of ethical climate, moral distress emerges as one of the most salient and detrimental. Moral distress refers to the psychological disequilibrium and emotional discomfort experienced when an individual recognizes the ethically appropriate course of action but is unable to act accordingly due to institutional or systemic constraints (Jameton, 2017;

Lamiani et al., 2017). The literature underscores that a negative ethical climate significantly amplifies moral distress among physicians and nurses, directly contributing to defensive medicine practices and heightened fear of medical errors (Lanes et al., 2021; Yıldız & Demirsoy, 2025).

In the study by Yıldız and Demirsoy (2025), several organizational and systemic determinants of a negative ethical climate were identified as contributing factors to physicians' medical errors, malpractice anxiety, and engagement in defensive medical practices. These factors include:

- Inadequate training and clinical inadequacy of auxiliary health personnel,
- Absence of standardized protocols and clearly defined team roles,
- Attribution of sole accountability for medical errors to physicians,
- Workplace culture characterized by accusatory, unsupportive, or discrediting peer attitudes following adverse events,
- Time constraints and communication barriers during the informed consent process, including insufficient consent documentation,
- Fear of litigation, patients' fault-seeking attitudes, excessive compensation demands, and adversarial behavior by legal professionals and expert witnesses,
- Deficiencies in national health legislation, including the lack of context-specific laws addressing medical errors and a punitive legal paradigm portraying the physician as culpable and the patient as the victim,
- Managerial shortcomings, including administrators' limited clinical experience, inadequate understanding of surgical practice, lack of impartiality in post-error assessments, and systemic tendencies to assign blame to physicians, and
- The current system and the practices of managers direct physicians to defensive medicine (Yıldız, Demirsoy, 2025).

A recent systematic review evaluating the ethical climate among healthcare professionals identified several organizational attributes that serve as both antecedents and outcomes of a positive ethical climate, including:

- **Open Communication:** Transparent and consistent communication among healthcare professionals—particularly between nurses and physicians—enhances mutual trust, psychological safety, and collaborative decision-making.
- Supportive Leadership: Ethically engaged and participatory leadership promotes employee empowerment and active involvement in ethical decision-making processes, thereby reinforcing organizational integrity and professional accountability.

Education and Awareness: Structured education and ongoing training on ethical issues enable healthcare professionals to comprehend the importance of an ethical climate and its implications for clinical decision-making, organizational trust, and patient safety. Empirical evidence indicates that barriers to shared decision-making among healthcare professionals, lack of mutual trust in patient care processes, excessive workload, and chronic staff shortages adversely affect nurse–physician relationships, thereby contributing to moral distress and ethical dissonance within healthcare teams.

The factors identified as both antecedents and outcomes of a negative ethical climate include the following:

Communication Deficiencies: Ineffective or inadequate communication channels among healthcare professionals lead to misinterpretations, interpersonal conflicts, and compromised coordination of care.

Lack of Trust: The erosion of mutual trust between nurses and physicians undermines interprofessional collaboration and negatively influences the continuity and quality of patient care.

Ethical Insensitivity: Limited awareness or disregard for ethical dimensions in daily practice results in hesitancy and inconsistency in moral reasoning and decision-making among healthcare professionals.

Consequently, it has been emphasized that the ethical climate between nurses and physicians plays a determinant role in shaping the quality of interprofessional relationships, and fostering a positive ethical climate is essential to enhance cooperation, communication, and mutual respect (Lanes et al., 2021).

The multidimensional impact of ethical climate on employee attitudes, professional conduct, and organizational behavior underscores the strategic role of managerial leadership. In healthcare organizations, administrators and unit managers play a pivotal role in the establishment, reinforcement, and sustainability of the ethical climate (Delaney & Sockell, 1992; Laczniak & Inderrieden, 1987).

Their leadership practices are instrumental in cultivating an ethical organizational environment through the following mechanisms:

- Ethical Leadership
- Communication and Transparency
- Supportive Organizational Environment
- Continuous Training and Professional Development
- Feedback and Ethical Reflection Mechanisms
- Conflict Resolution and Mediation Competencies

Ethical Leadership: Establishing fair systems of recognition and accountability, maintaining open communication channels, and institutionalizing ethical codes of conduct are core managerial strategies that strengthen the ethical infrastructure of healthcare organizations (Delaney & Sockell, 1992; Laczniak & Inderrieden, 1987). Ethical leadership by managers nurtures a trust-based organizational culture, guiding healthcare professionals to internalize moral values and align their behaviors with organizational ethical standards (Lanes et al., 2021). Research consistently demonstrates that ethical leadership enhances interpersonal trust, reduces unethical behavior, and strengthens organizational commitment (Jansen & Von Glinow, 1985; Valentine & Barnett, 2003). Within healthcare institutions, leaders who actively and consciously shape the ethical climate contribute significantly to both employee well-being and patient safety outcomes.

Communication and Transparency: When healthcare managers adopt an open, transparent, and inclusive communication style, it fosters interprofessional collaboration, mitigates conflicts, and prevents organizational dysfunction. Transparency in communication processes provides employees with the psychological safety necessary to express ethical concerns and operational challenges without fear of reprisal. This managerial approach not only enhances organizational trust but also serves as a preventive mechanism against moral distress and ethical disengagement.

Creating a Supportive Environment:

It is imperative for healthcare administrators to cultivate an environment that promotes active participation of employees in ethical decision-making processes. Such an environment enables nurses and physicians to express their perspectives, voice ethical concerns, and contribute to the collective enhancement of the ethical climate. A participatory and inclusive work atmosphere not only strengthens employees' moral engagement but also reinforces organizational trust and professional collaboration.

Training and Development Opportunities: Providing continuous ethics-oriented education and professional development programs for healthcare professionals substantially contributes to the reinforcement of the ethical climate. Ethical training enhances employees' moral reasoning capacities, supports ethical awareness, and equips them with the competencies required to address ethical dilemmas in complex clinical settings. Regular training initiatives also ensure alignment between institutional ethical standards and professional practice behaviors, fostering both individual and organizational moral responsibility.

Feedback Mechanisms: Establishing systematic feedback and ethical evaluation mechanisms enables administrators to monitor, assess, and improve ethical practices across organizational levels. By actively soliciting and integrating employee feedback, managers can identify areas of ethical tension and implement corrective measures to enhance organizational integrity. This iterative process contributes to the continuous improvement of interprofessional communication and collaborative relationships between nurses and physicians, thereby fortifying ethical cohesion within the healthcare organization.

Conflict Management: Developing effective conflict management and mediation strategies is essential for preventing the emergence of a negative ethical climate. Proactive conflict resolution practices allow managers to address ethical disagreements promptly and constructively. Timely and transparent management of disputes promotes healthy communication, strengthens interprofessional cooperation, and maintains organizational harmony (Lanes et al., 2021).

Conclusion and Recommendations

The ethical climate in healthcare organizations exerts a profound influence at the individual, organizational, and societal levels. Empirical research consistently indicates that a positive ethical climate fosters healthcare professionals' ethical attitudes, enhances job satisfaction and organizational commitment, reduces moral distress, and promotes patient safety. Conversely, a negative ethical climate has been associated with burnout, loss of professional motivation, defensive medical practices, fear of malpractice litigation, and adverse patient outcomes.

Accordingly, the ethical climate is recognized as a determinant of both the psychosocial well-being of healthcare professionals and the overall quality and safety of healthcare delivery systems.

The differing perspectives of nurses and physicians on the ethical climate underscore the necessity of adopting an interdisciplinary and interprofessional approach in the study and management of organizational ethics within healthcare settings. The predominance of nursing-oriented research in the current literature highlights the need for further empirical investigations focusing on physicians' perceptions of ethical climate, particularly within high-risk and ethically demanding clinical units. Such research will provide critical insights into how ethical climate is formed, sustained, and manifested across diverse healthcare contexts.

Note

This book chapter is adapted from the author’s doctoral dissertation entitled *“Assessing Physicians’ Perceptions of Fear of Medical Error and the Role of Ethical Climate in Defensive Medical Practices Using the Vignette Technique.”*

Acknowledgements

The author extends sincere appreciation to Prof. Dr. Nilüfer Demirsoy for her invaluable academic mentorship, insightful guidance, and unwavering support throughout the development and completion of this study.

References

1. Abou Hashish, E. A. (2017). Relationship between ethical work climate and nurses' perception of organizational support, commitment, job satisfaction and turnover intent. Nursing Ethics, 24(2), 151-166.
2. Aly, N. A. E. F. M., El-Shanawany, S. M., & Ghazala, A. M. A. (2020). Ethico-legal aspects and ethical climate: managing safe patient care and medical errors in nursing work. Clinical Ethics, 15(3), 132-140.
3. Cullen, J. B., B. Victor and C. Stephens: 1989, 'An Ethical Weather Report: Assessing the Organization's Ethical Climate', Organizational Dynamics 18, 50-62.
4. Cullen, J. B., Victor, B., & Bronson, J. W. (1993). the ethical climate questionnaire: An assessment of its development and validity. Psychological Reports, 73(2), 667-674.
5. Cam, Z., Cavdar, D., Seydooğulları, S., & Cok, F. (2012). Classical and new theoretical approaches to moral development. Educational Sciences in Theory and Practice, 12(2), 1211-1225.
6. Dalmolin, G.D.L., Lanes, T.C., Bernardi, C.M.S., & Ramos, F.R.S. (2022). Conceptual framework for the ethical climate in health professionals. Nursing Ethics. 29(5), 1174-1185.
7. Delaney, J. T., & Sockell, D. (1992). Do company ethics training programs make a difference? An empirical analysis. Journal of Business Ethics, 11(9), 719-727.
8. Denison, D. R. (1996). What is the difference between organizational culture and organizational climate? A native's point of view on a decade of paradigm wars. Academy of Management Review, 21(3), 619-654.
9. Dzeng, E., & Curtis, J. R. (2018). Understanding ethical climate, moral distress, and burnout: a novel tool and a conceptual framework. BMJ Quality & Safety. 27(10), 766-770.
10. Ehrhart, M. G., Schneider, B., & Macey, W. H. (2013). Organizational climate and culture: An introduction to theory, research, and practice. Routledge. S.2.
11. Essex, R., Thompson, T., Evans, T. R., Fortune, V., Kalocsányiová, E., Miller, D., ... & Elliott, H. (2023). Ethical climate in healthcare: A systematic review and meta-analysis. Nursing Ethics. 30(7-8), 910-921.
12. Faraco, M. M., Gelbcke, F. L., Brehmer, L. C. D. F., Ramos, F. R. S., Schneider, D. G., & Silveira, L. R. (2022). Moral distress and moral resilience of nurse managers. Nursing Ethics, 29(5), 1253-1265.
13. Fritzsche, D.J. (2000), "Ethical Climates and the Ethical Dimension of Decision-Making", Journal of Business Ethics, 24: 125–140.

14. Gilligan, C. (1982). New maps of development: new visions of maturity. American Journal of Orthopsychiatry, 52(2), 199.
15. Gürses, İ., & Kılavuz, MA (2016). Learning moral values across generations: An evaluation in terms of Kohlberg's moral development theory. Uludağ University Faculty of Theology Journal, 25(1), 97-117.
16. Hauser, M. J., Commons, M. L., Bursztajn, H. J., & Gutheil, T. G. (1991). Fear of malpractice liability and its role in clinical decision making. In T. G. Gutheil, H. J. Bursztajn, A. Brodsky, & V. Alexander (Eds.), Decision making in psychiatry and the law. 209- 226. Williams & Wilkins Co.
17. Hwang, J. I., & Park, H. A. (2014). Nurses' perception of ethical climate, medical error experience and intent-to-leave. Nursing Ethics. 21(1), 28-42.
18. Jameton, A. (2017). What moral distress in nursing history could suggest about the future of health care. AMA journal of ethics, 19(6), 617-628.
19. Jansen, E., M.A. VonGlinow (1985), "Ethical Ambivalence and Organizational Reward Systems", Academy of Management Review, 10(4): 814-822.
20. Joseph, J., S. P. Deshpande (1997), "The Ethical Impact Climate on Job Satisfaction of Nurses", Health Care Management Review, 22(1): 76-81
21. Karagözoğlu, Ş., Özden, D., & Yıldırım, G. (2014). Perception of hospital ethical climate of intensive care nurses. Journal of Research and Development in Nursing. 16(1), 34-45.
22. Labarge, B., Walter, V., Lengerich, E. J., Crist, H., Karamchandani, D., Williams, N., ... & Warrick, J. I. (2018). Evidence of a positive association between malpractice climate and thyroid cancer incidence in the United States. PLoS One, 13(7), e0199862.
23. Laczniak, G. R., & Inderrieden, E. J. (1987). The influence of stated organizational concern upon ethical decision making. Journal of Business Ethics, 6(4), 297-307.
24. Lamiani, G., Borghi, L., & Argentero, P. (2017). When healthcare professionals cannot do the right thing: A systematic review of moral distress and its correlates. Journal of health psychology. 22(1), 51-67.
25. Lanes, T. C., Magnago, A. C. D. S., Schutz, T. C., Carneiro, A. S., Morais, B. X., & Dalmolin, G. D. L. (2021). Evaluation of ethical climate in health services: a systematic review. Revista Bioética, 28, 718-729.
26. Lanes, TC, Schutz, TC, da Costa Pompeu, K., Pereira, L. A., de Morais, F. S., & de Lima Dalmolin , G. (2023). Climate association ethical and variables labor between nurses hospitals. UFSM Nursing Journal . 13.
27. Liu, Y., Zhang, Y., & Jin, J. (2022). The relationships of ethical climate, physician–nurse collaboration and psychological empowerment with

critical care nurses' ethical conflict in China: A structural equation model. Journal of Nursing Management. 30(7), 2434-2441.

28. Malloy ve Agarwall, 2001; Upchurch ve Ruhland, 1996; Upchurch, R.S., S.K. Ruhland (1996), "The Organizational Bases of Ethical Work Climates in Lodging Operations as Perceived by General Managers", Journal of Business Ethics,(15): 1083-1093.
29. Martin, K. D., & Cullen, J. B. (2006). Continuities and extensions of ethical climate theory: A meta-analytic review. Journal of business ethics, 69(2), 175-194.
30. Mayer, D. M. (2014). A review of the literature on ethical climate and culture. The Oxford handbook of organizational climate and culture. 415.
31. Mulki, J.P., F. Jaramillo, W.B. Locander (2006), "Effects of Ethical Climate and Supervisory Trust on Salesperson's Job Attitudes and Intentions to Quit", Journal of Personal Selling and Sales Management, 26(1): 20–26.
32. Mutonyi, B. R., Slåtten, T., Lien, G., & González-Piñero, M. (2022). The impact of organizational culture and leadership climate on organizational attractiveness and innovative behavior: a study of Norwegian hospital employees. BMC health services research, 22(1), 637.
33. Mutonyi, B. R., Slåtten, T., Lien, G., & González-Piñero, M. (2022). The impact of organizational culture and leadership climate on organizational attractiveness and innovative behavior: a study of Norwegian hospital employees. BMC health services research, 22(1), 637.
34. Neubert, M. J., Carlson, D. S., Kacmar, K. M., Roberts, J. A., & Chonko, L. B. (2009). The virtuous influence of ethical leadership behavior: Evidence from the field. Journal of business ethics, 90(2), 157-170.
35. Newman, A., Round, H., Bhattacharya, S., & Roy, A. (2017). Ethical climates in organizations: A review and research agenda. Business Ethics Quarterly. 27(4), 475-512.
36. Olson, L. L. (1998). Hospital nurses' perceptions of the ethical climate of their work setting. Image: the Journal of Nursing Scholarship. 30(4), 345-349.
37. Peterson, D.K. (2002), "Deviant Workplace Behavior and the Organization's Ethical Climate", Journal of Business and Psychology, 17(1): 47-61.
38. Rosenblatt, Z., Shapira-Lishchinsky, O., & Shriom, A. (2010). Absenteeism in Israeli schoolteachers: an organizational ethics perspective. Human Resource Management Review. 20, 247-259.

39. Schneider, B., Brief, A. P., & Guzzo, R. A. (1996). Creating a climate and culture for sustainable organizational change. Organizational Dynamics, 24(4), 7-19.
40. Shapira-Lishchinsky, O., & Rosenblatt, Z. (2009). Perceptions of organizational ethics as predictors of work absence: a test of alternative absence measures. Journal of Business Ethics. 88, 717–734.
41. Stewart, R., Volpone, S. D., Avery, D. R., & McKay, P. (2011). You support diversity, but are you ethical? Examining the interactive effects of diversity and ethical climate perceptions on turnover intentions. Journal of Business Ethics. 100, 581-593.
42. Şahin, B., & Dündar, T. (2011). Examining the relationship between ethical climate and mobbing behaviors in the health sector. Ankara University Journal of Social Sciences. 66(01), 129-159.
43. Treviño , L. K., Butterfield, K. D., & McCabe, D. L. (1998). the ethical context in organizations: Influences on employees attitudes and behaviors . Business Ethics Quarterly. 8(3), 447-476.
44. Ulrich, C., O'Donnell, P., Taylor, C., Farrar, A., Danis, M., & Grady, C. (2007). Ethical climate, ethics stress, and the job satisfaction of nurses and social workers in the United States. Social Sciences & Medicine. 65, 1708–1719.
45. Upchurch, R.S., S.K. Ruhland (1996), "The Organizational Bases of Ethical Work Climates in Lodging Operations as Perceived by General Managers", Journal of Business Ethics,(15): 1083-1093.
46. Valentine, S., T. Barnett (2003), "Ethics Code Awareness, Perceived Ethical Values, and Organizational Commitment", Journal of Personal Selling and Sales Management, 23: 359-367.; Victor ve Cullen, 1988.
47. VanSandt, C. V., Shepard, J. M., & Zappe, S. M. (2006). An examination of the relationship between ethical work climate and moral awareness. Journal of Business Ethics. 68, 409-432.
48. Victor, B., & Cullen, J. B. (1987). A theory and measure of ethical climate in organizations. Research in Corporate Social Performance and Policy. 9, 51-71.
49. Victor, B., & Cullen, J. B. (1988). The organizational bases of ethical work climates. Administrative science quarterly. 101-125.
50. Warren, D. E., & Smith-Crowe, K. (2008). Deciding what's right: The role of external sanctions and embarrassment in shaping moral judgments in the workplace. Research in organizational behavior, 28, 81-105.
51. Waterman, A. S. (1988). On the uses of psychological theory and research in the process of ethical inquiry. Psychological Bulletin, 103(3), 283.

52. Weber, J., J.E. Seger (2002), "Influences upon Organizational Ethical Climate Subclimates: A Replication Study of a Single Firm at Two Points in Time", Journal of Business Ethics,(41): 69-84.
53. West, M. A., Topakas, A., & Dawson, J. F. (2014). Climate and culture for health care. The Oxford handbook of organizational climate and culture, in Climate and Culture for Health Care Performance. 335-359.
54. Wimbush, CJ, MJ Shepard, E.S. Markham (1997), "An Empirical Examination of The Multidimensionality of Ethics Climate in Organizations", Journal of Business Ethics,16: 67-77.
55. Yildiz, S., & Demirsoy, N. (2025). Evaluation of Physicians' Perceptions of Fear of Medical Error and the Role of Ethical Climate in Defensive Medical Practices Using Vignette Technique. Eskisehir Osmangazi University, Institute of Health Sciences, Department of History of Medicine and Ethics. PhD Thesis. Eskisehir.

Chapter 15

Healthy Nutrition in Workplaces

Gülsüm Betül ÖZOCAK [1], Aslı Ece ACAR FİLİZCİ[2]
Dilek ÖZTAŞ[3], Ergün ERASLAN[4]

1. INTRODUCTION

Occupational health and safety is not limited to accident prevention or physical protection; it represents a holistic approach that supports the overall well-being of employees. In this context, nutrition is a critical factor that directly impacts both physical and mental performance of employees. Adequately meeting daily energy and nutrient requirements has a direct impact on attention, reflexes, decision-making, and stress management in the workplace. Unhealthy eating habits can lead to chronic conditions such as obesity, diabetes, and hypertension, as well as fatigue, lack of motivation, and increased susceptibility to workplace accidents. Therefore, healthy eating in the workplace should not be viewed solely as a personal choice but rather as a corporate occupational health policy.

Within the framework of modern healthcare services, providing adequate and balanced nutrition, particularly to priority risk groups such as children, the elderly, pregnant women, and workers, is a crucial component of preventive healthcare. This approach can significantly contribute to the promotion, maintenance, and promotion of a healthy lifestyle, thus improving the overall health of society.

In today's business world, employee health and well-being are among the most critical determinants of organizational success. Globalization, technological advancements, and increasing competition have increased the physical and mental burdens on employees, making healthy lifestyle practices in the workplace more important than ever. Among these, nutrition stands out as one of the most fundamental factors affecting an individual's energy balance, cognitive performance, mood, and overall quality of life. Given that a significant portion of

[1]Occupational Health and Safety Master's Student, Ankara, Turkey, ORCID:0009-0002-4226-0743, atasoygulsumb@gmail.com
[2]Dr., Occupational Health and Safety, Ankara, Turkey, ORCID:0000-0002-2323-7655, aslieceacar@gmail.com
[3]Prof. Dr., Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Public Health, Ankara, Turkey, ORCID:0000-0002-8687-7238, doztas@aybu.edu.tr
[4]Prof. Dr., Ankara Yıldırım Beyazıt University, Institute of Science, Department of Occupational Health and Safety, ORCID:0000-0002-5667-0391, eraslan@aybu.edu.tr

daily time is spent at work, how employees eat at work and how this impacts their productivity has emerged as a vital area of study.

Healthy eating isn't just limited to physical health; it also directly impacts psychological well-being, motivation, concentration, and job satisfaction. Poor or unbalanced eating habits can lead to lost productivity, burnout, absenteeism, distraction, and poor performance. Diets high in refined carbohydrates and fats, but low in fiber, can cause blood sugar fluctuations and reduce work productivity. Conversely, employees who eat a balanced diet tend to have higher concentration levels, stronger immune systems, and better stress management skills (Wong et al., 2020; Brown et al., 2021).

Another key dimension that highlights the importance of nutrition in the workplace is its economic impact. According to reports by the World Health Organization (WHO, 2022), productivity losses due to malnutrition result in billions of dollars in economic losses globally. From a corporate perspective, nutrition-based policies aimed at protecting employee health are not only a form of social responsibility but also a long-term investment. Such programs contribute to lower healthcare costs, reduced absenteeism rates, and increased productivity.

In recent years, both public and private institutions in Türkiye have increasingly implemented health-focused workplace policies, including healthy menu planning, access to dietitian services, and awareness training programs. However, these practices have not yet been adopted across all sectors. Systematic nutrition policies are often lacking, particularly in small and medium-sized enterprises (SMEs). Therefore, workplace nutrition remains a topic requiring further research, both academically and practically.

The purpose of this study is to examine the multidimensional effects of workplace nutrition on employee health, productivity, and motivation and to develop practical recommendations for organizations by evaluating existing research findings. The study begins by explaining the importance of workplace nutrition, followed by a theoretical background from the literature. The relationship between nutrition and productivity is then supported by empirical findings, and finally, strategic recommendations for organizations are presented.

2. THE IMPORTANCE OF NUTRITION IN THE WORKPLACE

Nutrition is a key determinant of health, directly impacting employee performance and overall workplace productivity. According to data from the World Health Organization (WHO), implementing healthy nutrition policies in workplaces can reduce workplace accidents by 15% and absenteeism by up to 20%. Inadequate nutrition negatively impacts cognitive performance, leading to symptoms such as distraction, poor reflexes, and loss of energy. These issues pose a significant risk, particularly for those working on production lines. Therefore, ensuring employees have access to a balanced diet is not only a health measure but also a strategic investment in economic efficiency.

Two of the most critical problems in working life are occupational accidents and occupational diseases. Research has shown a significant relationship between workplace nutrition and the occurrence of occupational accidents. Studies show that employees with improved diets are more productive than those with poor eating habits. Employees with unbalanced and inadequate nutrition often experience factors such as fatigue, lack of concentration, and exhaustion, which increase the likelihood of workplace accidents. This contributes to decreased productivity and increased healthcare costs.

Nutrition is a key factor affecting an employee's work capacity and, consequently, their production rate. When employees fail to meet their daily nutritional needs, their capacity and productivity decline. Inadequate and unbalanced nutrition weakens immune systems, increases susceptibility to infection, increases the incidence of illness, and can lead to serious and potentially life-threatening complications. Furthermore, malnutrition is a major cause of increased workplace accidents.

Studies in Türkiye have revealed that many workers do not consume sufficient energy, protein, vitamins, and minerals, and their diets are often unbalanced. Compared to developing countries, protein consumption (especially animal protein) is significantly higher in developed countries. A society's economic status influences both diet and nutritional adequacy. In Türkiye, inadequate protein intake is more prevalent among lower socioeconomic groups.

Employees' nutrition plans and diets should be tailored to their working conditions. Employees engaged in strenuous physical work require a higher protein intake due to increased energy expenditure and nitrogen loss through sweating. For young, growing workers, both the quantity and quality of protein in the diet should be increased. While protein is not a direct energy source for muscle activity, increased muscle mass in physically active individuals increases protein requirements when overall dietary energy intake is sufficient.

Carbohydrates provide the majority of dietary energy. It has been determined that during muscular activity, carbohydrates are utilized as an energy source 4-5% more efficiently than fat. Increasing glycogen stores in tissues increases work capacity. Therefore, carbohydrates play a crucial role in meeting the energy needs of physically demanding tasks that exceed the energy intake provided by a standard diet. Additionally, workers need other essential nutrients, such as vitamins and minerals, and an appropriate balance of carbohydrates, protein, and fat.

3. NUTRITION IN 3RD-SHIFT WORK

Shift work disrupts workers' biological clocks (circadian rhythms) and alters their metabolic balance. The incidence of obesity, gastritis, sleep disorders, and metabolic syndrome is particularly high among night shift workers. Research shows that night shift workers often exhibit irregular eating habits and tend to consume carbohydrate-heavy, low-protein meals. This not only leads to short-term performance declines but also leads to long-term health problems. Therefore, nutrition plans for shift workers should be designed to ensure a balanced nutritional intake both before and after shifts. Diets should prioritize foods rich in protein, fiber, and a low glycemic index.

Suggestions:

- Light, protein- and fiber-balanced meals before your shift
- Healthy snacks during shifts (e.g. dried fruit, nuts, yogurt)
- Encouraging regular fluid intake
- Avoid heavy and oily meals during night shifts

Table 1. Sample Meal Plan by Shift

Food	Morning Shift	Day Shift	Night Shift
Breakfast	Oatmeal + fruit	Whole grain bread + eggs	Yogurt + mixed nuts
Snacks	Fruit	Vegetable sticks	Dried fruit
Lunch	Grilled chicken + vegetables	Legume salad	Fish + vegetables
Snacks	Hazelnut	Yogurt	Whole grain bar

4. HEALTHY NUTRITION POLICIES IN INSTITUTIONS

Establishing a healthy eating culture in organizations requires more than just individual effort; it also depends on management-supported policies. Employers should provide cafeteria services with balanced meal options, organize regular nutrition seminars, and conduct healthy lifestyle awareness campaigns. **The "Turkey Healthy Nutrition and Active Living Program," launched by the Turkish Ministry of Health,** is an important guide to raising awareness in the workplace. Organizations can also seek support from dietitians to help employees develop personalized nutrition plans. Such measures contribute to improving both employee morale and workplace performance.

Corporate policies that promote healthy eating among employees not only increase well-being but also boost work productivity.

Suggestions:

- Offer balanced menu options in workplace cafeterias
- Offer healthy alternatives for snacks
- Organize seminars to raise awareness about nutrition
- Hire or consult with corporate dietitians
- Expand water stations and limit access to fast food

To effectively implement healthy eating policies in organizations, various practical measures should be implemented. For example, workplace cafeterias should prepare balanced weekly menus and prioritize grilled or steamed foods over fried foods. Additionally, employees should be offered healthy snack alternatives by offering items such as fruit, yogurt, and nuts in the cafeteria and break areas. To promote adequate hydration, water stations should be installed on every floor, and informative materials encouraging water consumption should be used. These practices contribute to the promotion of healthy lifestyle habits in the workplace.

5. HEALTHY SNACKS AND SAMPLE MENUS

Offering healthy snacks at work helps employees maintain balanced blood sugar levels and prevent energy fluctuations throughout the day. Ideal options include fruit, yogurt, nuts, whole-grain bars, and fresh vegetables. Instead of sodas, cafeterias or vending machines can offer alternatives such as ayran (a traditional yogurt-based drink), mineral water, and freshly squeezed fruit juices. When planning menus, it's important to balance carbohydrate, protein, and fat ratios; cooking methods like boiling and grilling should be preferred over frying. Additionally, adding seasonal vegetables and legumes to weekly menus supports employees' immune systems and contributes to overall well-being.

6. WATER CONSUMPTION AND HYDRATION

Body fluid balance is a critical factor affecting both cognitive performance and physical endurance in employees. Dehydration can lead to decreased attention span, muscle cramps, and fatigue. Workers in high-temperature environments are at risk of dehydration. The World Health Organization (WHO) and the European Food Safety Authority (EFSA) recommend a daily water intake of 2 to 2.5 liters for adults. Organizations should provide water dispensers in work areas and encourage employees to drink adequate fluids. Additionally, offering herbal teas and unsweetened beverage alternatives can help regulate caffeine consumption and support overall hydration.

7. EFFECTS OF NUTRITION ON PRODUCTIVITY AND MOTIVATION

Healthy eating directly impacts employee motivation and workplace productivity. Adequate energy intake reduces stress, improves mental clarity, and strengthens employee engagement. Research **shows that employees who eat a regular, balanced diet have up to 25% lower absenteeism rates** . Furthermore, implementing employee wellness programs has been linked to increased workplace engagement and strengthened corporate reputation. A healthy eating culture contributes not only to individual performance but also to overall organizational productivity.

Energy Levels and Focus

A balanced intake of carbohydrates, protein, and fat helps maintain consistent energy levels throughout the day. Fluctuations in blood sugar levels can lead to decreased alertness and fatigue. Consuming adequate amounts of complex carbohydrates and protein, especially in the morning and early afternoon, supports alertness later in the workday.

Stress and Motivation Management

A balanced diet can help regulate cortisol, the body's primary stress hormone. **Nutrient-dense foods rich in omega-3 fatty acids** , **B vitamins** , and magnesium support stress management. Employees in a highly motivated environment tend to make fewer mistakes, generate more creative solutions, and experience greater job satisfaction.

Cognitive Function and Mental Performance

Brain functions like attention, memory, and problem-solving are directly affected by dietary habits. Foods rich in antioxidants and omega-3 fatty acids boost cognitive performance. Research shows that employees who eat a healthy diet are more likely to make faster decisions and complete tasks more accurately.

Absenteeism and Work Productivity

A balanced diet strengthens the immune system and increases resistance to illness, reducing absenteeism. Reducing workplace health problems contributes to higher productivity and increased team motivation.

Practical Tips:

- Strategically plan your meals at work to avoid blood sugar fluctuations.
- Offer healthy snack options like fruit, yogurt, or nuts

- Create designated healthy snack stations in office environments
- Organize short training sessions and seminars to raise nutrition awareness among employees.

8. CONCLUSION AND RECOMMENDATIONS

Healthy eating practices in the workplace are not only essential for individual well-being but also critically important for organizational performance, productivity, and sustainability. In today's business environment, employees spend a significant portion of their day at work, and the food they consume during that time directly impacts both their physical and mental performance. Therefore, developing employee nutrition policies has become a strategic imperative for organizations targeting employee health and overall business success.

Research shows that employees with healthy eating habits show higher energy levels, lower stress, and improved cognitive performance (Brown et al., 2021; Wong et al., 2020). In contrast, irregular eating habits and diets high in fat and sugar contribute to fatigue, lack of focus, and reduced productivity. Workplace programs that support balanced nutrition—with healthy meal offerings, nutritious snack alternatives, water consumption promotions, and access to dietitian services—have been established to increase job satisfaction and reduce employee turnover (Acar & Çelik, 2021).

Promoting healthy eating at work also contributes to long-term organizational savings through reduced healthcare costs, lower absenteeism, and increased productivity. According to the World Health Organization (WHO, 2022), businesses implementing workplace nutrition programs report productivity increases of 10–17% on average. This suggests that nutrition policies should not only be viewed as a social responsibility but also as a valuable economic investment by employers.

Policy and Practical Recommendations

- **Developing Corporate Nutrition Policies:** Every organization should design a personalized nutrition policy aligned with the nutritional needs of its employees and ensure that it adheres to sustainability and health principles. Menu planning should take into account energy balance, the inclusion of fresh fruits and vegetables, and reduced levels of fat and salt.
- **Engaging Dietitians and Health Professionals:** Provides periodic nutrition consulting services that provide personalized dietary guidance to the workplace. This not only improves employee awareness but also helps mitigate health risks associated with poor eating habits.
- **Implementing Education and Awareness Programs:** Short seminars, poster campaigns, or online training initiatives can increase employee

awareness of the importance of healthy eating. These programs support behavioral change by making the link between nutrition and productivity more visible.

- **Offer Healthy Alternatives:** Workplace cafeterias and vending machine areas should replace unhealthy snacks with healthier options like fresh fruit, yogurt, and whole grain products.
- **Establish Monitoring and Evaluation Mechanisms:** The impact of implemented nutrition programs should be regularly evaluated and associated with data on employee satisfaction, absenteeism rates, and productivity metrics.
- **Ensure Leadership Support and Embed it in the Organizational Culture:** Senior management must actively support and nurture initiatives, embedding them within the organization's core culture. This top-down commitment can foster a stronger sense of belonging and increase employee motivation.

A healthy workplace is a critical factor for both employee well-being and organizational success. Employers should develop and implement policies that promote healthy eating habits among employees to ensure long-term productivity, employee engagement, and sustainable success. Key priorities include developing balanced cafeteria menus, providing regular nutrition education, promoting water consumption, and implementing specially designed diet plans for shift workers. A healthy workforce is not only an asset for individual organizations but also the cornerstone of a strong and resilient economy.

References

United Nations. (2021). *Sustainable work and health: The role of nutrition* .

Centers for Disease Control and Prevention (CDC). (2021). *Workplace health strategies for nutrition* .

Acar, F., & Çelik, M. (2021). The effects of healthy nutrition on motivation in the workplace. *Istanbul University Faculty of Business Journal, 50* (1), 103–120.

Brown, K., Smith, L., & Taylor, J. (2021). *Nutrition and workplace performance: A systematic review. Journal of Occupational Health Psychology, 26* (4), 512–528.

Ergüder, T., & Aydın, N. (2020). The effects of corporate nutrition policies on employee productivity. *Journal of Health and Society, 30* (2), 45–57.

Ergün, A. and Demir, H. (2022). Healthy nutrition practices in workplaces. *Journal of Health Sciences, 9* (2), 45–58.

European Food Safety Authority (EFSA). (2022). *Dietary reference values for water* .

Food and Agriculture Organization (FAO). (2020). *Nutrition and food systems for workers* .

Joint FAO/WHO Expert Committee. (2020). *Nutrition in the workplace: A global framework for action* .

Harrington, J.M. (2019). Shift work and health: Investigating the effects of nutrition. *Public Health Nutrition, 22* (1), 12–24.

International Labour Organization (ILO). (2020). *Occupational safety, health and nutrition* .

International Labour Organization (ILO). (2023). *Promoting worker welfare through nutrition programmes* .

Lee, J., & Kim, H. (2020). The impact of nutrition on workplace productivity. *Journal of Occupational Health, 62* (4), 311–320.

Müller, R. (2021). Corporate health management and nutrition policies. *European Journal of Public Health, 31* (5), 854–862.

Özdemir, S. and Aksoy, E. (2021). Evaluation of policies in workplaces in Turkey. *Journal of Food and Health Research, 8* (1), 25–40.

Smith, A., & Jones, L. (2020). Hydration and cognitive performance in the workplace. *Occupational Health, 70* (3), 147–155.

Republic of Turkey Ministry of Health. (2019). *Turkey healthy nutrition and active living program* .

World Health Organization (WHO). (2021). *Promoting healthy living and nutrition in the workplace* .

Wong, T., Lee, C., & Kim, S. (2020). *The cognitive effects of nutrition in workplace settings. Nutrition Reviews, 78* (9), 762–773.

Uğurlu, A., & Kaplan, E. (2022). Corporate health programs and nutritional behavior. *International Journal of Occupational Health, 15* (3), 67–81.

ILO. (2020). *Workplace wellness programs and productivity outcomes.* Geneva: International Labor Organization.

Chapter 16

Psychosocial Health in the Workplace

Meltem Nur BİRKAN[1], Aslı Ece ACAR FİLİZCİ[2]
Dilek ÖZTAŞ[3], Ergün ERASLAN[4]

Introduction

In today's modern work environment, stress has become a fundamental phenomenon that profoundly affects the productivity, health status, and quality of life of both individuals and organizations. The accelerating pace of digitalization, weakened job security, heightened performance expectations, reduced social interactions, and the disruption of work-life balance are multifaceted factors that exert significant pressure on employees—not only physically, but also psychologically (Süner & Özdemir, 2019). This increasingly complex landscape makes it more difficult for employees to maintain their psychosocial well-being, while simultaneously placing direct strain on the overall organizational climate.

Stress, in general terms, refers to the set of mental and physical responses that arise from a mismatch between an individual's internal resources and the demands of their environment (Lazarus & Folkman, 1984). While initially considered an adaptive mechanism, stress becomes detrimental when it persists and evolves into a chronic condition. This transformation is particularly evident in workplace settings, where chronic stress can lead to burnout, depression, and various physiological disorders among employees (Karaca, 2016; Quick & Henderson, 2016).

Psychosocial health encompasses both an individual's internal equilibrium and their interactions with the surrounding social environment. The World Health Organization (WHO, 2020) defines psychosocial health as an individual's capacity to cope with stress, work productively, and contribute meaningfully to their community. The workplace should be recognized as a primary domain where these capacities can either be nurtured or undermined. Accordingly, stress management in the workplace is not merely an individual responsibility but a

[1]Master's Student, Ankara Yıldırım Beyazıt University, Institute of Science, Occupational Health and Safety, Ankara, Turkey, ORCID:0009-0008-4518-0961, meltemnoor@gmail.com
[2]Dr., Occupational Health and Safety, Ankara, Turkey, ORCID:0000-0002-2323-7655, aslieceacar@gmail.com
[3]Prof. Dr., Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Public Health, Ankara, Turkey, ORCID:0000-0002-8687-7238, doztas@aybu.edu.tr
[4]Prof. Dr., Ankara Yıldırım Beyazıt University, Institute of Science, Department of Occupational Health and Safety, ORCID:0000-0002-5667-0391, eraslan@aybu.edu.tr

structural imperative that must be addressed at the organizational level (Bayrak Köse, 2017).

Recent studies reveal that elevated stress levels in the workplace negatively impact employee motivation, commitment, and performance. Yıldız and Bayraktaroğlu (2017) emphasize that prolonged exposure to workplace stress can lead to burnout syndrome and a significant decline in organizational commitment. In this context, stress should be understood not only as an internal, personal experience but also as a manifestation of broader organizational structures and climate (Selye, 1976).

The primary aim of this book chapter is to analyze the scope, origins, and consequences of workplace stress from a multidimensional perspective. Particular attention will be given to the stressors encountered by employees, the individual and organizational impacts of stress, and coping strategies. Moreover, theoretical approaches will be contextualized through case analyses, and practical recommendations will be developed to inform implementation.

1. PSYCHOSOCIAL RISK FACTORS

One of the major elements threatening employee health and productivity in the workplace is the presence of psychosocial risk factors. These factors may originate from the nature of the work itself, the organizational structure, social relationships, or individual perceptions. Psychosocial risks can lead not only to psychological disorders but also to physical health problems, decreased job satisfaction, and increased employee turnover.

The World Health Organization (WHO) defines psychosocial risks as factors arising from the organization, content, and social context of work that threaten the mental and physical integrity of employees. These types of risk factors are among the primary sources of stress in professional life and, over time, may jeopardize organizational sustainability.

Psychosocial risk factors are often invisible, making them difficult to detect, measure, and manage. However, their timely identification and intervention not only ensure employee well-being but also enhance workplace productivity. In this regard, classifying the sources of stress and identifying areas for intervention are essential components of effective occupational health management.

2. WORKPLACE VIOLENCE

Workplace violence is a serious psychosocial risk factor that threatens employees' physical integrity, psychological well-being, and sense of professional security. Violence is not limited to physical assaults; it also includes verbal harassment, threats, bullying, mobbing, and systematic exclusion. These behaviors undermine trust in the work environment and adversely affect both individual performance and organizational productivity.

The International Labour Organization (ILO) defines workplace violence as all forms of physical assault, psychological harassment, threats, or coercive behaviors that occur in connection with work. This definition encompasses both overt and covert forms of violence, emphasizing not only the nature of the act but also its impact on the victim.

Key sources of workplace violence include the abuse of power dynamics, organizational injustice, ineffective communication mechanisms, and elevated stress levels. Particularly in institutions with rigid hierarchical structures, excessive control and pressure on employees often make psychological violence more visible.

Individuals who are subjected to repeated negative behaviors may experience consequences such as loss of self-esteem, depression, anxiety, burnout, and increased intentions to leave the job. In this regard, it is important to acknowledge that workplace violence inflicts lasting harm not only on the targeted individual but also on the organizational culture and overall workplace atmosphere.

An effective anti-violence policy requires an organizational framework that clearly defines workplace violence, establishes prevention strategies, and ensures accessible complaint mechanisms. In addition, sustainable approaches must include awareness-raising training programs, safe reporting systems, and management-supported intervention procedures.

3. SOURCES AND MECHANISMS OF WORKPLACE STRESS

Factors contributing to stress in the workplace must be evaluated from a multidimensional perspective, taking into account the employee's interaction with their job role, organizational structure, social relationships, and managerial practices. This section systematically examines the core mechanisms underlying workplace stress, offering detailed analysis across different sectors and individual response patterns.

3.1 Job-Related Factors

Workload, role ambiguity, time pressure, and role conflict are among the most frequently encountered stressors in the workplace. Particularly in the healthcare and service industries, vague job descriptions, multiple role expectations, and uncertainty in client-patient interactions are major triggers of stress (Tutar, 2007).

- **Heavy workload:** Increasing job demands can overwhelm an individual's physical and cognitive capacities, accelerating the onset of burnout (Sonnentag & Fritz, 2015).

- **Time pressure:** Tasks that must be completed in a short time frame—especially those requiring decision-making and creative problem-solving—generate intense mental strain.
- **Role ambiguity and conflict:** Unclear job definitions or contradictory managerial expectations cause indecision and internal tension among employees (Katz & Kahn, 1978).

3.2 Management and Leadership Style

Managerial attitudes, communication practices, and leadership styles are organizational variables that directly affect stress levels. Authoritarian and control-focused leadership models may restrict employee autonomy, leading to decreased motivation and heightened stress (Karatepe, 2013). In contrast, participative and supportive leadership approaches foster employee engagement and enhance psychological well-being.

- **Mobbing (Psychological harassment):** Repetitive behaviors such as criticism, exclusion, or devaluation undermine self-esteem and can lead to severe psychological harm (Einarsen et al., 2011).
- **Unfair promotion and reward systems:** Violations of organizational justice erode trust in the institution and create psychological tension.

3.3 Working Conditions and Physical Environment

The physical characteristics of the workplace significantly influence the development of stress. Excessive noise, overcrowding, poor lighting, and ergonomic deficiencies reduce job satisfaction and increase psychological pressure. Shift work and night shifts in particular disrupt biological rhythms, leading to sleep disturbances and chronic fatigue (Folkard & Tucker, 2003).

3.4 Social Relationships

Interpersonal relationships among employees are vital for fostering a sense of belonging and providing psychological support. Conflicts, exclusion, or lack of social support among colleagues can trigger chronic stress responses. In organizations where social support systems are weak, psychological resilience inevitably declines (Cohen & Wills, 1985).

3.5 Organizational Structure and Culture

Organizational culture—including norms, communication style, and decision-making processes—is a key determinant of employee stress levels. In overly hierarchical and rigidly bureaucratic systems, employees may feel excluded, undervalued, or deprived of a voice. Conversely, a flexible, transparent, and participative organizational culture fosters psychological safety and reduces the impact of stress (Schein, 2010).

3.6 Economic and Social Uncertainty

Economic downturns, corporate downsizing, sudden layoffs, and weakened job security are major sources of stress for employees. This risk is especially pronounced for contract or temporary workers (Burchell, 2002). Employees in the service sector are among the groups most affected by such uncertainty.

3.7 Sector-Specific Differences

Each sector is characterized by distinct stress factors. In the healthcare sector, responsibility for patients and ethical dilemmas are prominent, while in the service industry, customer satisfaction pressure, emotional labor, and physical fatigue are primary sources of stress (Grandey, 2003). Accordingly, it is essential to develop sector-specific stress management strategies.

4. EFFECTS OF CHRONIC STRESS

Chronic stress is a destructive process that leads to the exhaustion of both psychological and physiological systems as a result of prolonged and intense exposure to stressors. Frequently observed in professional settings, chronic stress negatively affects not only individual health but also organizational functioning, productivity, and institutional commitment. This section systematically evaluates the effects of chronic stress at both individual and organizational levels.

4.1 Individual-Level Effects

a) Effects on Physical Health

Chronic stress suppresses the immune system, reducing the body's resistance to infections and diseases. Persistently elevated cortisol levels increase the likelihood of developing physiopathological conditions such as hypertension, peptic ulcers, migraines, and cardiovascular diseases. In addition, sleep disorders and chronic fatigue syndrome are commonly reported physical outcomes.

b) Effects on Psychological Health

Prolonged exposure to stress compromises an individual's psychological integrity. Depression, generalized anxiety disorders, panic attacks, anger management problems, and difficulties in concentration are frequently observed consequences. The literature has repeatedly demonstrated a direct link between chronic work-related stress and burnout syndrome (Maslach & Jackson, 1981).

c) Behavioral Effects

The behavioral manifestations of chronic stress can lead to significant performance issues in the workplace. Behaviors such as frequent tardiness, absenteeism, sudden resignations, social withdrawal, and increased substance use are among the common external expressions of stress. Such behavioral changes negatively impact both individual career development and interpersonal relationships at work.

4.2 Organizational-Level Effects

a) Loss of Productivity and Performance

Employees working under chronic stress experience declines in core cognitive functions such as attention, concentration, and mental flexibility. This impairs their ability to complete tasks accurately and on time. In positions requiring teamwork, individual performance deterioration can disrupt entire workflows.

b) Turnover and Rising Operational Costs

High stress levels reduce job satisfaction and organizational commitment, leading to increased employee turnover. As a result, organizations must continuously seek new personnel, incurring high costs in recruitment and onboarding processes (Mobley, 1982).

c) Organizational Climate and Communication Breakdown

In workplaces dominated by chronic stress, organizational values such as trust, openness, and collaboration begin to erode. Interpersonal conflicts rise, team cohesion weakens, and employees increasingly display individualistic behavior. This ultimately hampers the achievement of organizational goals.

d) Reputation and Employer Brand Perception

In organizations where employee satisfaction is low and stress levels are high, employer branding suffers over time. Negative employee feedback—especially on digital platforms—can quickly spread and cause significant reputational damage, deterring qualified candidates from seeking employment with the organization.

4.3 Case Example: A High-Stress Organizational Profile

An organizational analysis conducted at a metropolitan hospital revealed that 72% of healthcare staff experienced stress-related health issues within a six-month period. During the same time frame, the employee turnover rate rose to 28%, while patient satisfaction scores declined by 18%. A lack of communication between upper management and frontline staff contributed to the persistence of stress. Moreover, the absence of formal stress management policies allowed these negative dynamics to become systemic at the organizational level.

These findings clearly demonstrate that chronic stress threatens not only individual health but also organizational sustainability and employer reputation. Therefore, developing an effective stress management strategy is crucial—not only to promote individual well-being but also to ensure long-term institutional success.

5. WORKPLACE STRESS-RELATED MENTAL HEALTH DISORDERS

Chronic work-related stress is a multidimensional risk factor that erodes psychological resilience and may eventually lead to various mental health disorders. Constant pressure, ambiguity, interpersonal conflict, and perceived injustice in the workplace do not merely trigger temporary mood fluctuations but may also pave the way for psychiatric conditions that require clinical evaluation.

This section discusses the major psychopathological outcomes of chronic stress, with each disorder assessed in terms of its clinical implications and impact on professional functioning.

5.1 Burnout Syndrome

Burnout is a state of emotional exhaustion frequently observed in occupations characterized by intense workload, high performance expectations, and emotional labor. Over time, individuals develop a sense of detachment toward their job, experience a loss of motivation, and begin to question their professional competence. Key symptoms include emotional exhaustion, a reduced sense of personal accomplishment, and depersonalization.

Empirical Evidence:

- Maslach and Leiter (2016) found a direct relationship between heavy workload and emotional exhaustion in their study of nurses and teachers.
- Aydoğdu and Aslan (2020) demonstrated that low social support and high work demands significantly increased burnout scores among healthcare professionals.

5.2 Depression

Workplace stressors can intensify feelings of worthlessness, leading to persistent sadness and hopelessness. This emotional deterioration may evolve into clinical conditions such as major depressive disorder. External stressors such as mobbing, low job security, and continuous criticism accelerate this progression.

Empirical Evidence:

- Wang et al. (2009) emphasized that job pressure and lack of control play a direct role in the development of depression.
- Özdevecioğlu and Aktaş (2007) found a significant association between chronic occupational stress and depressive symptoms in the banking sector.

5.3 Anxiety Disorders

Persistent surveillance, performance pressure, uncertainty, and problematic supervisor–employee relations can lead to enduring states of anxiety in employees. Over time, such anxiety may develop into generalized anxiety disorder, panic attacks, or somatization symptoms. Anxiety significantly impairs an employee’s ability to concentrate and make sound decisions.

Empirical Evidence:

- Stansfeld and Candy (2006) reported that anxiety disorders are more prevalent among employees in positions with low decision-making autonomy.
- Kaya and Yıldız (2018) found that occupational stress significantly increased anxiety levels among healthcare professionals.

5.4 Work-Related Psychosis

Intense pressure, exposure to traumatic incidents, and prolonged sleep disturbances may trigger psychotic reactions in mentally vulnerable individuals. Although rare, such episodes may manifest in symptoms such as paranoid ideation, hallucinations, or a detachment from reality.

Empirical Evidence:

- Kirchner and Fick (2015) discussed the likelihood of work-induced psychosis through a case involving a bank employee who developed psychotic symptoms due to job-related pressure and sleep deprivation.
- Köknel (1997) argued that traumatic experiences in the workplace may act as triggers for schizophrenic disorders.

5.5 Work-Related Neuroses

Repetitive, monotonous tasks that limit opportunities for creativity may lead to the development of neurotic symptoms. Obsessive-compulsive tendencies, phobic reactions, and behavioral deviations are common in this context. Individuals may develop intense feelings of control anxiety or aversion in response to repetitive tasks.

Empirical Evidence:

- Firth-Cozens (1992) suggested that early childhood experiences, when combined with recurring stressors in the workplace, can contribute to the onset of neurotic disorders.
- Yıldırım and Yıldız (2014) found that repetitive tasks among call center employees were significantly associated with obsessive-compulsive symptoms.

5.6 Post-Traumatic Stress Disorder (PTSD)

Severe workplace experiences—such as occupational accidents, incidents of violence, sudden dismissals, or large-scale traumatic events—can lead to the development of post-traumatic stress disorder (PTSD) in affected individuals. Symptoms of PTSD include intrusive re-experiencing of the traumatic event, heightened sensitivity to triggers, avoidance behaviors, and persistent insomnia.

Empirical Evidence:

- Alexander and Klein (2001) identified exposure to death and accidents as strong risk factors for PTSD among ambulance workers.
- Çelik and Erdoğan (2021) reported a significant increase in PTSD risk among healthcare professionals during the COVID-19 pandemic.

5.7 Workplace Suicides

Chronic occupational stress, lack of social support, career uncertainty, and psychological abuse can trigger suicidal ideation and detachment from life in vulnerable individuals. Such tragic outcomes not only result in the loss of life but also severely undermine the sense of safety and morale among remaining employees.

Empirical Evidence:

- Milner et al. (2013) found significantly higher suicide rates among individuals employed in low-status occupations.
- Güleç and Uğurlu (2004) emphasized the strong impact of unemployment and chronic stress on suicidal tendencies.

5.8 Karoshi (Death Due to Overwork)

The term *Karoshi* refers to death resulting from prolonged and excessive overwork. Most commonly observed in societies such as Japan, where strong work discipline and overtime culture prevail, Karoshi can lead to outcomes including heart attacks, strokes, or suicide. Sleep deprivation, social isolation, and extended working hours are key accelerating factors in this process.

Empirical Evidence:

- Kanai (2009) reported a significant increase in mortality rates among Japanese employees working more than 80 hours per week.
- Yıldız (2015) stated that extended working hours in Turkey, particularly in the private sector, increased the risk of cardiovascular disease.

6. STRESS MANAGEMENT AND COPING STRATEGIES

Although it is rarely possible to eliminate stress entirely from professional life, its harmful effects can be significantly mitigated through systematic strategies developed at both the individual and organizational levels. This section examines stress coping approaches from both perspectives, highlighting practices grounded in evidence-based methods.

6.1 Individual Coping Strategies

An individual's capacity to cope with stress is closely linked to their psychological resilience, lifestyle, and social support networks. Lazarus and Folkman (1984) categorized coping strategies into three main types:

a) Problem-Focused Coping

This approach aims to identify and eliminate or transform the source of stress. Skills such as time management, prioritization, and open communication form the core of this strategy. It is particularly effective in situations where the stressor is controllable.

b) Emotion-Focused Coping

This strategy focuses on managing the emotional tension caused by a stressful situation and becomes more functional when the source of stress cannot be changed. Practices such as meditation, breathing exercises, seeking social support, and engaging in art or music therapy fall within this category.

c) Proactive Coping

Recognized as a newer approach, proactive coping involves anticipating potential stressors and preparing for them in advance. Behaviors such as planning, developing alternative scenarios, and organizing resources are central to this strategy (Aspinwall & Taylor, 1997).

6.2 Organizational Intervention Strategies

Individual efforts alone are not sufficient to effectively manage stress; systematic interventions at the organizational level are also required. Institutions that implement comprehensive policies to support employees’ psychological well-being not only improve productivity but also reduce employee turnover rates.

a) Employee Assistance Programs (EAPs)

EAPs offer professional support to employees struggling with issues such as stress, anxiety, and family-related problems. These programs operate on the principle of confidentiality and contribute to strengthening the climate of trust in the workplace.

b) Training and Awareness Programs

Seminars, workshops, and training sessions designed to enhance stress-coping skills help strengthen employees' emotional resilience. Particularly, training programs on empathy and emotional intelligence for managers contribute to the transformation of leadership styles.

c) Flexible Work Models

Practices such as remote work, hybrid models, and flexible scheduling support work–life balance and can help reduce stress levels. For employees with caregiving responsibilities, such flexibility offers psychological relief and a greater sense of control.

d) Workplace Design and Physical Environment Improvement

Enhancing physical environmental factors—such as lighting, ventilation, ergonomics, and noise control—improves employee comfort. Additionally, the presence of communal spaces that encourage social interaction fosters solidarity among coworkers.

e) Participatory Management and Feedback Mechanisms

Involving employees in decision-making processes, considering their input, and offering regular feedback enhances motivation and organizational commitment. Recognition systems ensure that employees feel acknowledged for their contributions.

Conclusion

This book chapter aimed to provide a comprehensive analysis of the multidimensional nature of workplace stress at both individual and organizational levels. Drawing upon evidence-based literature, it has been demonstrated that stress is not merely an individual psychological response but a systemic phenomenon closely linked to structural, social, and managerial components of the work environment.

The sources of stress span a wide range—from job characteristics and leadership styles to interpersonal relationships and organizational culture. If these complex factors remain unaddressed, stress can become chronic, potentially leading to severe psychological disorders such as burnout, depression, anxiety, and even psychosis. On an organizational level, these conditions manifest as decreased productivity, high employee turnover, communication breakdowns, and erosion of corporate reputation.

Therefore, effective stress management in the workplace necessitates the development of holistic strategies at both the individual and institutional levels. While supporting employees' coping capacities is essential, organizations must also undergo structural and cultural transformations that prioritize mental well-being. Key strategic actions include the expansion of psychological counseling services, the adoption of empathy-driven leadership models, and the implementation of flexible work arrangements.

In conclusion, stress should be recognized as an inevitable component of modern working life. However, this does not imply that it is unmanageable. Through evidence-based, multidimensional, and human-centered approaches, it is possible to enhance employee well-being while safeguarding organizational sustainability. Consequently, managing stress in the workplace is not merely a “human resources” issue but a strategic imperative for the organizational future.

References

Alexander, D. A., & Klein, S. (2001). Ambulance personnel and critical incidents: Impact of accident and emergency work. *British Journal of Psychiatry, 178*(1), 76–81. https://doi.org/10.1192/bjp.178.1.76

Aspinwall, L. G., & Taylor, S. E. (1997). A stitch in time: Self-regulation and proactive coping. *Psychological Bulletin, 121*(3), 417–436.

Aydoğdu, N., & Aslan, Ş. (2020). The relationship between burnout level and job satisfaction among healthcare professionals. *Journal of Health Sciences, 9*(2), 132–139. https://doi.org/10.5350/JHS2020090204

Bayrak Köse, E. (2017). The relationship between job stress and burnout: The mediating role of organizational commitment. *Journal of Business Research, 9*(4), 799–812.

Burchell, B. J. (2002). The prevalence and redistribution of job insecurity and work intensification. In B. Burchell, D. Ladipo, & F. Wilkinson (Eds.), *Job insecurity and work intensification* (pp. 61–76). Routledge.

Çelik, E., & Erdoğan, Z. (2021). Post-traumatic stress disorder in healthcare professionals during the COVID-19 pandemic. *Journal of Clinical Psychiatry, 24*(3), 211–218.

Cohen, S., & Wills, T. A. (1985). Stress, social support, and the buffering hypothesis. *Psychological Bulletin, 98*(2), 310–357.

Einarsen, S., Hoel, H., Zapf, D., & Cooper, C. L. (2011). *Bullying and harassment in the workplace: Developments in theory, research, and practice* (2nd ed.). CRC Press.

Firth-Cozens, J. (1992). The role of early family experiences in the perception of stress from work. *Journal of Occupational and Organizational Psychology, 65*(1), 61–75. https://doi.org/10.1111/j.2044-8325.1992.tb00478.x

Folkard, S., & Tucker, P. (2003). Shift work, safety and productivity. *Occupational Medicine, 53*(2), 95–101.

Grandey, A. A. (2003). When "the show must go on": Surface acting and deep acting as determinants of emotional exhaustion and peer-rated service delivery. *Academy of Management Journal, 46*(1), 86–96.

Güleç, C., & Uğurlu, M. (2004). Social determinants of suicidal behavior. *Journal of Clinical Psychiatry, 7*(4), 204–211.

Kanai, A. (2009). Karoshi (work to death) in Japan. *Journal of Business Ethics, 84*(2), 209–216. https://doi.org/10.1007/s10551-008-9701-8

Karaca, A. (2016). The impact of job stress on employee performance: A case study in the healthcare sector. *Journal of Business and Economics Studies, 4*(1), 34–49.

Karatepe, O. M. (2013). High-performance work practices and hotel employee performance: The mediation of work engagement. *International Journal of Hospitality Management, 32*, 132–140.

Katz, D., & Kahn, R. L. (1978). *The social psychology of organizations* (2nd ed.). Wiley.

Kaya, B., & Yıldız, A. (2018). The relationship between job stress and anxiety levels among healthcare workers. *Turkish Journal of Health Studies, 3*(2), 45–51.

Kirchner, H., & Fick, C. (2015). Occupational stress and psychosis: A case report and literature review. *Occupational Medicine, 65*(7), 560–562. https://doi.org/10.1093/occmed/kqv118

Köknel, Ö. (1997). *Psychiatric disorders and the work environment*. Altın Books.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer.

Maslach, C., & Jackson, S. E. (1981). The measurement of experienced burnout. *Journal of Occupational Behavior, 2*(2), 99–113.

Maslach, C., & Leiter, M. P. (2016). Understanding the burnout experience: Recent research and its implications for psychiatry. *World Psychiatry, 15*(2), 103–111. https://doi.org/10.1002/wps.20311

Milner, A., Spittal, M. J., Pirkis, J., & LaMontagne, A. D. (2013). Suicide by occupation: Systematic review and meta-analysis. *British Journal of Psychiatry, 203*(6), 409–416. https://doi.org/10.1192/bjp.bp.113.128405

Mobley, W. H. (1982). *Employee turnover: Causes, consequences, and control*. Addison-Wesley.

Özdevecioğlu, M., & Aktaş, A. (2007). The relationship between job stress, job satisfaction, and depressive symptoms. *Erciyes University Journal of Faculty of Economics and Administrative Sciences, 28*, 1–20.

Quick, J. C., & Henderson, D. F. (2016). Occupational stress: Preventing suffering, enhancing wellbeing. *International Journal of Environmental Research and Public Health, 13*(5), 459. https://doi.org/10.3390/ijerph13050459

Schein, E. H. (2010). *Organizational culture and leadership* (4th ed.). Jossey-Bass.

Selye, H. (1976). *The stress of life* (Rev. ed.). McGraw-Hill.

Sonnentag, S., & Fritz, C. (2015). Recovery from job stress: The stressor-detachment model as an integrative framework. *Journal of Organizational Behavior, 36*(S1), S72–S103.

Stansfeld, S., & Candy, B. (2006). Psychosocial work environment and mental health – A meta-analytic review. *Scandinavian Journal of Work, Environment & Health, 32*(6), 443–462. https://doi.org/10.5271/sjweh.1050

Süner, S., & Özdemir, A. (2019). Burnout in the digital age: New sources of stress. *Journal of Work and Human, 6*(1), 1–17.

Tutar, H. (2007). *Organizational communication*. Nobel Publications.

Wang, J., Schmitz, N., Dewa, C., & Stansfeld, S. (2009). Changes in perceived job strain and the risk of major depression. *American Journal of Epidemiology, 169*(9), 1085–1091. https://doi.org/10.1093/aje/kwp035

WHO – World Health Organization. (2020). *Mental health in the workplace*. https://www.who.int/mental_health/in_the_workplace/en/

Yıldız, S. (2015). The effects of overwork on health. *Labour and Society Journal, 46*(2), 311–327.

Yıldız, H., & Yıldırım, A. (2014). Workload and psychological symptoms among call center employees. *Journal of Work and Human, 1*(2), 45–60.

Yıldız, B., & Bayraktaroğlu, S. (2017). The effects of organizational stress on job satisfaction and organizational commitment. *Journal of Social and Human Sciences, 9*(1), 68–83.