GEÇİCİ KAPAK

Kapak tasarımı
devam ediyor.

BİDGE Yayınları

Emerging Concepts in Nutrition Science and Metabolic Medicine

Editor: GÖKHAN DEGE

ISBN: -

1st Edition
Page Layout By: Gözde YÜCEL
Publication Date: -
BİDGE Yayınları

Certificate No: 71374

www.bidgeyayinlari.com.tr - bidgeyayinlari@gmail.com
Krc Bilişim Ticaret ve Organizasyon Ltd. Şti.
Güzeltepe Mahallesi Abidin Daver Sokak Sefer Apartmanı No: 7/9 Çankaya / Ankara

PREFACE

Nutrition science is undergoing a period of remarkable transformation, driven by advances in molecular biology, microbiome research, metabolic medicine, and personalized nutrition. As our understanding of the complex interactions between diet, metabolism, and human health continues to expand, novel nutritional strategies and bioactive compounds are increasingly being integrated into both preventive and therapeutic healthcare approaches.

This book, *Frontiers in Metabolic Regulation and Therapeutic Nutrition*, brings together contemporary topics that reflect some of the most promising developments in nutritional and metabolic research. The chapters included in this volume explore the physiological and clinical significance of glucagon-like peptide-1 (GLP-1), the emerging role of postbiotics as bioactive agents, and the scientific foundations and health implications of fasting-mimicking diets. Although these topics originate from different areas of nutrition science, they collectively contribute to a deeper understanding of metabolic regulation and evidence-based nutritional interventions.

GLP-1 has gained considerable attention due to its critical role in glucose homeostasis, appetite regulation, and the management of metabolic diseases. Similarly, postbiotics have emerged as a novel class of functional bioactive compounds with promising applications in gut health, immune modulation, and chronic disease prevention. Meanwhile, fasting-mimicking diets represent an innovative nutritional strategy that seeks to harness the physiological benefits of fasting while maintaining nutritional adequacy and feasibility in clinical practice.

By integrating these emerging concepts, this book aims to provide researchers, healthcare professionals, academics, and students with a comprehensive overview of current scientific evidence and future directions in metabolic and clinical nutrition. It is our hope that this volume will contribute to ongoing scientific dialogue and inspire further research in the rapidly evolving fields of nutrition and metabolic health.

I would like to express my sincere gratitude to all contributing authors for sharing their expertise and scholarly efforts. Their valuable contributions have made this book possible. I also extend my appreciation to the publisher for supporting the dissemination of scientific knowledge.

I hope this book serves as a useful resource for researchers, clinicians, dietitians, and students interested in advancing the science and practice of nutrition.

Asst. Prof. Dr. Gökhan DEGE

CONTENTS

CHAPTER 0

GLP-1: ITS ROLE IN METABOLIC REGULATION AND CLINICAL APPLICATIONS

MERVE KILIÇ[1]

Introduction

The global rise in obesity, type 2 diabetes, and related metabolic disturbances has intensified interest in the biological systems that coordinate nutrient handling and energy balance. In this framework, the gastrointestinal tract is now understood not only as an organ of digestion and absorption, but also as an endocrine network that actively shapes whole-body metabolism. Rather than acting solely as a classical incretin, GLP-1 can be considered part of a broader regulatory network that integrates nutrient sensing with systemic metabolic responses. Following food intake, it is secreted from intestinal L cells and contributes to glycemic control through coordinated modulation of insulin and glucagon secretion in a glucose-dependent manner. This regulatory mechanism enables fine adjustment of postprandial metabolism without significantly increasing hypoglycemia risk (Holst, 2007:1409).

[1] Öğr. Gör., Doğuş Üniversitesi Meslek Yüksekokulu, Anestezi, İstanbul/Türkiye, ORCID ID: 0000-0003-2351-2511, mersah06@gmail.com

GLP-1 is an incretin hormone secreted predominantly by intestinal L cells after food intake. Once released, it promotes insulin secretion in a glucose-dependent manner and suppresses glucagon release, thereby contributing to postprandial glucose control (Nauck & Meier, 2018:5). This coordinated endocrine response is one of the main reasons GLP-1 occupies a prominent place in metabolic physiology. In addition to its pancreatic actions, GLP-1 slows gastric emptying and influences appetite-regulating neural circuits, which together reduce caloric intake and help maintain energy balance (Müller et al., 2019:72). In parallel, GLP-1 influences gastrointestinal motility and central satiety pathways, linking peripheral metabolic signals with neural control of food intake. These combined effects highlight its role not only in glucose regulation but also in overall energy balance.

Over the last decade, the scientific view of GLP-1 has expanded considerably. It is no longer regarded solely as an incretin involved in glucose homeostasis. Experimental and clinical studies increasingly indicate that GLP-1 signaling may affect cardiovascular health, inflammatory tone, renal physiology, and even neuronal function (Drucker, 2018:740). Because of these broader actions, GLP-1 has become more than a physiological mediator; it has also emerged as a major therapeutic target. Indeed, the development of GLP-1 receptor agonists has changed the management of type 2 diabetes and obesity, while also opening new possibilities for other chronic disorders (Zheng et al., 2024:234).

This chapter discusses the structure and biosynthesis of GLP-1, the molecular basis of its actions, its systemic physiological effects, and the clinical implications of GLP-1–based therapies. In addition, current directions and future perspectives are considered in light of the rapidly evolving literature.

Biogenesis and Molecular Features of GLP-1

GLP-1 originates from the proglucagon gene, but the final peptide products derived from this precursor differ by tissue because proglucagon processing is cell-specific. In pancreatic α-cells, the precursor is mainly converted into glucagon, whereas in intestinal enteroendocrine L cells, an alternative processing pattern generates GLP-1 together with GLP-2, oxyntomodulin, and glicentin (Drucker, 2018:740). This distinction largely depends on the differential expression of prohormone convertases, particularly PC1/3 in the intestine and PC2 in the pancreas.

The biosynthetic process begins with transcription of the GCG gene and translation of proglucagon, a precursor peptide approximately 180 amino acids in length. In L cells, prohormone convertase 1/3 cleaves this precursor into several biologically active fragments, among which GLP-1 is one of the most important (Müller et al., 2019:72). The main active molecular forms are GLP-1 (7–37) and GLP-1 (7–36) amide, with the amidated form representing the predominant circulating isoform in humans (Holst, 2007:1409).

A defining biochemical feature of native GLP-1 is its very short half-life. After secretion, it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), which limits its persistence in the circulation to only a few minutes (Nauck & Meier, 2018:5). This rapid inactivation suggests that GLP-1 functions within a tightly controlled temporal window, enabling a highly dynamic response to nutrient ingestion.

Although meal ingestion is the principal trigger for GLP-1 secretion, the process is more complex than a simple luminal nutrient effect. Carbohydrates and lipids are particularly effective stimuli, yet vagal activity, enteric neural signaling, and endocrine feedback mechanisms also modulate L-cell secretion. Moreover, GLP-1 is not confined to the gastrointestinal tract. Smaller amounts are also produced in neurons of the nucleus tractus solitarius in the

brainstem, supporting the idea that GLP-1 signaling integrates peripheral and central metabolic responses (Zheng et al., 2024:234).

Molecular Mechanisms of GLP-1 Action

The actions of GLP-1 are mediated primarily through the GLP-1 receptor (GLP-1R), a G protein-coupled receptor expressed in several tissues, including pancreatic islets, the brain, the heart, and the vasculature. This signaling cascade does not operate in isolation but interacts with multiple intracellular pathways that coordinate hormone secretion and cellular adaptation. The increase in cAMP activates downstream effectors such as PKA and Epac, which together regulate insulin exocytosis and cellular responsiveness (Campbell & Drucker, 2013:819). This rise in cAMP triggers downstream signaling pathways such as protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac), both of which contribute to the cellular effects of GLP-1.

In pancreatic β-cells, GLP-1 amplifies insulin secretion only when glucose levels are elevated, which is one of its most clinically advantageous features. Mechanistically, it modulates ATP-sensitive potassium channels, facilitates membrane depolarization, and promotes calcium influx through voltage-dependent calcium channels. The resulting increase in intracellular calcium supports the exocytosis of insulin granules (Seino et al., 2011:2118). At the same time, the cAMP/Epac pathway enhances the mobilization and priming of insulin-containing vesicles, thereby improving both the magnitude and coordination of insulin release.

GLP-1 signaling also appears to support β-cell integrity beyond its acute secretory effects. Experimental evidence suggests that receptor activation can mitigate endoplasmic reticulum stress, reduce apoptosis, and preserve β-cell function under adverse metabolic conditions (Yusta et al., 2006:391). These protective actions are particularly relevant in the context of chronic

hyperglycemia and lipotoxicity, where β-cell failure contributes to the progression of type 2 diabetes.

Outside the pancreas, GLP-1 influences additional signaling networks linked to vascular and metabolic health. In endothelial cells, receptor activation has been associated with enhanced nitric oxide production and improved endothelial responsiveness, findings consistent with vasoprotective effects (Ban et al., 2008:2340). GLP-1 may also interact with AMP-activated protein kinase (AMPK), a central regulator of energy balance. Through this pathway, GLP-1 signaling has been linked to increased lipid oxidation and improved metabolic efficiency, especially in states characterized by insulin resistance or excess adiposity (Zheng et al., 2024:234).

Another important dimension of GLP-1 biology relates to inflammation. Experimental studies indicate that GLP-1 receptor activation can attenuate inflammatory signaling and reduce cytokine production, suggesting a potential contribution to the control of chronic low-grade inflammation commonly observed in metabolic disease (Arakawa et al., 2010:1030). Taken together, these findings indicate that GLP-1 acts through an interconnected signaling network that links endocrine secretion, nutrient sensing, cellular stress responses, and inflammatory regulation.

Systemic Effects of GLP-1

The influence of GLP-1 extends well beyond glycemic regulation. Rather than functioning in isolation, GLP-1 participates in coordinated inter-organ communication that affects gastrointestinal activity, central appetite control, cardiovascular physiology, renal function, and overall energy homeostasis.

Within the gastrointestinal system, GLP-1 slows gastric emptying, delaying the movement of nutrients from the stomach into the small intestine. This delay in gastric emptying represents a key mechanism through which GLP-1 modulates postprandial glucose

excursions, ensuring a more gradual absorption of nutrients.This delay reduces the rate at which glucose enters the circulation after meals, thereby blunting postprandial glycemic excursions. In parallel, GLP-1 affects gastrointestinal motility through vagal and enteric neural pathways, indicating that its digestive effects are partly mediated by neurohumoral mechanisms (Marathe et al., 2011:1).

In the central nervous system, GLP-1 contributes importantly to satiety and feeding behavior. GLP-1 receptors are present in several brain regions involved in energy regulation, particularly the hypothalamus and brainstem. Activation of anorexigenic pathways, including proopiomelanocortin neurons, together with reduced orexigenic signaling such as neuropeptide Y activity, leads to lower appetite and decreased food intake (Blundell et al., 2017:1242). These central effects are among the main reasons GLP-1 receptor agonists have proven successful in obesity treatment.

Cardiovascular actions of GLP-1 have also become a major focus of recent research. Large clinical outcome trials have shown that GLP-1–based therapies can reduce major adverse cardiovascular events in high-risk populations. Although the underlying mechanisms are likely multifactorial, proposed contributors include improved endothelial function, reduced vascular inflammation, modest blood pressure reduction, and favorable effects on body weight and glycemic burden (Gerstein et al., 2019:121). There is also evidence suggesting that GLP-1 may improve myocardial energy handling and increase resistance to ischemic injury.

The kidney has emerged as another relevant target organ for GLP-1 action. Clinical studies indicate that GLP-1 receptor agonists may reduce albuminuria and slow the decline in renal function in some patient groups. These benefits may reflect a combination of hemodynamic, natriuretic, anti-inflammatory, and metabolic effects

(Mann et al., 2017:839). Given the strong overlap between diabetes, obesity, and chronic kidney disease, these observations have increased interest in GLP-1–based approaches in nephrometabolic medicine.

The effect of GLP-1 on body weight also involves more than appetite suppression alone. It reflects integrated changes in feeding behavior, caloric intake, insulin sensitivity, and adipose tissue metabolism. For this reason, GLP-1 is increasingly recognized as a broader regulator of energy balance rather than a hormone limited to glucose handling (Müller et al., 2022:201).

Emerging data additionally point to possible neuroprotective effects. Experimental studies have suggested that GLP-1 signaling may reduce oxidative stress, improve synaptic function, and modulate pathological processes associated with neurodegeneration. Although these findings are still evolving, they raise the possibility that GLP-1–based therapies could eventually find applications outside conventional metabolic disease management (Siddeeque et al., 2024:113537).

Overall, GLP-1 can be described as a pleiotropic hormone whose actions are distributed across multiple organ systems. Its biological relevance lies not only in its individual effects, but also in its capacity to coordinate metabolic adaptation at the whole-body level.

Therapeutic Development of GLP-1–Based Agents

The therapeutic use of GLP-1 was initially limited by the instability of the native peptide. One of the primary challenges in the clinical use of native GLP-1 is its rapid degradation by the DPP-4 enzyme, which results in a very short duration of action. To address this limitation, structurally modified GLP-1 receptor agonists have been developed with increased resistance to enzymatic breakdown, allowing for prolonged therapeutic effects. This challenge led to the

development of GLP-1 receptor agonists with greater resistance to enzymatic degradation and longer durations of action. These agents now occupy an important place in the treatment of type 2 diabetes and obesity.

GLP-1 receptor agonists are commonly divided into short-acting and long-acting compounds. These pharmacological agents differ mainly in their duration of activity. Short-acting formulations tend to exert stronger effects on postprandial glucose levels, whereas long-acting compounds provide more stable glycemic control throughout the day.By contrast, long-acting agents including liraglutide, semaglutide, and dulaglutide provide more sustained receptor stimulation and influence both fasting and postprandial glucose control (Meier, 2012:728). The introduction of once-daily and once-weekly regimens has also improved convenience and adherence in routine practice.

A major advantage of this drug class is that insulin secretion is enhanced in a glucose-dependent manner. As a result, the risk of hypoglycemia is lower than with several conventional antidiabetic therapies when GLP-1 receptor agonists are used without sulfonylureas or insulin. At the same time, these agents promote weight reduction, making them particularly useful in individuals with type 2 diabetes accompanied by overweight or obesity. Clinical trials have repeatedly shown meaningful improvements in glycemic control together with clinically significant reductions in body weight, especially with liraglutide and semaglutide (Wilding et al., 2021:989).

The cardiovascular dimension of GLP-1 therapy has further strengthened its clinical importance. Landmark trials such as LEADER and SUSTAIN-6 demonstrated that liraglutide and semaglutide, respectively, reduced major adverse cardiovascular events in patients with type 2 diabetes at elevated cardiovascular risk (Marso et al., 2016:1834). In parallel, the REWIND trial expanded

this evidence base for dulaglutide (Gerstein et al., 2019:121). These findings have influenced treatment guidelines, which increasingly recommend GLP-1 receptor agonists in patients who require cardiometabolic risk reduction in addition to glucose control.

In obesity management, GLP-1 analogs have moved from an adjunctive option to a central therapeutic strategy. Trials from the STEP program demonstrated that higher-dose semaglutide produced substantial and sustained weight loss in adults with overweight or obesity (Wilding et al., 2021:989). These benefits are explained not only by reduced appetite, but also by altered reward-related eating behavior and improved adherence to lower caloric intake over time.

As with all therapies, tolerability and safety remain important considerations. Gastrointestinal adverse effects, especially nausea, vomiting, early satiety, and occasionally diarrhea, are the most commonly reported problems. These effects are usually dose-related and often diminish with continued treatment. Less common but clinically relevant concerns include gallbladder-related events and pancreatitis, both of which continue to be monitored through long-term pharmacovigilance and post-marketing data (Davies et al., 2016:222).

Recent developments have widened the therapeutic landscape even further. Oral semaglutide has offered a non-injectable alternative, representing a major advance in patient acceptability. In addition, dual incretin-based therapies, particularly agents targeting both GLP-1 and GIP receptors, have shown impressive metabolic efficacy and may redefine future treatment strategies for obesity and type 2 diabetes.

In this sense, GLP-1 analogs are no longer viewed as drugs that simply lower blood glucose. They have become multifunctional agents capable of influencing body weight, cardiometabolic risk, and possibly a wider spectrum of chronic disease pathways.

Conclusion and Future Perspectives

GLP-1 has gradually shifted from being viewed as a hormone with a relatively narrow physiological role to a much broader regulatory signal that participates in complex metabolic coordination. Earlier interpretations largely emphasized its incretin effect, particularly its ability to enhance insulin secretion after meals. However, accumulating evidence now indicates that its function cannot be confined to glycemic regulation alone. Instead, GLP-1 appears to operate at the intersection of multiple physiological systems, linking nutrient intake with hormonal, neural, and metabolic responses.

This expanded understanding is reflected in the clinical impact of GLP-1–based therapies. The benefits observed with GLP-1 receptor agonists extend beyond reductions in blood glucose levels. Improvements in body weight, appetite control, and cardiovascular outcomes suggest that these agents influence interconnected aspects of metabolic disease rather than targeting a single biochemical pathway. Such a profile is particularly relevant in conditions like obesity and type 2 diabetes, where dysregulation involves multiple overlapping mechanisms.

Despite these advances, important gaps in knowledge remain. One of the ongoing challenges is to clarify how much of the therapeutic effect is driven by direct receptor activation in specific tissues and how much arises from secondary systemic adaptations. In addition, clinical experience has shown that not all individuals respond similarly to GLP-1–based treatments. This variability points toward the potential influence of genetic background, metabolic phenotype, and environmental factors, although these relationships are not yet fully defined.

Another area of growing interest is the development of therapies that act on more than one metabolic pathway

simultaneously. Agents that combine GLP-1 receptor activity with other incretin or metabolic targets, such as GIP, have demonstrated enhanced effects on both weight reduction and glycemic control. These findings suggest that future treatment approaches may increasingly move toward multi-target strategies designed to address the complexity of metabolic disorders in a more integrated manner.

Beyond traditional indications, there is also increasing curiosity about whether GLP-1 signaling may have relevance in other disease contexts. Preliminary data from experimental and clinical studies have raised the possibility that GLP-1–based interventions could play a role in neurodegenerative conditions, inflammatory processes, and kidney-related disorders. Although these areas are still under investigation, they highlight the potential for GLP-1 to be considered within a wider biomedical framework.

In this context, GLP-1 should not be regarded merely as a pharmacological target limited to glucose control. Rather, it represents a dynamic component of the metabolic network, capable of integrating signals across different organ systems. Future research that combines mechanistic insights with carefully designed clinical studies will be essential for clarifying its full range of actions. Such efforts may ultimately contribute to more individualized and effective therapeutic strategies, particularly in the management of complex, multifactorial diseases.

References

Arakawa, M., Mita, T., Azuma, K., Ebato, C., Goto, H., Nomiyama, T., Fujitani, Y., Hirose, T., Kawamori, R., & Watada, H. (2010). Inhibition of Monocyte Adhesion to Endothelial Cells and Attenuation of Atherosclerotic Lesion by a Glucagon-like Peptide-1 Receptor Agonist, Exendin-4. *Diabetes*, *59*(4), 1030–1037. https://doi.org/10.2337/db09-1694

Ban, K., Noyan-Ashraf, M. H., Hoefer, J., Bolz, S.-S., Drucker, D. J., & Husain, M. (2008). Cardioprotective and Vasodilatory Actions of Glucagon-Like Peptide 1 Receptor Are Mediated Through Both Glucagon-Like Peptide 1 Receptor–Dependent and –Independent Pathways. *Circulation*, *117*(18), 2340–2350. https://doi.org/10.1161/CIRCULATIONAHA.107.739938

Blundell, J., Finlayson, G., Axelsen, M., Flint, A., Gibbons, C., Kvist, T., & Hjerpsted, J. B. (2017). Effects of once-weekly semaglutide on appetite, energy intake, control of eating, food preference and body weight in subjects with obesity. *Diabetes, Obesity and Metabolism*, *19*(9), 1242–1251. https://doi.org/10.1111/dom.12932

Campbell, J. E., & Drucker, D. J. (2013). Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. *Cell Metabolism*, *17*(6), 819–837. https://doi.org/10.1016/j.cmet.2013.04.008

Davies, M. J., Bain, S. C., Atkin, S. L., Rossing, P., Scott, D., Shamkhalova, M. S., Bosch-Traberg, H., Syrén, A., & Umpierrez, G. E. (2016). Efficacy and Safety of Liraglutide Versus Placebo as Add-on to Glucose-Lowering Therapy in Patients With Type 2 Diabetes and Moderate Renal Impairment (LIRA-RENAL): A Randomized Clinical Trial. *Diabetes Care*, *39*(2), 222–230. https://doi.org/10.2337/dc14-2883

Drucker, D. J. (2018). Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. *Cell Metabolism*, *27*(4), 740–756. https://doi.org/10.1016/j.cmet.2018.03.001

Gerstein, H. C., Colhoun, H. M., Dagenais, G. R., Diaz, R., Lakshmanan, M., Pais, P., Probstfield, J., Riesmeyer, J. S., Riddle, M. C., Rydén, L., Xavier, D., Atisso, C. M., Dyal, L., Hall, S., Rao-Melacini, P., Wong, G., Avezum, A., Basile, J., Chung, N., … Zigrang, W. (2019). Dulaglutide and

cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. *The Lancet*, *394*(10193), 121–130. https://doi.org/10.1016/S0140-6736(19)31149-3

Holst, J. J. (2007). The Physiology of Glucagon-like Peptide 1. *Physiological Reviews*, *87*(4), 1409–1439. https://doi.org/10.1152/physrev.00034.2006

Mann, J. F. E., Ørsted, D. D., Brown-Frandsen, K., Marso, S. P., Poulter, N. R., Rasmussen, S., Tornøe, K., Zinman, B., & Buse, J. B. (2017). Liraglutide and Renal Outcomes in Type 2 Diabetes. *New England Journal of Medicine*, *377*(9), 839–848. https://doi.org/10.1056/NEJMoa1616011

Marathe, C. S., Rayner, C. K., Jones, K. L., & Horowitz, M. (2011). Effects of GLP-1 and Incretin-Based Therapies on Gastrointestinal Motor Function. *Experimental Diabetes Research*, *2011*, 1–10. https://doi.org/10.1155/2011/279530

Marso, S. P., Bain, S. C., Consoli, A., Eliaschewitz, F. G., Jódar, E., Leiter, L. A., Lingvay, I., Rosenstock, J., Seufert, J., Warren, M. L., Woo, V., Hansen, O., Holst, A. G., Pettersson, J., & Vilsbøll, T. (2016). Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *New England Journal of Medicine*, *375*(19), 1834–1844. https://doi.org/10.1056/NEJMoa1607141

Meier, J. J. (2012). GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology*, *8*(12), 728–742. https://doi.org/10.1038/nrendo.2012.140

Müller, T. D., Blüher, M., Tschöp, M. H., & DiMarchi, R. D. (2022). Anti-obesity drug discovery: advances and challenges. *Nature*

Reviews Drug Discovery, *21*(3), 201–223. https://doi.org/10.1038/s41573-021-00337-8

Müller, T. D., Finan, B., Bloom, S. R., D'Alessio, D., Drucker, D. J., Flatt, P. R., Fritsche, A., Gribble, F., Grill, H. J., Habener, J. F., Holst, J. J., Langhans, W., Meier, J. J., Nauck, M. A., Perez-Tilve, D., Pocai, A., Reimann, F., Sandoval, D. A., Schwartz, T. W., … Tschöp, M. H. (2019). Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism*, *30*, 72–130. https://doi.org/10.1016/j.molmet.2019.09.010

Nauck, M. A., & Meier, J. J. (2018). Incretin hormones: Their role in health and disease. *Diabetes, Obesity and Metabolism*, *20*(S1), 5–21. https://doi.org/10.1111/dom.13129

Seino, S., Shibasaki, T., & Minami, K. (2011). Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *Journal of Clinical Investigation*, *121*(6), 2118–2125. https://doi.org/10.1172/JCI45680

Siddeeque, N., Hussein, M. H., Abdelmaksoud, A., Bishop, J., Attia, A. S., Elshazli, R. M., Fawzy, M. S., & Toraih, E. A. (2024). Neuroprotective effects of GLP-1 receptor agonists in neurodegenerative Disorders: A Large-Scale Propensity-Matched cohort study. *International Immunopharmacology*, *143*, 113537. https://doi.org/10.1016/j.intimp.2024.113537

Wilding, J. P. H., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L. F., Lingvay, I., McGowan, B. M., Rosenstock, J., Tran, M. T. D., Wadden, T. A., Wharton, S., Yokote, K., Zeuthen, N., & Kushner, R. F. (2021). Once-Weekly Semaglutide in Adults with Overweight or Obesity. *New England Journal of Medicine*, *384*(11), 989–1002. https://doi.org/10.1056/NEJMoa2032183

Yusta, B., Baggio, L. L., Estall, J. L., Koehler, J. A., Holland, D. P., Li, H., Pipeleers, D., Ling, Z., & Drucker, D. J. (2006). GLP-1

receptor activation improves β cell function and survival following induction of endoplasmic reticulum stress. *Cell Metabolism*, *4*(5), 391–406. https://doi.org/10.1016/j.cmet.2006.10.001

Zheng, Z., Zong, Y., Ma, Y., Tian, Y., Pang, Y., Zhang, C., & Gao, J. (2024). Glucagon-like peptide-1 receptor: mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy*, *9*(1), 234. https://doi.org/10.1038/s41392-024-01931-z

CHAPTER 0

POSTBİOTİCS AS BİOACTİVE AGENTS: MECHANİSMS AND CLİNİCAL USES

OSMAN EREN[1]

Abstract

Postbiotics are defined as bioactive compounds derived from the metabolic activity of live microorganisms, exerting beneficial effects on the host organism without requiring the administration of viable cells. These compounds encompass cell wall components, enzymes, peptidoglycans, short-chain fatty acids, exopolysaccharides, and diverse secondary metabolites. Mechanistically, postbiotics modulate host immunity, inhibit pathogenic microorganisms, attenuate inflammatory responses, reinforce intestinal barrier integrity, and mitigate oxidative stress, reflecting a multifaceted spectrum of biological activities. Production is achieved through controlled fermentation of selected probiotic strains, followed by isolation of cell-free supernatants or inactivated microbial fractions, thereby ensuring stability, safety, and reproducibility without the challenges

[1] Dr, Hazine ve Maliye Bakanlığı-Ağrı Defterdarlığı, Orcid: 0000-0002-2016-7671

associated with live microbial administration. In clinical contexts, postbiotics exhibit promising potential in managing gastrointestinal disorders, enhancing immune competence, modulating metabolic pathways, and mitigating chronic inflammatory states. Their antimicrobial and antiviral properties further position them as adjunctive agents in infection risk reduction. The inherent physicochemical stability of postbiotics confers advantages in storage, formulation, and targeted delivery, distinguishing them from traditional probiotics. Accumulating evidence underscores their capacity as next-generation biotherapeutics, offering high bioavailability, safety, and mechanistic specificity. Consequently, postbiotics represent a transformative paradigm in microbiome-based interventions, bridging the gap between microbial metabolites and precision therapeutics in contemporary clinical and translational medicine.

Postbiotic Concept

The conceptual framework of postbiotics is rooted in the etymological fusion of the Greek terms post (“after”) and bios (“life”), reflecting the transition from microbial viability to biological functionality independent of cellular life. In its contemporary usage, the term encompasses a diverse array of inanimate microbial preparations, including structurally intact but non-viable cells, cell wall fragments, surface-associated molecules, and fermentation-derived metabolites, all of which retain the capacity to modulate host physiology through defined mechanistic pathways. Historically, the field has been marked by considerable terminological heterogeneity, with descriptors such as paraprobiotics, non-viable probiotics, ghost biotics, and heat-inactivated probiotics appearing throughout the literature. However, the taxonomic and functional ambiguity associated with these alternative terms has led to the predominance of postbiotic as the preferred descriptor in contemporary microbial

therapeutics. A major conceptual refinement was introduced by the International Scientific Association for Probiotics and Prebiotics (ISAPP), which defines postbiotics as “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.” This definition is deliberately inclusive: it removes the requirement for the source microorganism to meet probiotic criteria and rejects the long-standing assumption that postbiotic effects are confined to the gastrointestinal tract. Instead, it positions postbiotics as a mechanistically heterogeneous class of bioactive agents capable of engaging host pathways across multiple organ systems, including mucosal, metabolic, neuroimmune, and systemic axes. By decoupling microbial viability from functional efficacy, the ISAPP definition expands the conceptual landscape of host-microbe interactions, enabling the integration of postbiotics into broader frameworks of microbial-derived therapeutics, immune modulation, and precision microbiome interventions. Consequently, postbiotics now occupy a distinct and increasingly influential niche within microbiome science, bridging the gap between classical probiotic theory and next-generation microbial therapeutics (Anandharaj et al., 2014; İçier et al., 2022; Vinderola et al., 2022).

Postbiotic preparations consist of bioactive, soluble products that are predominantly located in the supernatant fraction. This molecular repertoire may include microbial enzymes, vitamins, complex polysaccharides, short-chain fatty acids (SCFAs), cell-surface associated proteins, various organic acids, peptidoglycan derivatives, and structurally or functionally diverse exopolysaccharides. However, ISAPP explicitly emphasizes that although such metabolites may be present within a postbiotic formulation, they do not qualify as postbiotics when considered in isolation. This position provides a more stringent and

mechanistically coherent framework in which the postbiotic concept is not reduced merely to free microbial metabolites,but is instead anchored in the biological activity derived from inanimate microbial structures and their composite preparations (Figure 1) (Moradi et al., 2020; Vinderola et al., 2022).

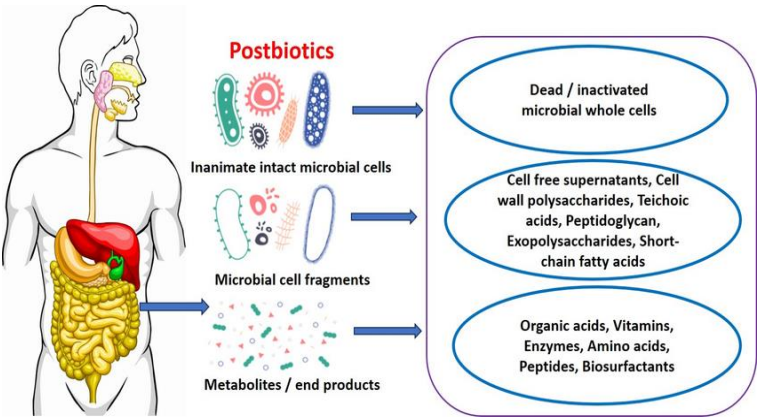

Figure 1. Postbiotics

The Function and Biomedical Relevance of Postbiotics

The importance of intestinal health has become increasingly evident, accompanied by a rapid expansion of research focused on the gut ecosystem. Over the past several decades, accumulating evidence has demonstrated that the gut and its resident microbiome exert profound short and long term influences on host physiology, with particularly strong mechanistic links emerging between the microbiome and the immune system. Within this conceptual landscape, probiotics have traditionally been regarded as the primary agents capable of modulating gut health. According to the World Health Organization (WHO), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." However, emerging scientific evidence indicates that the beneficial effects attributed to

probiotic bacteria are not exclusively dependent on microbial viability. Instead, bacterial metabolites, structural components, and products of microbial lysis increasingly appear to constitute the primary mediators of the health-promoting outcomes historically ascribed to probiotics. Consequently, current research has shifted toward non viable cellular fractions, including cell lysates and metabolite rich preparations, thereby positioning postbiotics as a compelling alternative within the broader domain of microbiome-derived therapeutics. Postbiotics exhibit several biotechnological and physiological advantages over live probiotic microorganisms. Their superior absorption, metabolic handling, and systemic distribution enable more predictable pharmacokinetic and pharmacodynamic profiles. In contrast, probiotics as living biological entities are subject to numerous limitations, including challenges in storage stability, viability during food processing, survival through the gastrointestinal tract, and the ability to effectively colonize the host environment. Furthermore, viable microorganisms pose inherent risks such as translocation across the intestinal epithelium, especially in immunocompromised hosts, and the potential acquisition or transfer of antibiotic resistance genes. By circumventing these viability-associated risks, postbiotics offer enhanced safety, significantly longer shelf life, and stability across broad ranges of salinity, pH, and temperature. Their inability to produce biogenic amines (BAs), lack of transferable antibiotic resistance determinants, structural reliability, and ease of formulation and storage collectively place them at a clear advantage over conventional probiotics for both food and pharmaceutical applications. Accumulating evidence indicates that postbiotics possess a wide spectrum of therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, antiproliferative, and immunomodulatory activities. Together, these biofunctional attributes underscore the growing recognition of postbiotics as

next-generation microbial-derived interventions with significant potential in precision microbiome modulation and host-targeted therapeutic strategies (Sanders et al., 2019; Moradi et al.,2020; İçier et al., 2022; Liang & Xing, 2023; Rahman et al.,2025). Postbiotics exert a far broader spectrum of biological activities than those initially recognized. Numerous studies have demonstrated that postbiotic preparations inhibit pathogenic microorganisms, exhibit pronounced anti-inflammatory properties and modulate immune responses through both immunosuppressive and immunoregulatory mechanisms. They have been shown to attenuate the production of pro-inflammatory cytokines, induce interferon-γ secretion, and contribute to antiviral defense pathways. Moreover, postbiotics participate in host energy metabolism by suppressing lipogenesis, reducing cholesterol ester and triacylglycerol levels, and improving dysregulated gene expression profiles, thereby contributing to the control of carcinogenic processes. Additional evidence indicates that postbiotics may exert neurohormonal effects, possess antibiofilm activity, and even hold potential applications in dermatological care. Experimental findings further reveal that postbiotic preparations retain antimicrobial efficacy against clinically relevant pathogens such as *Escherichia coli*, *Salmonella Typhimurium*, and *Staphylococcus aureus*, maintaining their bioactivity for up to one month at both 4°C and 20°C. Collectively, these attributes underscore the substantial advantages of postbiotics over traditional probiotics, positioning them as highly stable, mechanistically versatile, and therapeutically robust alternatives within the expanding field of microbiome-derived interventions (Bleau et al., 2010; Nikolic et al., 2012; Kareem et al., 2014; Murofushi et al., 2015; Makino et al., 2016; Khmaladze et al., 2019; Aguilar-Toalá et al., 2020; Teame et al., 2020; Engevik et al., 2021; Yeşilyurt et al., 2021; Rahman et al., 2025). Advances in fermentation technology and

contemporary bioengineering methodologies provide substantial opportunities for the tailored synthesis of diverse postbiotic preparations. However, because postbiotics represent a comparatively novel class of microbiome-derived agents relative to prebiotics and probiotics, their consumption necessitates careful consideration of potential risk factors, supported by rigorous safety assessments. Ultimately, postbiotics are reactive biological products derived from living microorganisms; therefore, improper formulation, contamination, or inadequate purification may theoretically precipitate adverse outcomes, including sepsis or various gastrointestinal disturbances. As such, the development and application of postbiotics must be guided by stringent production standards, comprehensive toxicological evaluations, and evidence-based regulatory frameworks to ensure their safe integration into biomedical and nutritional interventions (figure-2) (Aggarwal et al., 2022).

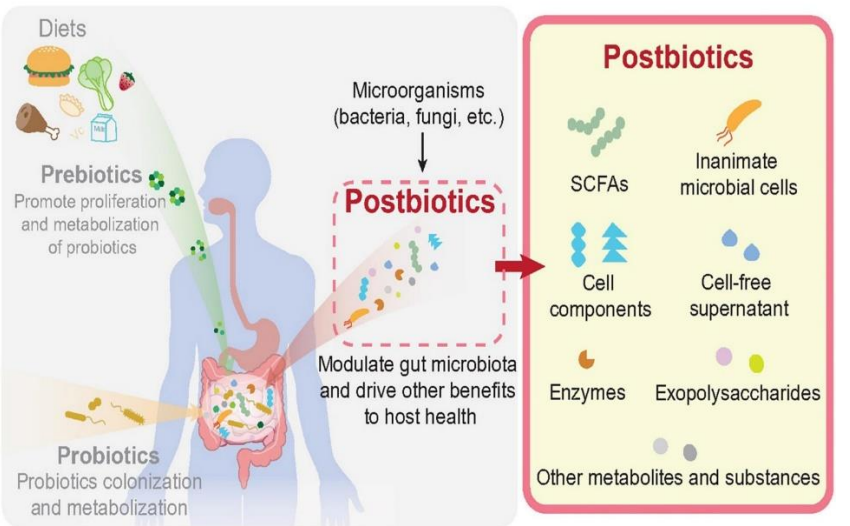

Figure-2. Prebiotics,probiotics and postbiotics

Mechanisms of Action of Postbiotics

Although the physiological benefits attributed to postbiotics have been increasingly recognized, their mechanistic underpinnings remain only partially elucidated. Nevertheless, several plausible biological pathways have been proposed. These include modulation of the resident gut microbiota, reinforcement of epithelial barrier integrity, enhancement of both local and systemic immune responses, regulation of systemic metabolic processes, and the transmission of signals via neural circuits implicated in the gut-brain axis. Postbiotics not only preserve the functional architecture of the intestinal mucosa but also constrain the colonization and expansion of pathogenic microorganisms. In doing so, they contribute to the maintenance of intestinal microflora homeostasis while simultaneously exerting beneficial influences on intracellular signaling cascades and neurohormonal communication pathways. Emerging evidence further suggests that postbiotic components can interact with pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain containing receptors (NODs), thereby modulating downstream transcriptional programs that orchestrate inflammatory tone and epithelial regeneration. Additionally, their structural constituents including peptidoglycan fragments, lipoteichoic acids, exopolysaccharides, and surface-layer proteins may engage host signaling networks that regulate tight junction assembly, mucus secretion, and antimicrobial peptide production. Through these multifaceted interactions, postbiotics act as potent modulators of host microbe crosstalk, integrating metabolic, immunological, and neuroendocrine pathways into a unified framework of therapeutic activity (Figure-2) (Salminen et al., 2021; Kumar et al., 2024)

Postbiotic Production

In recent years, the development of synthetic culture media and the incorporation of sucrose-enriched formulations have markedly enhanced both the yield and efficiency of postbiotic production. Postbiotic biosynthesis is predominantly achieved using anaerobic *Lactobacillus* species and/or yeasts such as *Saccharomyces cerevisiae*, as well as lactic acid bacteria more broadly. In addition to conventional substrates, the fermentation of industrial by-products including whey, milk permeate, and lignocellulosic biomass has emerged as a sustainable and economically advantageous strategy, simultaneously valorizing waste streams while mitigating their ecological impact. The type and yield of postbiotics are strongly influenced by multiple process parameters, including the composition of the growth medium, the isolation and inactivation methodology (e.g., thermal treatment, sonication, centrifugation, enzymatic digestion, hyperbaric processing, or exposure to chemical solvents), as well as physicochemical factors such as pH and temperature. Optimization of these variables is critical, as subtle modifications can significantly alter the metabolic output of the microbial cultures, the structural integrity of inactivated cells, and the resultant bioactivity profile of the postbiotic preparations. Collectively, these considerations underscore the necessity of rationally designed, reproducible, and scalable production protocols to ensure both the functional consistency and industrial applicability of postbiotic formulations (Lee et al., 2019; Amiri et al., 2021; Duarte et al., 2022; Oliveira et al., 2023; Khakpour et al., 2024;).

For postbiotic production, bacterial cultures are initially fermented in small-scale bioreactors, typically ranging from 1 to 10 liters (IT-01). Once the cultures reach the exponential growth phase, these primarily probiotic microorganisms are transferred

into larger bioreactors with anaerobic conditions (STR-01). After sufficient biomass accumulation, the fermentation supernatant is harvested and directed to subsequent downstream processing. The recovery of postbiotics can be achieved through two distinct, yet mechanistically related, approaches that share many downstream operations but differ in their initial steps: (i) conventional biomass removal, and (ii) direct cell lysis followed by the elimination of cellular debris.

The conventional biomass removal process typically commences with centrifugation (C-01), resulting in a cell-free supernatant (HSN) that can subsequently be filtered (F-01) and transferred to a homogenization tank (HT-01). Alternatively, the HSN may be directed to a cell inactivation tank for secondary extraction (CIT-02), during which one or a combination of methods such as ultrasonication, hyperbaric treatment, thermal processing, enzymatic digestion, or exposure to chemical solvents may be applied. Following these treatments, the fermentation fluid is transferred to an extraction tank (ET-02), after which the residual biomass is finally conveyed to the homogenization tank. The extraction tank is typically designed with enhanced solvent capabilities to efficiently remove undesired components. In certain workflows, the HSN is transferred directly to the cell inactivation tank without prior filtration, with subsequent processing proceeding as described above. In such cases, the fluid biomass is sequentially conveyed first to the extraction tank and subsequently to the homogenization tank.

An alternative approach for postbiotic recovery involves the direct transfer of the fermentation broth to a cell disruption tank (CIT-02) without prior centrifugation. In this methodology, high-pressure treatment, ultrasonication, elevated temperature, enzymatic processing, and chemical solvents are employed to disintegrate the microbial cell wall, thereby releasing both cell

wall constituents and cytosolic compounds into the solution. The resultant mixture then undergoes centrifugation (C-02) and filtration (F-02) to remove larger cellular debris. A notable limitation of this approach is the requirement for more extensive centrifugation and filtration steps. Post-processing, the clarified fluid may be transferred either directly to the homogenization tank or, in some protocols, first to an extraction tank and subsequently to homogenization.

To ensure product homogeneity, the homogenization tank constitutes the terminal stage in all workflows. When a solid postbiotic formulation is desired (P-01), spray-drying units (SD-01) are commonly employed. Although high-temperature air is used in spray dryers, this process is generally less deleterious to postbiotic activity than the inactivation methods applied earlier. Moreover, protective excipients such as glucose, lactose, or maltodextrin are typically incorporated to enhance product stability.

Comprehensive compositional analysis of the recovered postbiotic metabolites is imperative. Although postbiotics pose lower inherent risks than live probiotics due to the absence of viable cells, rigorous quality control must still be performed to assess toxicity, anti-pathogenic activity, functional efficacy, and the presence of residual viable cells. Further characterization of postbiotics is recommended, encompassing the evaluation of saccharides, organic acids, antibiotics, bacteriocins, proteins, and enzymatic constituents, thereby ensuring a thorough understanding of their bioactive profile (Figure-3) (Ácsová et al., 2022; Cuevas-González et al., 2020; da Silva Vale et al., 2023; de Melo Pereira et al., 2018; Hernández-Granados & Franco-Robles, 2020; D. Kumar et al., 2021; Nam et al., 2021; Tantratian & Pradeamchai, 2020).

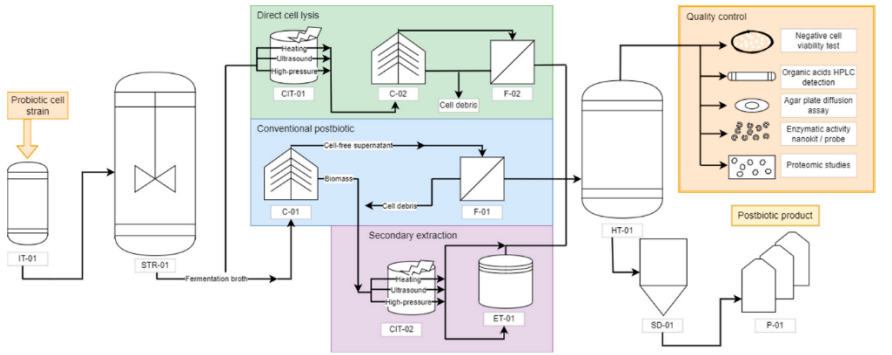

Figure 3. Stages of Postbiotic Production (da Silva Vale et al., 2023)

Flowchart of postbiotic production. IT = inoculum tank; STR = stirred tank reactor; C = centrifuge; F = filter; CIT = cell inactivation tank; ET = extraction tank; HT = homogenizing tank; SD = spray dryer; P = packaging.

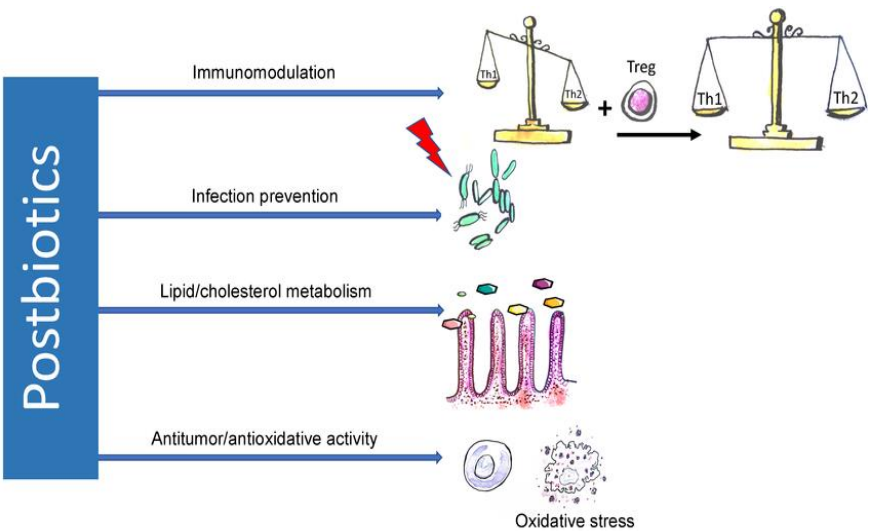

Figure 4. Therapeutic Effects of Postbiotics

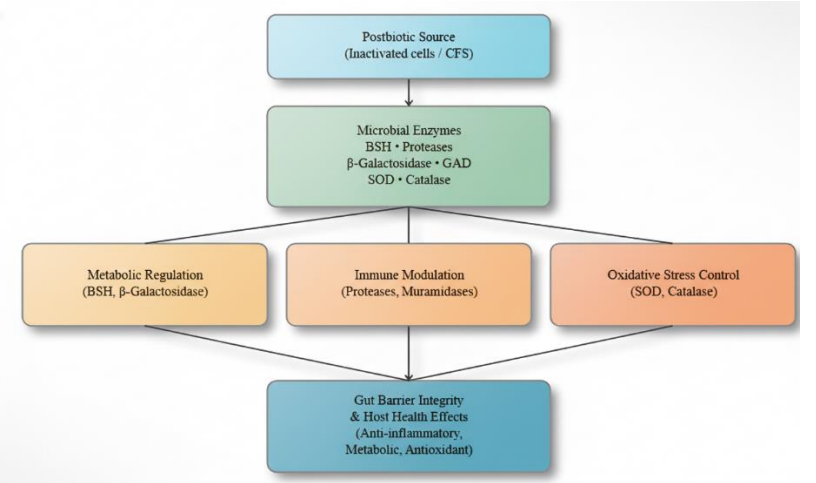

Figure 5. The modes of action of postbiotic enzymes

Therapeutic Effects of Postbiotics

Postbiotics Modulate the Gut Microbiota

In a recent study, heat-inactivated postbiotic *Bifidobacterium longum* CECT 7347 (HT-ES1) was administered to human participants. The findings indicated that HT-ES1 supplementation promoted the proliferation of butyrate-producing commensal bacteria, maintained fecal calprotectin levels within physiological ranges, and contributed to a reduction in serum cholesterol concentrations. Collectively, these outcomes suggest that HT-ES1 may exert a beneficial modulatory effect on intestinal homeostasis, supporting gut barrier integrity and systemic metabolic health through microbiota-mediated mechanisms (Naghibi et al., 2024). Irritable Bowel Syndrome (IBS), affecting approximately 4-11% of the global population,

represents not only a prevalent gastrointestinal disorder but also one of the most frequently encountered conditions associated with dysregulated gut-brain axis interactions that profoundly impair quality of life. IBS is inherently psychosomatic in nature and is closely modulated by psychological, social, and environmental determinants. Clinically, IBS is classified into three principal subtypes: constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), and mixed-type IBS (IBS-M), with some experts additionally recognizing an unclassified subtype (IBS-U) for cases that do not conform to the established categories. The etiology and pathophysiology of IBS remain incompletely understood, and current diagnostic and therapeutic approaches are largely symptomatic. Emerging evidence suggests that targeted modulation of the gut microbiome may offer a promising therapeutic strategy to ameliorate IBS-associated dysfunctions, underscoring the potential of microbiota-centered interventions in the management of this multifactorial disorder (Gralnek et al., 2000; Lovell & Ford, 2012; Aggeletopoulou & Triantos, 2024; Li et al., 2024; Ng et al., 2024). Constipation-predominant IBS (IBS-D) is a chronic gastrointestinal disorder characterized by abnormal stool frequency or form, accompanied by recurrent abdominal pain and bloating. Several studies have demonstrated that individuals with this chronic condition exhibit a gut microbiota composition distinct from that of healthy controls. In a similar investigation, researchers evaluated the efficacy of the probiotic *Bifidobacterium longum* CECT 7347 (ES1) and the heat-inactivated postbiotic form of the same strain (HT-ES1) in adults with diarrheal -predominant irritable bowel syndrome (IBS-D). The study aimed to assess primary outcomes related to symptom severity, as well as secondary outcomes including stool consistency, quality of life, abdominal pain intensity, and anxiety levels. Over the course of 84 days, both ES1 and HT-ES1 elicited statistically and clinically significant

improvements compared to placebo. This trial holds particular significance in the literature, as it represents the first study to evaluate the same bacterial strain in both probiotic and postbiotic formats, thereby providing critical insights into the therapeutic potential of non-viable microbial interventions in IBS-D management. (Naghibi et al., 2024). A high-fat diet (HFD) disrupts gut microbiota homeostasis, promotes systemic inflammation, and compromises intestinal barrier integrity, ultimately contributing to the development of insulin resistance. Researchers have investigated the effects of postbiotics, specifically cell-free supernatants (CFS), on HFD-induced metabolic dysfunctions and gut-liver regulatory mechanisms. The study demonstrated that CFS administration ameliorated insulin resistance, suppressed pro-inflammatory bacterial taxa, enriched beneficial microbial genera, reduced lipopolysaccharide translocation, and preserved intestinal barrier integrity in obese murine models. These findings underscore the potential of postbiotic interventions to modulate host-microbiome interactions and counteract diet-induced metabolic derangements through integrated immunometabolic and gut-liver axis mechanisms (Sun et al., 2025).

Various studies have reported that both live bacterial strains and their derived postbiotics confer ameliorative effects on symptomatology and biochemical markers in murine models of chronic ulcerative colitis. These interventions have been shown to modulate gut microbial composition, attenuate inflammatory responses, and promote intestinal homeostasis, thereby highlighting the therapeutic potential of postbiotics as microbiota-targeted modulators in the context of chronic intestinal inflammation (Jin et al., 2024; Peng et al., 2024). However, therapeutic interventions and dietary strategies aimed at modulating the gut microbiota have yielded heterogeneous

outcomes. Consequently, establishing microbiota-targeted modulation as a valid and reliable treatment modality for IBS and other gastrointestinal disorders necessitates robust evidence derived from well-powered, randomized, and long-term clinical studies (Li et al., 2024).

Postbiotics as Potential Interventions for Obesity

A study investigating the efficacy of *Bifidobacterium bifidum* DS0908 (DS0908), *Bifidobacterium longum* DS0950 (DS0950), and their derived postbiotics in obese murine models reported favorable outcomes. The intervention was associated with the induction of brown and beige adipocyte marker expression, concomitant improvements in insulin sensitivity and glucose uptake, reductions in triglycerides, low-density lipoprotein, and total cholesterol levels, as well as overall amelioration of plasma lipid profiles. These findings underscore the potential of postbiotics to modulate adipose tissue function and systemic metabolic parameters, highlighting their promise as microbiota-derived therapeutics in obesity management (Shamim Rahman et al., 2023). In a study investigating the therapeutic effects of the probiotic *Lactobacillus reuteri* and the postbiotic butyrate in preventing programmed hepatic steatosis induced by a perinatal high-fat diet in offspring of Sprague-Dawley rats, *L. reuteri* was found to be more efficacious than postbiotic butyrate. The findings demonstrated that *L. reuteri* positively modulated maternal and fetal metabolic profiles, mitigated obesity-related parameters, and effectively prevented fetal hepatic lipid accumulation, highlighting its superior therapeutic potential in the context of diet-induced metabolic programming (Yu et al., 2022). Postbiotics derived from *Lacticaseibacillus plantarum* have been demonstrated to possess anti-obesity potential. Postbiotics obtained through the combined application of heat and ultrasonication were reported to significantly reduce key obesity-

related parameters in murine models, including body weight, circulating lipid levels, and adipokine concentrations. Furthermore, these interventions were associated with attenuation of white and brown adipose tissue damage, alongside an enrichment of beneficial genera such as Lactobacillus and Bifidobacterium. However, it was also noted that the abundance of Faecalibacterium, a taxon implicated in anti-obesity effects, was concomitantly reduced, suggesting nuanced and strain-specific impacts on gut microbial composition (Miao et al., 2024). In a randomized, double-blind clinical trial involving 66 overweight and obese participants, subjects were randomly assigned to one of three groups and administered either yogurt enriched with *Akkermansia muciniphila* postbiotics (AMY), yogurt enriched with *Lactobacillus rhamnosus* postbiotics, or low-fat yogurt as a placebo. The study reported that the *Akkermansia muciniphila* postbiotic-enriched dairy product exerted beneficial effects on weight management and liver enzyme levels in overweight and obese individuals. In contrast, the *Lactobacillus rhamnosus* postbiotic-enriched yogurt did not demonstrate statistically significant improvements compared to the placebo, highlighting a strain-specific differential impact on metabolic outcomes (Aalipanah et al., 2025). The study primarily underscores that not all postbiotics exert functional effects under every condition, a finding that represents a particularly valuable aspect of this investigation. Although the eight-week intervention provided meaningful insights into weight management, it does not capture potential long-term outcomes. Furthermore, the small sample size comprising data from only 22 individuals per group limits the statistical power and generalizability of the findings to the broader population. Nonetheless, as an early-phase clinical study, it constitutes a pioneering and valuable contribution, laying the groundwork for more extensive analyses and future investigations.

Researchers evaluated the effects of heat-inactivated *Ligilactobacillus salivarius* strain 189 postbiotics over a four-week period in 48 swine. The study demonstrated that this postbiotic preparation elicited significant modulatory effects on the gut microbiota and suggested its potential applicability as an anti-obesity intervention (Ryu et al., 2022). Upon further evaluation, it is evident that the study was limited by both the short duration and the small sample size. Consequently, these factors constrain the interpretation and generalizability of the findings to the broader population.

Researchers investigating the effects of a combined postbiotic and prebiotic formulation in mice subjected to a high-fat diet observed that weight gain was significantly lower in the treatment group compared to controls. Furthermore, a positive correlation was reported between the administered dose of the formulation and the reduction in weight gain. The study also indicated an increase in intestinal probiotic bacterial populations and enhanced fecal lipid excretion. These findings suggest substantial potential for combating obesity, as postbiotics derived from kefir administered to high-fat diet-induced mice were associated with marked reductions in body weight gain, adipose tissue mass, serum triglycerides, and insulin resistance (Seo et al., 2022; Zhao et al., 2023).

Multiple studies have reported that postbiotic supplementation in high-fat diet-fed mice leads to reduced weight gain, decreased white adipose tissue mass and adipocyte size, and reductions in cholesterol and lipoprotein levels (Lim et al., 2022). Additionally, administration of heat-inactivated *Akkermansia muciniphila* in obese individuals was associated with improvements in insulin sensitivity, insulinemia, and plasma total cholesterol (Depommier et al., 2019). In normoweight murine models, this postbiotic was found to reduce body weight, liver mass, and white adipose tissue

mass (Ashrafian et al., 2021). Moreover, a specific protein derived from this bacterium was reported to enhance the reduction of fat mass development, insulin resistance, and dyslipidemia in mice (Plovier et al., 2017).

Although the body of evidence generally indicates that postbiotics confer beneficial effects on weight gain and energy metabolism in obese individuals, certain postbiotics have been reported to lack functional efficacy. To establish standardized guidelines and delineate the overall therapeutic potential, there is a critical need for an expansion of both preclinical and clinical studies, with increased sample sizes sufficient to permit robust generalizable conclusions.

Postbiotics Exhibit Anticarcinogenic Effects

Colorectal cancer (CRC), which encompasses both colon and rectal cancers, is the leading cause of cancer-related deaths among men under 50 years of age. In 2022, more than 1.9 million CRC cases were reported globally, resulting in approximately 904,000 deaths. Risk factors such as smoking, alcohol consumption, obesity, family history, and chronic inflammation significantly contribute to CRC development. Additionally, bacterial infections caused by organisms including *Bacteroides fragilis*, *Fusobacterium nucleatum*, and *Helicobacter pylori* have been implicated in increasing this risk (Adedayo & Riethmacher, 2025).

In a reported study, the combination of immunotherapy with postbiotics derived from gut microbiota metabolic outputs such as phytosphingosine was shown to enhance HLA class I expression in solid tumors, thereby rendering carcinogenic cells more susceptible to cytotoxic T lymphocyte mediated lysis (Ferrari et al., 2023).

Researchers reported that putrescine, a postbiotic metabolite isolated from the probiotic *Escherichia coli* Nissle 1917, inhibited the growth of colibactin-producing *E. coli* strains implicated in colorectal carcinogenesis under *in vitro* conditions, and, in chemically induced murine models, significantly reduced both the number and size of colonic tumors, concomitantly attenuating the release of pro-inflammatory cytokines within the colonic lumen (Oliero et al., 2024)

It has been reported that the oral administration of extracellular vesicles derived from Lactobacillus rhamnosus GG synergistically enhanced the efficacy of anti–PD-1 immunotherapy against colorectal cancer, modulated intestinal immune responses, and increased the serum levels of biomarkers known to be associated with antitumor activity (Lu et al., 2023).

Despite promising *in vitro* findings in cancer therapeutics, *in vivo* outcomes have unfortunately remained suboptimal. To enhance *in vivo* efficacy, researchers encapsulated the lysate of *Lacticaseibacillus paracasei* GMNL-133 (SGMNL-133). They reported that, through encapsulation, the lysate was protected from gastric acid degradation, effectively interacted with carcinogenic cells, and consequently exhibited enhanced therapeutic efficacy in tumor treatment (Huang et al., 2024).

Helicobacter pylori, owing to increasing antibiotic resistance, can lead to gastric ulcers, gastritis, and even gastric cancer. Given their inherent advantages, postbiotics have consequently emerged as valuable tools in H. pylori–related research. Researchers reported that Lactobacillus rhamnosus MN45 exhibited the strongest co-aggregation capacity with *H. pylori*, and markedly suppressed *H. pylori* induced urease activity, virulence factor expression, and the associated inflammatory response (Hua et al., 2025).

In another study, the anticancer activities of postbiotics obtained from the post-fermentation media (PFM) and cell extracts (CM) of 39 different lactic acid bacteria (LAB) strains were evaluated against colon and cervical cancer cell lines. The results demonstrated that both PFM and CM exerted pronounced inhibitory effects on cancer cell viability, with particularly strong activity against colon cancer cells. Notably, PFM exhibited superior anticancer efficacy compared with CM, and the study reported that these postbiotics induced apoptosis via a mitochondrial signaling pathway through the generation of oxidative stress (Nowak et al., 2022). According to the data obtained from this study, different postbiotics may exhibit variable activities against different cancer cell types. This *in vitro* study represents a significant step toward establishing standards and identifying effective treatments through preclinical and clinical research outcomes

In a study where the supernatant of active cultures was utilized as postbiotics, researchers observed a positive correlation between the concentration of postbiotics and heat-inactivated paraprobiotics and the toxicity against colorectal carcinoma cell lines (Avci et al., 2024). In another study, researchers utilized a cell-free supernatant derived from *Saccharomyces cerevisiae* var. *boulardii*. According to the report, this postbiotic exhibited pronounced antigenotoxic and cytotoxic effects and induced apoptotic responses in HT-29 cancer cells (Abbasi et al., 2023).

Across multiple independent studies, it has been reported that postbiotics derived from bacterial sources induce apoptosis and suppress proliferation in colorectal carcinogenic cells (Zhong et al., 2024), promote apoptotic pathways in human pancreatic cancer cell lines while markedly reducing stromatogenesis in *in vivo* models (Panebianco et al., 2022), and that short-chain fatty acids originating from microbiota-associated bacteria enhance

antitumoral cellular activity (Pérez et al., 2024). Additionally, a yeast-derived supernatant postbiotic has been shown to exert inhibitory effects against gastric adenocarcinoma cell lines (Pakbin et al., 2023).

The findings derived from carcinoma cell lines and *in vivo* experimental models suggest that postbiotics hold considerable potential as future therapeutic agents, either as standalone interventions or in combination with existing treatment modalities to achieve synergistic effects. However, a substantial proportion of the available evidence is still based on *in vitro* and preclinical investigations. While these studies provide critical reference points for the design of clinical trials, further comprehensive research is required. Despite the encouraging laboratory-based outcomes, results obtained in animal models and at the clinical stage may differ due to the inherent complexity of living organisms. Consequently, although these therapeutic approaches are promising, they necessitate more extensive investigation and robust clinical validation.

Postbiotics exhibit antimicrobial properties.

In a study employing lactic acid bacteria used for fermentation—*Lactobacillus sakei* and a *Lactobacillus curvatus/Pediococcus acidilactici* combination—researchers reported that the cell-free supernatant demonstrated a 4.84-fold increase in antimicrobial activity (Foudjing et al., 2023).

In a randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of postbiotics containing inactivated *Limosilactobacillus reuteri* DSM 17648 as an adjuvant therapy for *Helicobacter pylori* eradication, the eradication rate was reported to be 96.7% in the postbiotic group compared with 86.0% in the placebo group. Furthermore, patients receiving the postbiotic exhibited a lower incidence of adverse

effects relative to the placebo group (Ivashkin et al., 2024)Although a statistically significant improvement in eradication efficacy was observed (P = 0.039), the level of significance remains modest. Therefore, stronger and more robust evidence is required to support the effective clinical application of the postbiotic evaluated in this study. Moreover, while statistically meaningful results were obtained from a total cohort of 129 participants, the relatively limited sample size constrains the extrapolation of these findings to broader populations.

Klebsiella pneumoniae is a clinically significant pathogen characterized by multidrug resistance and is considered a major cause of recurrent infections in immunocompromised populations. In response, researchers evaluated the antimicrobial activity of the cell-free supernatant (CFS) derived from *Lactobacillus gasseri* 1A-TV against clinical isolates of *K. pneumoniae*. The findings indicated that this CFS represents a valuable bioproduct for the prevention and control of *K. pneumoniae* colonization and exhibited strong aggregation activity against certain strains (Vertillo Aluisio et al., 2024). A closer examination of the study reveals that the aggregation activity exhibited by the CFS was not statistically significant across all strains. In this context, it can be inferred that distinct postbiotics or alternative therapeutic strategies may need to be developed for different *K. pneumoniae* strains to achieve effective and targeted intervention.

Researchers investigated the antifungal activity of the cell-free supernatants (CFS) derived from six LAB strains belonging to the *Enterococcus* and *Leuconostoc* genera against filamentous fungi. The researchers observed notable antifungal activities of LAB-derived compounds and suggested that these metabolites could be considered as potential biocontrol agents. Additionally, they reported that the functionality of these metabolites was reduced

under conditions of pH alteration as well as exposure to elevated temperatures and hyperbaric environments (Fugaban et al., 2023). This study is of particular importance, as it not only contributes to the establishment of standards and the identification of effective therapeutic approaches but also delineates critical exclusionary factors that may limit efficacy

Foodborne pathogens colonizing the biofilm matrix represent a major cause of food spoilage. In this context, researchers evaluated the antibiofilm efficacy of *Lacticaseibacillus rhamnosus* YT and its cell surface extract against biofilms produced by *Bacillus subtilis* and *Salmonella enterica*, with *S. enterica* being a particularly significant pathogen. The study reported a linear correlation between the biomass of the cell extract and antibacterial activity, along with a broad antimicrobial spectrum. Furthermore, abiotic environmental factors were shown to modulate antimicrobial activity, while antibiofilm efficacy remained stable under extreme conditions (Guan et al., 2023). To enable more effective utilization of the obtained findings, it is considered necessary to strengthen the study through *in vivo* analyses and to conduct antimicrobial evaluations against a broader range of bacterial genera and species.

It has been reported that the cell-free supernatant of *Bacillus* thuringiensis exhibits dose-dependent, linearly correlated antibiofilm activity against clinically important pathogens such as Staphylococcus aureus, while not affecting the planktonic growth of *S. aureus* (Ray et al., 2023).

Researchers evaluated the activities of cell-free supernatants (CFS) derived from three different non-pathogenic *Acanthamoeba* species against multiple *Staphylococcus aureus* strains. It was reported that the CFS obtained from the reference *Acanthamoeba* strain exerted a particularly strong inhibitory

effect against methicillin-resistant *S. aureus* (MRSA), a clinically significant pathogen. Moreover, CFS from different aquatic amoebae were shown to inhibit distinct *S. aureus* strains to varying extents, ranging from 0% to 100% (Özcan Aykol & Zeybek, 2024). An evaluation of the study indicates that investigating the efficacy of postbiotic products derived from microorganisms other than bacteria is highly valuable, as these organisms may represent a rich source of potent antimicrobial agents. However, for postbiotic products originating from amoebae belonging to the protozoan group to be considered for clinical application, substantially more evidence is required, along with systematic and long-term assessments of all potential risks most notably cytotoxic and genotoxic effect

Researchers investigating the antibacterial activity of the cell-free supernatant (CFS) derived from *Lactobacillus paracasei* CH88 against *Gardnerella vaginalis* reported that the CFS effectively inhibited both the growth and biofilm formation of *G. vaginalis*. The compound was found to be thermostable, and the authors suggested that this CFS could be a potential therapeutic option for the treatment of bacterial vaginosis, a condition characterized by the overgrowth of anaerobes such as *Gardnerella vaginalis* spp. The metabolite was reported to be effective at both *in vitro* and *in vivo* levels (Moon et al., 2022).

Postbiotics exert immunomodulatory effects on the host immune system.

Immune system integrity is of vital importance for the maintenance of host health and constitutes the primary line of defense against infectious agents. Accordingly, certain therapeutic strategies have been designed to directly target and enhance immune system function. Alcohol consumption disrupts the gut microbiota and thereby contributes to the pathogenesis of

alcohol-associated liver disease (ALD). To mitigate these effects, researchers have explored the use of postbiotic metabolites. Yin et al. (2025) reported that postbiotics derived from *Lactobacillus johnsonii* can attenuate ALD-associated pathology by modulating the intestinal immune system. In their study, immune responses elicited by heat-inactivated *L. johnsonii* in ALD-induced murine models inhibited ethanol-driven alterations in the gut microbiota, including the overgrowth of opportunistic pathogens, while preserving butyrate-producing bacterial populations. Through the restoration of intestinal microbial homeostasis, these *L. johnsonii* derived postbiotics were identified as promising therapeutic candidates for the management of ALD.

Multidrug-resistant non-typhoidal salmonellosis (NTS) represents a significant public health concern. In a study evaluating the combined use of postbiotics alongside para and probiotics, it was reported that these bi-combined biotics directly inhibited the growth of *Salmonella* spp. Conducted *in vitro*, the study proposed that *Lactiplantibacillus plantarum* KUNN19-2 and its derived postbiotics may serve as a potential alternative therapeutic strategy against *Salmonella* infections (S. Li et al., 2025).

A commercial postbiotic known as “Symbiota®” has been reported to induce antitumor responses in xenograft mouse models by modulating the gut microbiota. Mechanistically, this product was shown to inhibit the growth of colon and lung carcinoma cells by promoting $CD8^+$ T-cell expansion and reducing PD-1 expression (Lee et al., 2024). Although the findings in the murine model indicate a potential postbiotic capable of inducing immune responses, the authors’ disclosure of conflicts of interest with the company producing the product necessitates further validation of these results through additional, independent studies.

Cutaneous immunity constitutes a crucial first-line barrier, and the integrity of the epidermal barrier is directly linked to effective cutaneous immune defense. To induce cutaneous immune responses, researchers investigated the effects of the postbiotic derived from *Latilactobacillus curvatus* BGMK2-41. The study demonstrated that BGMK2-41 upregulated the expression of antimicrobial response genes in keratinocytes against *Staphylococcus aureus* colonization, increased the expression of the anti-inflammatory cytokine IL-10, and accelerated re-epithelialization, thereby promoting the restoration of epidermal barrier integrity. (Dinić et al., 2024).

Type 2 helper T (Th2) cell–mediated immune responses, a $CD4^+$ T helper cell subset of the adaptive immune system, play a central role in the pathogenesis of type I hypersensitivity reactions. Modulation of these responses with pro and postbiotics has been reported as a potential strategy to prevent or alleviate disease symptoms. In this context, *Bifidobacterium breve* DSM 32583 (Bb), isolated from human breast milk, and *Limosilactobacillus fermentum* CECT5716 (Lf) were shown to modulate Th2-induced immune responses, resulting in a reduction of the Th2/Th1 ratio. This *in vitro* study indicated efficacy in symptom alleviation; however, the authors emphasized that *in vivo* investigations are required to further substantiate these findings and clarify their clinical relevance (Amar et al., 2024). This study also underscores the importance of human breast milk in protecting against hypersensitivity reactions during the neonatal and infant periods. Moreover, it provides valuable insights for improving the composition of infant formulas developed for breastfed-deprived infants. The researchers propose the incorporation of postbiotics into pediatric diets as adjunctive therapeutic tools for the management of allergic reactions (Burgos et al., 2022).

It has been reported that a paraprobiotic obtained through heat inactivation of *Bacillus velezensis* GV1 elicited immune responses and exerted positive modulatory effects on the gut in immunosuppressed murine models (H. J. Lee et al., 2024); that allergic responses induced by air pollution were partially attenuated in genetically modified mice following administration of a postbiotic-containing combination (Chiu et al., 2023); that postbiotics were capable of upregulating immune function in Lateolabrax maculatus fed a high soybean meal diet (Z. Y. Liu et al., 2024); and that, in mice with 2,4-dinitrochlorobenzene induced hypersensitivity, the exaggerated Th2-skewed immune response was significantly reduced, accompanied by downregulation of transcription factors associated with Th2 and Th17 cell lineages (Kim et al., 2024). An evaluation of the available studies indicates that immune responses can be either induced or suppressed by postbiotics in a context-dependent manner. In this regard, it can be inferred that postbiotics may hold therapeutic potential in conditions characterized by both immune suppression and excessive immune activation.

Postbiotics can be utilized as anti-inflammatory agents.

Researchers investigating the anti-inflammatory effects of postbiotics derived from *Escherichia coli* A0 34/86 (EcO83), specifically extracellular vesicles (EcO83-EV), reported based on an *ex vivo* study conducted in human nasal epithelial cells that EcO83-EVs modulated inflammatory responses by altering the expression of inflammation associated proteins (Razim et al., 2024)

Gut dysbiosis can trigger inflammatory bowel disease (IBD). Researchers investigated the effects of postbiotic metabolites derived from *Lactiplantibacillus argentoratensis* BBLB001 using both cell culture experiments and *in vivo* animal models. Based

on the obtained data, the postbiotic metabolites were reported to reduce the levels of inflammatory cytokines and to elicit significant improvements in other inflammation-associated biomarkers (Itoh et al., 2024).

It has been demonstrated that thioredoxin (Trx), a postbiotic derived from *Saccharomyces boulardii,* exhibits anti-inflammatory activity, including the attenuation of inflammation, preservation of intestinal barrier integrity, suppression of apoptosis, and reduction of oxidative stress (Qin et al., 2024).

Researchers obtained a cell-free supernatant (CFS) from *Lactiplantibacillus plantarum* EIR/IF-1. The study demonstrated that these postbiotic metabolites significantly reduced the production of pro-inflammatory cytokines, including IL-8, IL-6, and IL-1β, while concurrently inducing a marked upregulation of the anti-inflammatory cytokine IL-10 (Demirhan et al., 2025).

Researchers investigated the anti-inflammatory properties of *Lactococcus lactis* HF08 (HF08) and its derived postbiotic (P-HF08). The results demonstrated that P-HF08 exerted a significantly stronger inhibitory effect than HF08 on the pro-inflammatory cytokines IL-6 and IL-1β, as well as on IL-10, and that this effect was mediated through the downregulation of key proteins in the TLR4/NF-κB signaling pathway (Liu et al., 2024).

Researchers investigated the anti-inflammatory properties of a postbiotic (Pa JY062) derived from *Lactobacillus paracasei* JY062 isolated from a fermented dairy product. The study demonstrated that Pa JY062 increased the levels of the anti-inflammatory cytokine IL-10 while concurrently suppressing pro-inflammatory cytokines, including IL-6, IL-17α, and TNF-α (Guo et al., 2025).

It has been reported that anti-inflammatory responses in microglial cells against neuroinflammation can be enhanced by metabolites derived from gut microbiota–associated bacteria (Chiano et al., 2024); that postbiotics obtained from *Lactobacillus delbrueckii* CIDCA 133 exhibit pronounced anti-inflammatory properties (ADS et al., 2024); and that postbiotics derived from *Streptococcus thermophilus*, commonly used in milk fermentation, exert significant anti-inflammatory effects (Allouche et al., 2024). Furthermore, postbiotics derived from *Lactiplantibacillus plantarum* have been shown to display anti-inflammatory activity in the treatment of atopic dermatitis (AD), a condition driven by chronic inflammation, and have been proposed for use in individuals with AD (Lee et al., 2025). Collectively, these findings indicate that postbiotics obtained from diverse bacterial sources, owing to their anti-inflammatory properties, may be effectively utilized in the treatment of inflammatory conditions and in the attenuation of inflammation-associated symptoms (Rezaie et al., 2024, 2025; J. Wu et al., 2025).

Postbiotics are functionally active as antioxidants

Researchers reported that broiler chickens subjected to heat stress and fed with the postbiotic RI11 exhibited increased levels of antioxidant enzymes and compounds, including plasma glutathione peroxidase, catalase, and glutathione. Based on these findings, the authors suggested that postbiotics could be utilized as antioxidant agents in poultry nutrition (Humam et al., 2021).

Accumulated oxidative stress and dysbiosis accelerate the aging process. Researchers have reported that probiotics such as *Lactobacillus* and *Bifidobacterium*, along with their fermented metabolites (postbiotics), exhibit antioxidant activities that regulate oxidative stress and protect cells against oxidative

damage (Lin et al., 2022). In this study, antioxidant effects in middle-aged mice were assessed exclusively using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Within this context, although the findings suggest that postbiotics possess antioxidant activity and may be recommended for promoting health in aging populations, stronger evidence supported by a broader range of antioxidant assays is still required. Nevertheless, the direct application of the test in experimental animals represents a notable strength of the study.

It has been reported that heat-inactivated cells of *Lactiplantibacillus plantarum* S1, isolated from whey, along with their produced metabolites, reduce oxidative stress and exhibit free radical scavenging activity (Kostelac et al., 2022).

In another study, the antioxidant effects of the cell-free supernatant (CFS) derived from *Akkermansia muciniphila* were evaluated in *Caenorhabditis elegans*. The findings indicated that the CFS not only enhanced antioxidant capacity but also improved locomotor activity and promoted improvements in glucose and lipid metabolism (Z. Q. Wu et al., 2023).

Postbiotics derived from *Lactobacillus rhamnosus* 1.0320 have been reported to confer multiple benefits, including protection against oxidative stress, alleviation of colitis symptoms, restoration of intestinal integrity, and a reduction in myeloperoxidase (MPO) activity in bone marrow associated tissues (Zhang et al., 2024). Although decreased MPO activity has been associated with reduced tissue damage and improvements in pulmonary structure and functional outcomes (Teng et al., 2021), pronounced suppression of MPO activity may pose potential risks, particularly in immunosuppressed individuals. MPO is an enzyme expressed predominantly in neutrophils, as well as in other immune cells, and plays a critical role in host defense

mechanisms. MPO deficiency can render individuals more susceptible to severe infections and chronic inflammatory conditions (Mousavi et al., 2024). Therefore, the use of such metabolites in immunosuppressed populations should be carefully evaluated, with particular consideration given to the balance between anti-inflammatory benefits and potential impairment of innate immune defenses.

In another study, it was reported that viable *Bifidobacterium bifidum* reduced oxidative stress, whereas heat-inactivated cells and their metabolites contrary to the prevailing literature were ineffective in mitigating oxidative damage (Yin et al., 2025). Within this context, it becomes evident that not all postbiotics consistently exert their intended biological functions under specific conditions and may therefore remain functionally inactive. The predominance of studies reporting positive outcomes in the postbiotic literature suggests the presence of a certain degree of 'positive bias' in the field. Accordingly, the transparent and unbiased reporting of neutral findings, as observed in the study by J. Yin et al. (2025), provides a valuable contribution to both scientific transparency and the accurate understanding of the true biological potential of postbiotics, thereby enhancing the study's originality. In doing so, this work offers a differentiating perspective relative to the repeatedly positive findings in the literature and emphasizes that the effects of postbiotics can vary depending on context, microbial species, dosage, and target tissue.

In conclusion, to definitively determine whether postbiotics possess therapeutic potential as direct antioxidant biomolecules or modulators of oxidative stress, there is a need for advanced *in vivo* studies that are rigorously designed with high methodological power, validated across diverse models, and incorporate in-depth mechanistic investigations

Postbiotics May Alleviate Constipation

In a study, the postbiotic Probio-Eco was reported to be potentially effective in alleviating constipation in both humans and mice. A randomized, double-blind, placebo-controlled crossover trial involving 110 adults with chronic constipation (Rome IV criteria) was conducted over a period of three weeks. The results indicated that Probio-Eco intervention significantly improved constipation symptoms, reduced straining during defecation, and lowered anxiety scores (Ma et al., 2025). The mechanistic validation performed in murine models represents a methodological strength of the study, whereas the small sample size, limited placebo control, and short duration are aspects requiring improvement.

In another study, the effects of postbiotics derived from milk fermented with *Lactobacillus helveticus* CP790 on fecal microbiota and gut health were investigated. The authors reported that the postbiotic product improved stool consistency and reduced straining during defecation, while significantly enhancing overall mood compared to placebo. Based on these findings, the researchers concluded that this postbiotic could ameliorate chronic constipation symptoms and positively influence mood (Tanihiro et al., 2024). Although the inclusion of a placebo control represents a methodological advantage, the limited sample size (n = 60) and short intervention period (4 weeks) restrict the generalizability of these findings to a broader population with chronic constipation. Therefore, cautious interpretation is warranted, and the results should be corroborated by larger-scale, long-term studies.

In a randomized, double-blind, placebo-controlled trial with 51 participants in the control group and 53 in the treatment group, the effects of heat-inactivated *Bifidobacterium longum* CLA8013

on bowel movements were evaluated. The authors reported that inactivated B. longum CLA8013 improved bowel function in healthy individuals prone to constipation, without any observed safety concerns (Okada et al., 2023). However, the small sample size (n = 104) and short intervention duration (2 weeks) limit the generalizability of the findings.

A review of the literature reveals that studies investigating the effects of postbiotics on chronic constipation remain limited. While current research generally reports positive outcomes, the small number of studies restricts the ability to generalize these results to the wider population. Moreover, several studies are limited by small sample sizes, short intervention periods, and, in some cases, the absence of placebo controls, representing important methodological limitations. Future research should aim to increase sample sizes, extend intervention and follow-up durations, implement placebo-controlled designs, and ensure sample heterogeneity through multicenter and international trials. To obtain stronger mechanistic evidence, comprehensive validation studies in vitro and in animal models are also warranted.

Conclusion and Future Perspectives

Postbiotics, defined by ISAPP as “inactivated microorganisms and/or their components that confer a health benefit,” represent a new generation of biotic agents. A key advantage of these metabolites and cellular fractions is their minimal risk of adverse events such as translocation, infection, or excessive immune activation, which can be associated with live probiotics. Consequently, scientific interest in postbiotics has markedly increased in recent years, leading to numerous studies examining their effects across various physiological systems.

Studies conducted over the past decade indicate that postbiotics are not only safe and stable biological products but also possess the potential to exert multifunctional bioactive properties, including maintenance of intestinal barrier integrity, immune modulation, inflammation control, oxidative stress reduction, antimicrobial activity, and intervention in cancer biology.

Nevertheless, a substantial portion of the available evidence remains based on in vitro or animal models, and mechanistic explanations are often limited to indirect assessments. Heterogeneity in study designs, lack of standardized postbiotic formulations, and limited clinical human data complicate the accurate characterization of true biological efficacy and clinical relevance.

Therefore, although current evidence strongly supports the anti-inflammatory, antioxidant, antimicrobial, and metabolic regulatory potential of postbiotics, their safe translation into clinical practice requires standardized production methods, well-designed, long-term human studies with larger sample sizes, and multilayered integrative approaches to strengthen mechanistic validation

References

Aalipanah, E., Askarpour, M., Eskandari, M. H., Zare, M., Famouri, M., Bedeltavana, A., Mohsenpour, M. A., & Sohrabi, Z. (2025). Comparing the effects of yogurt containing Akkermansia muciniphilia postbiotic with yogurt containing Lactobacillus rhamnosus postbiotic on body composition, biochemical indices, appetite, and depression scores in overweight or obese adults: A randomized…. *Clinical Nutrition ESPEN*, *68*, 438–446. https://doi.org/10.1016/j.clnesp.2025.05.045

Abbasi, A., Rad, A. H., Maleki, L. A., Kafil, H. S., & Baghbanzadeh, A. (2023). Antigenotoxicity and Cytotoxic Potentials of Cell-Free Supernatants Derived from Saccharomyces cerevisiae var. boulardii on HT-29 Human Colon Cancer Cell Lines. *Probiotics and Antimicrobial Proteins*, *15*(6), 1583–1595. https://doi.org/10.1007/S12602-022-10039-1

Ácsová, A., Hojerová, J., & Martiniaková, S. (2022). Efficacy of postbiotics against free radicals and UV radiation. *Chemical Papers 2021 76:4*, *76*(4), 2357–2364. https://doi.org/10.1007/S11696-021-02018-7

Adedayo, S. I., & Riethmacher, E. (2025). Emerging New Treatments for Colon Cancer. *Current Medicinal Chemistry*, *32*. https://doi.org/10.2174/0109298673372776250505155945

Ads, F., Fal, B., Gm, C., Mf, A., Rcds, V., Gc, G., Kd, V., Soa, F., Rdo, C., J, J., Apgds, M., E, F., Fs, M., Jg, L., G, J., V, A., & Lcl, De J. (2024). Exploring the anti-inflammatory effects of postbiotic proteins from Lactobacillus delbrueckii CIDCA 133 on inflammatory bowel disease model. *International Journal of Biological Macromolecules*, *277*(Pt 2). https://doi.org/10.1016/J.IJBIOMAC.2024.134216

Aggarwal, S., Sabharwal, V., Kaushik, P., Joshi, A., Aayushi, A., & Suri, M. (2022). Postbiotics: From emerging concept to application. *Frontiers in Sustainable Food Systems*, *6*, 887642. https://doi.org/10.3389/FSUFS.2022.887642/FULL

Aggeletopoulou, I., & Triantos, C. (2024). Microbiome Shifts and Their Impact on Gut Physiology in Irritable Bowel Syndrome. *International Journal of Molecular Sciences 2024, Vol. 25, Page 12395*, *25*(22), 12395. https://doi.org/10.3390/IJMS252212395

Aguilar-Toalá, J. E., Hall, F. G., Urbizo-Reyes, U. C., Garcia, H. S., Vallejo-Cordoba, B., González-Córdova, A. F., Hernández-Mendoza, A., & Liceaga, A. M. (2020). In Silico Prediction and In Vitro Assessment of Multifunctional Properties of Postbiotics Obtained From Two Probiotic Bacteria. *Probiotics and Antimicrobial Proteins*, *12*(2), 608–622. https://doi.org/10.1007/S12602-019-09568-Z/TABLES/5

Allouche, R., Hafeez, Z., Dary-Mourot, A., Genay, M., & Miclo, L. (2024). Streptococcus thermophilus: A Source of Postbiotics Displaying Anti-Inflammatory Effects in THP 1 Macrophages. *Molecules (Basel, Switzerland)*, *29*(7). https://doi.org/10.3390/MOLECULES29071552

Amar, Y., Grube, J., Köberle, M., Schaubeck, M., Biedermann, T., & Volz, T. (2024). Bifidobacterium breve DSM 32583 and Limosilactobacillus fermentum CECT5716 postbiotics attenuate S. aureus and IL-33-induced Th2 responses. *Microbiological Research*, *289*. https://doi.org/10.1016/j.micres.2024.127913

Amiri, S., Rezazadeh-Bari, M., Alizadeh-Khaledabad, M., Rezaei-Mokarram, R., & Sowti-Khiabani, M. (2021). Fermentation Optimization for Co-production of Postbiotics by Bifidobacterium lactis BB12 in Cheese Whey. *Waste and Biomass Valorization 2021*

12:11, *12*(11), 5869–5884. https://doi.org/10.1007/S12649-021-01429-7

Anandharaj, M., Sivasankari, B., & Rani, R. P. (2014). Effects of Probiotics, Prebiotics, and Synbiotics on Hypercholesterolemia: A Review. *Chinese Journal of Biology*, *2014*(1), 572754. https://doi.org/10.1155/2014/572754

Ashrafian, F., Keshavarz Azizi Raftar, S., Shahryari, A., Behrouzi, A., Yaghoubfar, R., Lari, A., Moradi, H. R., Khatami, S., Omrani, M. D., Vaziri, F., Masotti, A., & Siadat, S. D. (2021). Comparative effects of alive and pasteurized Akkermansia muciniphila on normal diet-fed mice. *Scientific Reports 2021 11:1*, *11*(1), 17898-. https://doi.org/10.1038/s41598-021-95738-5

Avci, G. A., Yilmaz, Ü. İ., & Avci, E. (2024). Efficacy of probiotics, paraprobiotics, and postbiotics in colorectal cancer cell line and their role in immune response. *Revista Da Associacao Medica Brasileira (1992)*, *70*(6). https://doi.org/10.1590/1806-9282.20240226

Bleau, C., Monges, A., Rashidan, K., Laverdure, J. P., Lacroix, M., Van Calsteren, M. R., Millette, M., Savard, R., & Lamontagne, L. (2010). Intermediate chains of exopolysaccharides from Lactobacillus rhamnosus RW-9595M increase IL-10 production by macrophages. *Journal of Applied Microbiology*, *108*(2), 666–675. https://doi.org/10.1111/J.1365-2672.2009.04450.X

Burgos, F., Herrero, T., Martínez, J., Tabacco, O., & Vinderola, G. (2022). Immunomodulation properties of biotics and food allergy in pediatrics. *Archivos Argentinos de Pediatria*, *120*(4), 274–280. https://doi.org/10.5546/AAP.2022.ENG.274

Chiu, Y. H., Chiu, H. P., & Lin, M. Y. (2023). Synergistic effect of probiotic and postbiotic on attenuation of PM2.5-induced lung damage and allergic response. *Journal of Food Science*, *88*(1), 513–522. https://doi.org/10.1111/1750-3841.16398

Cuevas-González, P. F., Aguilar-Toalá, J. E., García, H. S., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A. (2020). Protective Effect of the Intracellular Content from Potential Probiotic Bacteria against Oxidative Damage Induced by Acrylamide in Human Erythrocytes. *Probiotics and Antimicrobial Proteins 2020 12:4*, *12*(4), 1459–1470. https://doi.org/10.1007/S12602-020-09636-9

da Silva Vale, A., de Melo Pereira, G. V., de Oliveira, A. C., de Carvalho Neto, D. P., Herrmann, L. W., Karp, S. G., Soccol, V. T., & Soccol, C. R. (2023). Production, Formulation, and Application of Postbiotics in the Treatment of Skin Conditions. *Fermentation*, *9*(3), 264. https://doi.org/10.3390/FERMENTATION9030264/S1

de Melo Pereira, G. V., de Oliveira Coelho, B., Magalhães Júnior, A. I., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, *36*(8), 2060–2076. https://doi.org/10.1016/J.BIOTECHADV.2018.09.003

Demirhan, H. K., Omer Oglou, E., Aksoy, Z. B., & Kiran, F. (2025). Evaluation of the anti-inflammatory, antioxidant and regenerative effects of microbiota-derived postbiotics in human periodontal ligament mesenchymal stromal cells. *Clinical Oral Investigations*, *29*(5). https://doi.org/10.1007/S00784-025-06341-1

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N. M., de Barsy, M., Loumaye, A., Hermans, M. P., Thissen, J. P., de Vos, W. M., & Cani, P. D. (2019). Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nature Medicine 2019 25:7*, *25*(7), 1096–1103. https://doi.org/10.1038/S41591-019-0495-2

Di Chiano, M., Rocchetti, M. T., Spano, G., Russo, P., Allegretta, C., Milior, G., Gadaleta, R. M., Sallustio, F., Pontrelli, P., Gesualdo, L., Avolio, C., Fiocco, D., & Gallone, A. (2024). Lactobacilli Cell-Free Supernatants Modulate Inflammation and Oxidative Stress in Human Microglia via NRF2-SOD1 Signaling. *Cellular and Molecular Neurobiology*, *44*(1). https://doi.org/10.1007/S10571-024-01494-1

Dinić, M., Burgess, J. L., Lukić, J., Catanuto, P., Radojević, D., Marjanović, J., Verpile, R., Thaller, S. R., Gonzalez, T., Golić, N., Strahinić, I., Tomic-Canic, M., & Pastar, I. (2024). Postbiotic lactobacilli induce cutaneous antimicrobial response and restore the barrier to inhibit the intracellular invasion of Staphylococcus aureus in vitro and ex vivo. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *38*(14). https://doi.org/10.1096/FJ.202400054RR

Duarte, M., Oliveira, A. L., Oliveira, C., Pintado, M., Amaro, A., & Madureira, A. R. (2022). Current postbiotics in the cosmetic market an update and development opportunities. *Applied Microbiology and Biotechnology 2022 106:18*, *106*(18), 5879–5891. https://doi.org/10.1007/S00253-022-12116-5

Engevik, M. A., Ruan, W., Esparza, M., Fultz, R., Shi, Z., Engevik, K. A., Engevik, A. C., Ihekweazu, F. D., Visuthranukul, C., Venable, S., Schady, D. A., & Versalovic, J. (2021). Immunomodulation of dendritic cells by Lactobacillus reuteri surface components and metabolites. *Physiological Reports*, *9*(2), e14719. https://doi.org/10.14814/PHY2.14719

Ferrari, V., Lo Cascio, A., Melacarne, A., Tanasković, N., Mozzarelli, A. M., Tiraboschi, L., Lizier, M., Salvi, M., Braga, D., Algieri, F., Penna, G., & Rescigno, M. (2023). Sensitizing cancer cells to immune checkpoint inhibitors by microbiota-mediated

upregulation of HLA class I. *Cancer Cell*, *41*(10), 1717-1730.e4. https://doi.org/10.1016/j.ccell.2023.08.014

Foudjing, G. G. D., Sarmast, E., Allahdad, Z., Salmieri, S., & Lacroix, M. (2023). Influence of growth parameters on bacteriocin-like inhibitory substances (BLIS) production by lactic acid bacteria. *Letters in Applied Microbiology*, *76*(1). https://doi.org/10.1093/LAMBIO/OVAC013

Fugaban, J. I. I., Jung, E. S., Todorov, S. D., & Holzapfel, W. H. (2023). Evaluation of Antifungal Metabolites Produced by Lactic Acid Bacteria. *Probiotics and Antimicrobial Proteins*, *15*(5), 1447–1463. https://doi.org/10.1007/S12602-022-09995-5

Gralnek, I. M., Hays, R. D., Kilbourne, A. A., Naliboff, B., & Mayer, E. A. (2000). The impact of irritable bowel syndrome on health-related quality of life. *Gastroenterology*, *119*(3), 654–660. https://doi.org/10.1053/GAST.2000.16484

Guan, C., Zhang, W., Su, J., Li, F., Chen, D., Chen, X., Huang, Y., Gu, R., & Zhang, C. (2023). Antibacterial and antibiofilm potential of Lacticaseibacillus rhamnosus YT and its cell-surface extract. *BMC Microbiology 2023 23:1*, *23*(1), 12-. https://doi.org/10.1186/S12866-022-02751-3

Guo, J., Zhao, Y., Guo, W., Sun, Y., Zhang, W., Zhao, Q., Zhang, Y., & Jiang, Y. (2025). Effects of Lactobacillus paracei JY062 Postbiotic on Intestinal Barrier, Immunity, and Gut Microbiota. *Nutrients*, *17*(7). https://doi.org/10.3390/NU17071272

Hernández-Granados, M. J., & Franco-Robles, E. (2020). Postbiotics in human health: Possible new functional ingredients? *Food Research International*, *137*, 109660. https://doi.org/10.1016/J.FOODRES.2020.109660

Hua, Q., Hu, J., Zhang, X., He, J., Wang, P., Duan, Z., Cui, H., Yi, H., Zhang, L., & Zhang, Z. (2025). Postbiotics Suppress Helicobacter pylori Adhesion and Survival through a Coaggregation Mechanism. *Journal of Agricultural and Food Chemistry*, *73*(35), 21976–21986. https://doi.org/10.1021/ACS.JAFC.5C07004

Huang, H. L., Lai, C. H., Tsai, W. H., Chen, K. W., Peng, S. L., Lin, J. H., & Lin, Y. H. (2024). Nanoparticle-enhanced postbiotics: Revolutionizing cancer therapy through effective delivery. *Life Sciences*, *337*. https://doi.org/10.1016/j.lfs.2023.122379

Humam, A. M., Loh, T. C., Foo, H. L., Izuddin, W. I., Zulkifli, I., Samsudin, A. A., & Mustapha, N. M. (2021). Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. *Poultry Science*, *100*(3). https://doi.org/10.1016/j.psj.2020.12.011

İçier, S., Güzelcan, C., Hıdır, Ş., & Kaplan Türköz, B. (2022). Postbiotics and their applications in the food industry. *Gıda, 47*(2), 252–265. https://doi.org/10.15237/gida.gd21145

Itoh, T., Miyazono, D., Sugata, H., Mori, C., & Takahata, M. (2024). Anti-inflammatory effects of heat-killed Lactiplantibacillus argentoratensis BBLB001 on a gut inflammation co-culture cell model and dextran sulfate sodium-induced colitis mouse model. *International Immunopharmacology*, *143*(Pt 2). https://doi.org/10.1016/j.intimp.2024.113408

Ivashkin, V., Maev, I., Poluektova, E., Sinitsa, A., Avalueva, E., Mnatsakanyan, M., Simanenkov, V., Karpeeva, J., Kopylova, D., Kuprina, I., Kucheryavyy, Y., Lapina, T., Solovyeva, O., Soom, M., Cheremushkina, N., Maevskaya, E., & Maslennikov, R. (2024). Efficacy and Safety of Postbiotic Contained Inactivated

Lactobacillus reuteri (Limosilactobacillus reuteri) DSM 17648 as Adjuvant Therapy in the Eradication of Helicobacter pylori in Adults with Functional Dyspepsia: A Randomized Double-Blind Placebo-C…. *Clinical and Translational Gastroenterology*, *15*(9), e1. https://doi.org/10.14309/CTG.0000000000000750

Jin, Y., Wu, J., Huang, K., & Liang, Z. (2024). Heat-Killed Saccharomyces boulardii Alleviates Dextran Sulfate Sodium-Induced Ulcerative Colitis by Restoring the Intestinal Barrier, Reducing Inflammation, and Modulating the Gut Microbiota. *Nutrients*, *16*(5). https://doi.org/10.3390/NU16050702

Kareem, K. Y., Ling, F. H., Chwen, L. T., Foong, O. M., & Anjas Asmara, S. (2014). Inhibitory activity of postbiotic produced by strains of Lactobacillus plantarum using reconstituted media supplemented with inulin. *Gut Pathogens*, *6*(1), 1–7. https://doi.org/10.1186/1757-4749-6-23/TABLES/2

Khakpour, M., Mohsenzadeh, M., & Salari, A. (2024). Feasibility of lactiplantibacillus plantarum postbiotics production in challenging media by different techniques. *AMB Express 2024 14:1*, *14*(1), 1–8. https://doi.org/10.1186/S13568-024-01704-5

Khmaladze, I., Butler, É., Fabre, S., & Gillbro, J. M. (2019). Lactobacillus reuteri DSM 17938—A comparative study on the effect of probiotics and lysates on human skin. *Experimental Dermatology*, *28*(7), 822–828. https://doi.org/10.1111/EXD.13950

Kim, G. Il, Jeong, H. Y., Kim, I. S., Lee, S. H., Kim, S. H., Moon, Y. S., & Cho, K. K. (2024). Interconnection of the Gut-Skin Axis in NC/Nga Mouse with Atopic Dermatitis: Effects of the Three Types of Bifidobacterium bifidum CBT-BF3 (Probiotics, Postbiotics, and Cytosine-Phosphate-Guanine Oligodeoxynucleotide) on T Cell Differentiation and Gut Mi…. *Food Science of Animal Resources*, *44*(6), 1417–1439. https://doi.org/10.5851/KOSFA.2024.E100

Kostelac, D., Gerić, M., Gajski, G., & Frece, J. (2022). Probiotic and paraprobiotic derivates exhibit anti-inflammatory and genoprotective effects during induced stress. *Journal of Applied Microbiology*, *133*(2), 819–829. https://doi.org/10.1111/JAM.15595

Kumar, A., Green, K. M., & Rawat, M. (2024). A Comprehensive Overview of Postbiotics with a Special Focus on Discovery Techniques and Clinical Applications. *Foods 2024, Vol. 13, Page 2937*, *13*(18), 2937. https://doi.org/10.3390/FOODS13182937

Kumar, D., Kumar, S., & Kumar, A. (2021). Extraction and characterization of secondary metabolites produced by bacteria isolated from industrial wastewater. *Journal of Water Process Engineering*, *40*, 101811. https://doi.org/10.1016/J.JWPE.2020.101811

Lee, F. H., Wan, S. Y., Foo, H. L., Loh, T. C., Mohamad, R., Rahim, R. A., & Idrus, Z. (2019). Comparative Study of Extracellular Proteolytic, Cellulolytic, and Hemicellulolytic Enzyme Activities and Biotransformation of Palm Kernel Cake Biomass by Lactic Acid Bacteria Isolated from Malaysian Foods. *International Journal of Molecular Sciences 2019, Vol. 20, Page 4979*, *20*(20), 4979. https://doi.org/10.3390/IJMS20204979

Lee, H. J., Tran, M. T. H., Le, M. H., Justine, E. E., & Kim, Y. J. (2024). Paraprobiotic derived from Bacillus velezensis GV1 improves immune response and gut microbiota composition in cyclophosphamide-treated immunosuppressed mice. *Frontiers in Immunology*, *15*. https://doi.org/10.3389/FIMMU.2024.1285063/PDF

Lee, P. J., Hung, C. M., Yang, A. J., Hou, C. Y., Chou, H. W., Chang, Y. C., Chu, W. C., Huang, W. Y., Kuo, W. C., Yang, C. C., Lin, K. I., Hung, K. H., Chang, L. C., Lee, K. Y., Kuo, H. P., Lu, K. M., Lai, H. C., Kuo, M. L., & Chen, W. J. (2024). MS-20 enhances the gut

microbiota-associated antitumor effects of anti-PD1 antibody. *Gut Microbes*, *16*(1). https://doi.org/10.1080/19490976.2024.2380061

Lee, Y. S., Noh, D. I., Lee, S. J., Jeon, M. H., Kim, Y. R., Jang, W. J., & Lee, E. W. (2025). Anti-inflammatory and barrier-restoring effects of heat-killed Lactiplantibacillus plantarum postbiotics in an in vitro model of atopic dermatitis. *Journal of Applied Microbiology*, *136*(10). https://doi.org/10.1093/JAMBIO/LXAF256

Li, J., Ghosh, T. S., Arendt, E., Shanahan, F., & O'Toole, P. W. (2024). Cross-Cohort Gut Microbiome Signatures of Irritable Bowel Syndrome Presentation and Treatment. *Advanced Science*, *11*(41), 2308313. https://doi.org/10.1002/ADVS.202308313;WGROUP:STRING:PUBLICATION

Li, S., Tantibhadrasapa, A., Buddhasiri, S., Boonpan, P., Sukjoi, C., Mongkolkarvin, P., Nakphaichit, M., Nitisinprasert, S., & Thiennimitr, P. (2025). Probiotic, Paraprobiotic, and Postbiotic Activities of Lactiplantibacillus plantarum KUNN19-2 Against Non-Typhoidal Salmonella Serovars. *International Journal of Molecular Sciences*, *26*(5). https://doi.org/10.3390/IJMS26051821

Liang, B., & Xing, D. (2023). The Current and Future Perspectives of Postbiotics. *Probiotics and Antimicrobial Proteins*, *15*(6), 1626–1643. https://doi.org/10.1007/S12602-023-10045-X/TABLES/4

Lim, J. J., Jung, A. H., Joo Suh, H., Choi, H. S., & Kim, H. (2022). Lactiplantibacillus plantarum K8-based paraprobiotics prevents obesity and obesity-induced inflammatory responses in high fat diet-fed mice. *Food Research International (Ottawa, Ont.)*, *155*. https://doi.org/10.1016/J.FOODRES.2022.111066

Lin, W. Y., Lin, J. H., Kuo, Y. W., Chiang, P. F. R., & Ho, H. H. (2022). Probiotics and their Metabolites Reduce Oxidative Stress in

Middle-Aged Mice. *Current Microbiology*, *79*(4). https://doi.org/10.1007/S00284-022-02783-Y

Liu, X., Ma, Y., Guan, K., Liu, R., Mao, K., Xu, X., Li, Q., & Wang, R. (2024). Intestinal barrier, immunity and gut microbiota-based protective effects of Lactococcus lactis HF08 and its postbiotic derivative on aging and aging colitis mice. *Food Research International*, *197*(Pt 1). https://doi.org/10.1016/j.foodres.2024.115164

Liu, Z. Y., Yang, H. L., Li, S., Cai, G. H., Ye, J. D., Zhang, C. X., & Sun, Y. Z. (2024). Paraprobiotic and postbiotic forms of Bacillus siamensis improved growth, immunity, liver and intestinal health in Lateolabrax maculatus fed soybean meal diet. *Fish and Shellfish Immunology*, *145*. https://doi.org/10.1016/j.fsi.2024.109370

Lovell, R. M., & Ford, A. C. (2012). Global Prevalence of and Risk Factors for Irritable Bowel Syndrome: A Meta-analysis. *Clinical Gastroenterology and Hepatology*, *10*(7), 712-721.e4. https://doi.org/10.1016/J.CGH.2012.02.029

Lu, S., Xu, J., Zhao, Z., Guo, Y., Zhang, H., Jurutka, P. W., Huang, D., Cao, C., & Cheng, S. (2023). Dietary Lactobacillus rhamnosus GG extracellular vesicles enhance antiprogrammed cell death 1 (anti-PD-1) immunotherapy efficacy against colorectal cancer. *Food & Function*, *14*(23), 10314–10328. https://doi.org/10.1039/D3FO02018E

Ma, T., Li, Y., Yang, N., Wang, H., Shi, X., Liu, Y., Jin, H., Kwok, L. Y., Sun, Z., & Zhang, H. (2025). Efficacy of a postbiotic and its components in promoting colonic transit and alleviating chronic constipation in humans and mice. *Cell Reports Medicine*, *6*(5). https://doi.org/10.1016/j.xcrm.2025.102093

Makino, S., Sato, A., Goto, A., Nakamura, M., Ogawa, M., Chiba, Y., Hemmi, J., Kano, H., Takeda, K., Okumura, K., & Asami, Y.

(2016). Enhanced natural killer cell activation by exopolysaccharides derived from yogurt fermented with Lactobacillus delbrueckii ssp. bulgaricus OLL1073R-1. *Journal of Dairy Science*, *99*(2), 915–923. https://doi.org/10.3168/JDS.2015-10376

Miao, C., Wang, L., Wang, H., Shen, Y., Man, C., Zhang, W., Zhang, Y., Zhao, Q., & Jiang, Y. (2024). Lacticaseibacillus plantarum postbiotics prepared by the combined technique of pasteurization and ultrasound: effective measures to alleviate obesity based on the SCFAs-GPR41/GPR43 signaling pathway. *Food & Function*, *15*(22), 11005–11019. https://doi.org/10.1039/D4FO03591G

Moon, E. C., Park, M. S., Lim, T., Kim, R. H., Ji, G. E., Kim, S. Y., & Hwang, K. T. (2022). Antibacterial effect of cell-free supernatant fraction from Lactobacillus paracasei CH88 against Gardnerella vaginalis. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-08808-7

Moradi, M., Kousheh, S. A., Almasi, H., Alizadeh, A., Guimarães, J. T., Yılmaz, N., & Lotfi, A. (2020). Postbiotics produced by lactic acid bacteria: The next frontier in food safety. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3390–3415. https://doi.org/10.1111/1541-4337.12613

Mousavi, S., Hosseini, M. H., Sarandili, S., & Nejati, B. (2024). Myeloperoxidase Deficiency: A Rare Case. *Cureus*, *16*(7), e63596. https://doi.org/10.7759/CUREUS.63596

Murofushi, Y., Villena, J., Morie, K., Kanmani, P., Tohno, M., Shimazu, T., Aso, H., Suda, Y., Hashiguchi, K., Saito, T., & Kitazawa, H. (2015). The toll-like receptor family protein RP105/MD1 complex is involved in the immunoregulatory effect of exopolysaccharides from Lactobacillus plantarum N14. *Molecular*

Immunology, *64*(1), 63–75. https://doi.org/10.1016/J.MOLIMM.2014.10.027

Naghibi, M., Pont-Beltran, A., Lamelas, A., Llobregat, L., Martinez-Blanch, J. F., Rojas, A., Álvarez, B., López Plaza, B., Arcos Castellanos, L., Chenoll, E., Vijayakumar, V., & Day, R. (2024). Effect of Postbiotic Bifidobacterium longum CECT 7347 on Gastrointestinal Symptoms, Serum Biochemistry, and Intestinal Microbiota in Healthy Adults: A Randomised, Parallel, Double-Blind, Placebo-Controlled Pilot Study. *Nutrients*, *16*(22). https://doi.org/10.3390/NU16223952

Nam, Y., Kim, J. H., Baek, J., & Kim, W. (2021). Improvement of Cutaneous Wound Healing via Topical Application of Heat-Killed Lactococcus chungangensis CAU 1447 on Diabetic Mice. *Nutrients 2021, Vol. 13, Page 2666*, *13*(8), 2666. https://doi.org/10.3390/NU13082666

Ng, Q. X., Yaow, C. Y. L., Moo, J. R., Koo, S. W. K., Loo, E. X. L., & Siah, K. T. H. (2024). A systematic review of the association between environmental risk factors and the development of irritable bowel syndrome. *Journal of Gastroenterology and Hepatology (Australia)*, *39*(9), 1780–1787. https://doi.org/10.1111/JGH.16587;SUBPAGE:STRING:FULL

Nikolic, M., López, P., Strahinic, I., Suárez, A., Kojic, M., Fernández-García, M., Topisirovic, L., Golic, N., & Ruas-Madiedo, P. (2012). Characterisation of the exopolysaccharide (EPS)-producing Lactobacillus paraplantarum BGCG11 and its non-EPS producing derivative strains as potential probiotics. *International Journal of Food Microbiology*, *158*(2), 155–162. https://doi.org/10.1016/J.IJFOODMICRO.2012.07.015

Nowak, A., Zakłos-Szyda, M., Rosicka-Kaczmarek, J., & Motyl, I. (2022). Anticancer Potential of Post-Fermentation Media and Cell

Extracts of Probiotic Strains: An In Vitro Study. *Cancers*, *14*(7). https://doi.org/10.3390/CANCERS14071853

Okada, K., Takami, D., Makizaki, Y., Tanaka, Y., Nakajima, S., Ohno, H., & Sagami, T. (2023). Effects of Bifidobacterium longum CLA8013 on bowel movement improvement: a placebo-controlled, randomized, double-blind study. *Bioscience of Microbiota, Food and Health*, *42*(3), 213–221. https://doi.org/10.12938/BMFH.2022-066

Oliero, M., Cuisiniere, T., Ajayi, A. S., Gerkins, C., Hajjar, R., Fragoso, G., Calvé, A., Rendos, H. V., Mathieu-Denoncourt, A., Dagbert, F., De Broux, É., Loungnarath, R., Schwenter, F., Sebajang, H., Ratelle, R., Wassef, R., Richard, C., Duperthuy, M., Gravel, A. E., … Santos, M. M. (2024). Putrescine Supplementation Limits the Expansion of pks+ Escherichia coli and Tumor Development in the Colon. *Cancer Research Communications*, *4*(7), 1777–1792. https://doi.org/10.1158/2767-9764.CRC-23-0355

Oliveira, A. L. S., Seara, M., Carvalho, M. J., de Carvalho, N. M., Costa, E. M., Silva, S., Duarte, M., Pintado, M., Oliveira, C., & Madureira, A. R. (2023). Production of Sustainable Postbiotics from Sugarcane Straw for Potential Food Applications. *Applied Sciences 2023, Vol. 13, Page 3391*, *13*(6), 3391. https://doi.org/10.3390/APP13063391

Özcan Aykol, Ş. M., & Zeybek, Z. (2024). Interaction between Acanthamoeba and Staphylococcus. *Journal of Basic Microbiology*, *64*(4). https://doi.org/10.1002/JOBM.202300551

Pakbin, B., Allahyari, S., Dibazar, S. P., Zolghadr, L., Chermahini, N. K., Brück, W. M., Brück, T. B., & Mahmoudi, R. (2023). Effects of Probiotic Saccharomyces boulardii Supernatant on Viability, Nano-Mechanical Properties of Cytoplasmic Membrane and Pro-Inflammatory Gene Expression in Human Gastric Cancer AGS

Cells. *International Journal of Molecular Sciences*, *24*(9). https://doi.org/10.3390/IJMS24097945

Panebianco, C., Villani, A., Pisati, F., Orsenigo, F., Ulaszewska, M., Latiano, T. P., Potenza, A., Andolfo, A., Terracciano, F., Tripodo, C., Perri, F., & Pazienza, V. (2022). Butyrate, a postbiotic of intestinal bacteria, affects pancreatic cancer and gemcitabine response in in vitro and in vivo models. *Biomedicine and Pharmacotherapy*, *151*. https://doi.org/10.1016/j.biopha.2022.113163

Peng, L., Yin, Q., Wang, X., Zhong, Y., Wang, Y., Cai, W., Zhou, R., Chen, Y., Hu, Y., Cheng, Z., Jiang, W., Yue, X., & Huang, L. (2024). Pasteurized Akkermansia muciniphila Ameliorates Preeclampsia in Mice by Enhancing Gut Barrier Integrity, Improving Endothelial Function, and Modulating Gut Metabolic Dysregulation. *Microorganisms*, *12*(12). https://doi.org/10.3390/MICROORGANISMS12122483

Pérez, M., Buey, B., Corral, P., Giraldos, D., & Latorre, E. (2024). Microbiota-Derived Short-Chain Fatty Acids Boost Antitumoral Natural Killer Cell Activity. *Journal of Clinical Medicine*, *13*(13). https://doi.org/10.3390/JCM13133885

Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., Chilloux, J., Ottman, N., Duparc, T., Lichtenstein, L., Myridakis, A., Delzenne, N. M., Klievink, J., Bhattacharjee, A., Van Der Ark, K. C. H., Aalvink, S., Martinez, L. O., Dumas, M. E., Maiter, D., … Cani, P. D. (2017). A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine*, *23*(1), 107–113. https://doi.org/10.1038/NM.4236;SUBJMETA

Qin, X., Zhao, Q., Zhao, Q., Yang, L., Li, W., Wu, J., Liu, T., Zhong, W., Jiang, K., Liu, W., Wang, B., Wang, S., & Cao, H. (2024). A Saccharomyces boulardii-derived antioxidant protein, thioredoxin,

ameliorates intestinal inflammation through transactivating epidermal growth factor receptor. *Pharmacological Research*, *208*. https://doi.org/10.1016/J.PHRS.2024.107372

Rahman, Md. M., Sazili, A. Q., Ahmad, S. A., Khalil, K. A., Ismail-Fitry, M. R., & Sarker, Md. S. K. (2025). Inhibitory efficacy, production dynamics, and characterization of postbiotics of lactic acid bacteria. *BMC Microbiology 2025 25:1*, *25*(1), 1–20. https://doi.org/10.1186/S12866-025-04123-Z

Ray, S., Jin, J. O., Choi, I., & Kim, M. (2023). Cell-Free Supernatant of Bacillus thuringiensis Displays Anti-Biofilm Activity Against Staphylococcus aureus. *Applied Biochemistry and Biotechnology*, *195*(9), 5379–5393. https://doi.org/10.1007/S12010-022-03971-Z

Razim, A., Zabłocka, A., Schmid, A., Thaler, M., Černý, V., Weinmayer, T., Whitehead, B., Martens, A., Skalska, M., Morandi, M., Schmidt, K., Wysmołek, M. E., Végvári, A., Srutkova, D., Schwarzer, M., Neuninger, L., Nejsum, P., Hrdý, J., Palmfeldt, J., … Schabussova, I. (2024). Bacterial extracellular vesicles as intranasal postbiotics: Detailed characterization and interaction with airway cells. *Journal of Extracellular Vesicles*, *13*(10). https://doi.org/10.1002/JEV2.70004

Rezaie, N., Aghamohammad, S., Haj Agha Gholizadeh Khiavi, E., Khatami, S., Sohrabi, A., & Rohani, M. (2024). The comparative anti-oxidant and anti-inflammatory efficacy of postbiotics and probiotics through Nrf-2 and NF-kB pathways in DSS-induced colitis model. *Scientific Reports*, *14*(1). https://doi.org/10.1038/S41598-024-62441-0

Rezaie, N., Aghamohammad, S., Khiavi, E. H. A. G., Talebi, M., Pourshafie, M. R., & Rohani, M. (2025). The Analysis and Comparison of Anti-Inflammatory and Antioxidant Characteristics of Postbiotic and Paraprobiotic Derived From Novel Native

Probiotic Cocktail in DSS-Induced Colitic Mice. *Food Science & Nutrition*, *13*(2). https://doi.org/10.1002/FSN3.70034

Ryu, S., Kyoung, H., Park, K. Il, Oh, S., Song, M., & Kim, Y. (2022). Postbiotic heat-killed lactobacilli modulates on body weight associated with gut microbiota in a pig model. *AMB Express*, *12*(1). https://doi.org/10.1186/S13568-022-01424-8

Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M. E., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature Reviews Gastroenterology and Hepatology*, *18*(9), 649–667. https://doi.org/10.1038/S41575-021-00440-6;SUBJMETA

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology and Hepatology*, *16*(10), 605–616. https://doi.org/10.1038/S41575-019-0173-3;SUBJMETA

Seo, K. H., Gyu Lee, H., Young Eor, J., Jin Jeon, H., Yokoyama, W., & Kim, H. (2022). Effects of kefir lactic acid bacteria-derived postbiotic components on high fat diet-induced gut microbiota and obesity. *Food Research International*, *157*. https://doi.org/10.1016/j.foodres.2022.111445

Shamim Rahman, M., Lee, Y., Park, D. S., & Kim, Y. S. (2023). Bifidobacterium bifidum DS0908 and Bifidobacterium longum DS0950 Culture-Supernatants Ameliorate Obesity-Related Characteristics in Mice with High-Fat Diet-Induced Obesity. *Journal of Microbiology and Biotechnology*, *33*(1), 96–105. https://doi.org/10.4014/JMB.2210.10046

Sun, Y., Liu, X., Zhao, L., Li, D., Guan, K. F., Ma, Y., Wang, R., & Li, Q. (2025). Lacticaseibacillus rhamnosus HF01 postbiotics reprogram gut microbial tryptophan metabolism to coordinate enterohepatic barrier-insulin signaling axis. *Current Research in Food Science*, *11*, 101111. https://doi.org/10.1016/J.CRFS.2025.101111

Tanihiro, R., Yuki, M., Sakano, K., Sasai, M., Sawada, D., Ebihara, S., & Hirota, T. (2024). Effects of Heat-Treated Lactobacillus helveticus CP790-Fermented Milk on Gastrointestinal Health in Healthy Adults: A Randomized Double-Blind Placebo-Controlled Trial. *Nutrients*, *16*(14). https://doi.org/10.3390/NU16142191

Tantratian, S., & Pradeamchai, M. (2020). Select a protective agent for encapsulation of Lactobacillus plantarum. *LWT*, *123*, 109075. https://doi.org/10.1016/J.LWT.2020.109075

Teame, T., Wang, A., Xie, M., Zhang, Z., Yang, Y., Ding, Q., Gao, C., Olsen, R. E., Ran, C., & Zhou, Z. (2020). Paraprobiotics and Postbiotics of Probiotic Lactobacilli, Their Positive Effects on the Host and Action Mechanisms: A Review. *Frontiers in Nutrition*, *7*, 570344. https://doi.org/10.3389/FNUT.2020.570344/FULL

Teng, R. J., Jing, X., Martin, D. P., Hogg, N., Haefke, A., Konduri, G. G., Day, B. W., Naylor, S., & Pritchard, K. A. (2021). N-acetyl-lysyltyrosylcysteine amide, a novel systems pharmacology agent, reduces bronchopulmonary dysplasia in hyperoxic neonatal rat pups. *Free Radical Biology and Medicine*, *166*, 73–89. https://doi.org/10.1016/j.freeradbiomed.2021.02.006

Vertillo Aluisio, G., Mezzatesta, M. L., Cafiso, V., Scuderi, R., Stefani, S., & Santagati, M. (2024). Cell-free supernatant of Lactobacillus gasseri 1A-TV shows a promising activity to eradicate carbapenem-resistant Klebsiella pneumoniae colonization. *Frontiers*

in Cellular and Infection Microbiology, *14*. https://doi.org/10.3389/FCIMB.2024.1471107/PDF

Vinderola, G., Sanders, M. E., & Salminen, S. (2022). The Concept of Postbiotics. *Foods 2022, Vol. 11, Page 1077*, *11*(8), 1077. https://doi.org/10.3390/FOODS11081077

Wegh, C. A. M., Schoterman, M. H. C., Vaughan, E. E., Belzer, C., & Benninga, M. A. (2017). The effect of fiber and prebiotics on children's gastrointestinal disorders and microbiome. *Expert Review of Gastroenterology & Hepatology*, *11*(11), 1031–1045. https://doi.org/10.1080/17474124.2017.1359539

Wu, J., Li, Z., Zhang, Z., Zhang, J., Hu, H., Lan, H., Hong, W., & Yang, Z. (2025). Characterization of a postbiotic exopolysaccharide produced by Lacticaseibacillus paracasei ET-22 with antioxidant and anti-inflammatory efficacy. *International Journal of Biological Macromolecules*, *306*(Pt 3). https://doi.org/10.1016/j.ijbiomac.2025.141608

Wu, Z. Q., Chen, X. M., Ma, H. Q., Li, K., Wang, Y. L., & Li, Z. J. (2023). Akkermansia muciniphila Cell-Free Supernatant Improves Glucose and Lipid Metabolisms in Caenorhabditis elegans. *Nutrients*, *15*(7). https://doi.org/10.3390/NU15071725

Yeşilyurt, N., Yılmaz, B., Ağagündüz, D., & Capasso, R. (2021). Involvement of Probiotics and Postbiotics in the Immune System Modulation. *Biologics 2021, Vol. 1, Pages 89-110*, *1*(2), 89–110. https://doi.org/10.3390/BIOLOGICS1020006

Yin, J., Chen, L., Lin, Y., Qiu, J., Liu, F., Wang, Y., & Dou, X. (2025). Bifidobacterium bifidum reduces oxidative stress and alters gut flora to mitigate acute liver injury caused by N-acetyl-p-aminophenol. *BMC Microbiology*, *25*(1). https://doi.org/10.1186/S12866-025-03775-1

Yin, R., Wang, T., Sun, J., Dai, H., Zhang, Y., Liu, N., & Liu, H. (2025). Postbiotics From Lactobacillus Johnsonii Activates Gut Innate Immunity to Mitigate Alcohol-Associated Liver Disease. *Advanced Science (Weinheim, Baden-Wurttemberg, Germany)*, *12*(2). https://doi.org/10.1002/ADVS.202405781

Yu, H. R., Sheen, J. M., Hou, C. Y., Lin, I. C., Huang, L. T., Tain, Y. L., Cheng, H. H., Lai, Y. J., Lin, Y. J., Tiao, M. M., & Tsai, C. C. (2022). Effects of Maternal Gut Microbiota-Targeted Therapy on the Programming of Nonalcoholic Fatty Liver Disease in Dams and Fetuses, Related to a Prenatal High-Fat Diet. *Nutrients*, *14*(19). https://doi.org/10.3390/NU14194004

Zhang, J., Duan, X., Chen, X., Qian, S., Ma, J., Jiang, Z., & Hou, J. (2024). Lactobacillus rhamnosus 1.0320 Postbiotics Ameliorate Dextran Sodium Sulfate-Induced Colonic Inflammation and Oxidative Stress by Regulating the Intestinal Barrier and Gut Microbiota. *Journal of Agricultural and Food Chemistry*, *72*(45), 25078–25093. https://doi.org/10.1021/ACS.JAFC.4C06303

Zhao, Y., Zheng, Y., Xie, K., Hou, Y., Liu, Q., Jiang, Y., Zhang, Y., & Man, C. (2023). Combating Obesity: Harnessing the Synergy of Postbiotics and Prebiotics for Enhanced Lipid Excretion and Microbiota Regulation. *Nutrients*, *15*(23). https://doi.org/10.3390/NU15234971

Zhong, B., Zhao, Y., Gao, L., Yang, G., Gao, Y., Li, F., & Li, S. (2024). Anticancer Effects of Weizmannia coagulans MZY531 Postbiotics in CT26 Colorectal Tumor-Bearing Mice by Regulating Apoptosis and Autophagy. *Life (Basel, Switzerland)*, *14*(10). https://doi.org/10.3390/LIFE14101334

CHAPTER 0

FASTING-MIMICKING DIET: SCIENTIFIC BASIS, NUTRITIONAL COMPOSITION, AND HEALTH IMPLICATIONS

Şule KALAN [1]
Gülperi DEMİR [2]

Introduction

Fasting, although it is an ancient practice applied for the purpose of physical and mental purification within different cultures and belief systems throughout history, is today being reconsidered scientifically in the context of metabolic regulation and the prevention of diseases (Fanti, Mishra, Longo, & Brandhorst, 2021; Longo & Mattson, 2014). Recent studies have revealed that fasting is not merely an approach based on the restriction of energy intake; rather, it exerts effects through numerous physiological mechanisms such as insulin sensitivity, metabolic flexibility, cellular stress responses, autophagy, and growth factor signaling pathways (Wilhelmi de Toledo, Grundler, Bergouignan, Drinada, & Michalsen, 2019; Anton , et al., 2018; Alirezaei, et al., 2010). Based on these biological responses, intermittent fasting and time-restricted feeding models developed aim to translate the physiology of fasting into practice through different protocols and are among modern

[1] Yüksek Lisans Öğrencisi, Selçuk Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik A.B.D., Orcid: 0009-0000-0338-3498
[2] Doç.Dr., Selçuk Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Orcid: 0000-0002-7362-3335

nutritional approaches (Visioli, Mucignat-Caretta, Anile, & Panaite, 2022). Among these approaches, the fasting-mimicking diet (FMD), which aims to mimic the metabolic effects that occur during fasting through specific macronutrient and micronutrient modifications, stands out due to its unique structure and scientifically based protocol.

The fundamental factors in the development of diets that protect and promote health and support healthy aging are the energy and nutrient content of the diet. In addition to the energy and nutrient content of the daily diet, the amount and frequency of meals and their effects on health have been the focus of research for many years. As a result of these regulations, they emerge as powerful tools used for pre-disease prevention, delaying the onset of disease, and slowing the aging process (Brandhorst, et al., 2015; Di Francesco, Di Germanio, Bernier , & de Cabo, 2018; Paoli, Tinsley, Bianco, & Moro, 2019).

Overnutrition, which is commonly observed in modern societies, plays a critical role in metabolic health in terms of characteristics such as total caloric intake, meal frequency, and meal timing. In particular, it is argued that changes in meal timing may influence the success of reducing obesity risk (Sutton, et al., 2018).

Overnutrition is considered one of the primary risk factors in the development of obesity-related problems. This condition plays a significant role in the emergence of numerous chronic diseases, including neurodegenerative diseases, metabolic disorders, cardiovascular diseases, and various types of cancer (Brandhorst, 2021).

In recent years, extensive research has been conducted to develop feasible strategies to combat overnutrition. Among the dietary approaches developed to promote healthy aging, prevent metabolic diseases, and extend lifespan, two main dietary strategies

stand out, particularly calorie-restricted diets and fasting-based diets (Tang, et al., 2023).

Calorie-restricted diets involve the continuous reduction of daily energy intake to below an individual's requirements, whereas fasting-based nutrition, unlike religious or cultural fasting practices, is a dietary strategy based on planned periods of fasting and feeding that targets metabolic and physiological effects. Growing evidence indicates that both calorie restriction and fasting-based nutrition exert beneficial effects on quality of life and lifespan by delaying the onset and slowing the progression of various diseases (Di Francesco, Di Germanio, Bernier , & de Cabo, 2018).

Fasting or the restriction of energy and nutrient intake is practiced worldwide based on traditional, cultural, or religious reasons and generally refers to the restriction of solid food intake for a certain period of time. In ancient medicine, fasting, applied as a therapeutic method since Hippocrates, was used by many early European medical schools for the treatment of acute and chronic diseases (Michalsen, 2010).

The restriction of fasting or energy and nutrient intake, practiced for thousands of years, has recently been shown to play roles in the molecular clock, the gut microbiome, and tissue homeostasis and function. Fasting slows aging and provides protection against various diseases, including neurodegenerative diseases, metabolic disorders, and cancers. Observations and findings accumulated over centuries indicate that the fasting state has the potential to delay aging, minimize the side effects caused by chronic dietary interventions, and prevent and treat diseases (Tang, et al., 2023). However, the dietary pattern adopted today involves excessive food consumption, prolonged periods of energy intake throughout the day, and short fasting intervals. Consequently, high-calorie diets and sedentary lifestyles adversely affect the body's

metabolism and increase the incidence of obesity, diabetes, cardiovascular diseases, and dementia (Wang & Wu, 2022).

Today, the increasing prevalence of obesity, diabetes, cardiovascular diseases, cancer, and neurodegenerative diseases has heightened interest in dietary strategies that directly target metabolic mechanisms. However, the literature often does not clearly distinguish intermittent fasting, time-restricted feeding, and fasting-mimicking diet approaches in conceptual and practical terms; in particular, the scientific mechanisms underlying the FMD and its nutritional composition are not sufficiently emphasized. Nevertheless, the fasting-mimicking diet is a distinctive dietary model that aims to controllably mimic the metabolic and cellular responses observed in classical fasting through protein and carbohydrate restriction, regulation of fat content, micronutrient supplementation, and a limited duration of application (Wei, et al., 2017). In this study, the scientific foundations, nutritional composition, and implementation characteristics of the fasting-mimicking diet are addressed; its relationship with various diseases is evaluated in light of current scientific evidence by comparing it with intermittent fasting and time-restricted feeding.

FASTING-BASED DIETARY MODELS

Fasting-oriented dietary patterns refer to nutritional approaches in which food consumption is confined to particular periods of the day, while abstinence from food is maintained during the remaining hours. Owing to their potential influence on energy metabolism, insulin responsiveness, and various cellular adaptation mechanisms, these patterns have increasingly attracted scientific attention in recent years (Wilhelmi de Toledo, Grundler, Bergouignan, Drinada, & Michalsen, 2019; Anton , et al., 2018; Alirezaei, et al., 2010).

Fasting-based nutrition, unlike religious or cultural fasting practices, is a dietary strategy based on planned periods of fasting and feeding that targets metabolic and physiological effects. It is a dietary approach in which individuals restrict food intake for specific periods within their daily or weekly eating patterns while implementing planned fasting periods. This model focuses on meal timing rather than nutrient composition and is referred to in the literature as intermittent fasting and time-restricted eating (Visioli, Mucignat-Caretta, Anile, & Panaite, 2022).

Intermittent Fasting

Intermittent fasting has become one of the most commonly practiced dietary models today due to its role in supporting weight loss and being a sustainable nutritional strategy (Conde-Pipó, et al., 2024). Intermittent fasting is defined as an eating pattern in which no calorie restriction is applied and which alternates between periods of fasting and eating. The basic principle of intermittent fasting is that it is an eating pattern with a specific time restriction during which no food is consumed for a defined period. The duration of the non-eating interval may range from 12 hours to several days (Moon, et al., 2020; Rynders, et al., 2019; Anic, et al., 2022). Although intermittent fasting diets have different modes of implementation, the most common forms are alternate-day fasting, modified fasting, and religious fasting practices (Templeman, Gonzalez, Thompson, & Betts, 2019; Patterson & Sears, Metabolic Effects of Intermittent Fasting, 2017).

Alternate-day fasting is a method in which individuals either do not consume energy-containing foods throughout the day or, in order to reduce the difficulty of its implementation, meet up to a maximum of 25% of their energy requirements, followed by a day on which they eat freely (Patterson, et al., 2015).

Modified fasting (the 5:2 diet) is a method in which individuals eat regularly and in a balanced manner on five days of the week, while on the other two days they follow restricted eating by consuming 20–25% of their daily energy intake (approximately below 600 kcal for men and 500 kcal for women) (Varady, Cienfuegos, Ezpeleta, & Gabel, 2022).

Intermittent fasting not only creates positive effects on health by reducing body weight in obese individuals, but can also improve metabolic parameters in normal-weight individuals. In addition, it plays a role in the maintenance of the circadian rhythm, which regulates the delicate balance between appetite, satiety, sleep, and wakefulness. However, although there are studies indicating potential benefits of intermittent fasting on cardiovascular diseases, diabetes, cancer, obesity, and neurological disorders, long-term clinical data remain contradictory (Bekar & Gerboğa, 2023).

Time-Restricted Eating

The time-restricted eating model is an approach that limits food intake during the day to an 8-hour or shorter time window and restricts food consumption outside this period, and is based on the regulation of eating timing rather than the amount of food consumed (Hatori, et al., 2012).

Time-restricted eating is not principally based on intentional calorie restriction. Instead, it prolongs the daily fasting interval by restricting the timing of food intake and concentrating meals within limited periods of the day. Currently applied time-restricted eating is limited to certain hours of the day without calorie restriction and generally refers to food consumption lasting between 4 and 12 hours. An individual's food intake is planned according to the hours during which the circadian rhythm is most active and is implemented through different protocols such as 16:8, 18:6, or 20:4. The most

common form is the 16:8 model, which includes an 8-hour eating period following a 16-hour fasting period (Upadhyay, et al., 2019).

A large number of animal studies conducted to date have demonstrated that time-restricted eating confers notable health benefits. These include reductions in body weight and food intake, as well as improvements in hyperlipidemia, ectopic fat accumulation, and inflammatory markers. Furthermore, beneficial effects such as a decreased risk of cardiovascular disease and cancer, along with extended lifespan, have been reported. In addition, time-restricted eating has been suggested to support brain health and may delay the onset of neurodegenerative diseases (Chaix, Manoogian, Melkani, & Panda, 2019).

Water-Only Fasting

Water-only fasting is defined as a therapeutic practice in which an individual voluntarily abstains from the consumption of all energy-containing foods and beverages except pure water, and which is typically conducted under medical supervision (Finnell, Saul, Alan, & Myers, 2018).

The intervention protocol consists of three fundamental phases that are meticulously planned. During the preparatory phase, which lasts at least two days, processed foods, animal products, alcohol, and caffeine are eliminated from the diet, and nutritional intake is restricted exclusively to fruits and vegetables. Subsequently, in the primary fasting phase, the individual consumes a minimum of 1.2 liters of distilled water per day, limits physical activity, and is monitored under continuous 24-hour medical supervision (Zeiler, et al., 2024; Finnell, Saul, Alan, & Myers, 2018). The process is safely concluded with a “refeeding” period lasting at least half the duration of the fasting phase, which aims to facilitate the gradual adaptation of the digestive system from liquid foods to solid, whole plant-based foods (Gabriel, et al., 2022).

Fasting-Mimicking Diet

Restriction of the energy and nutrient content of the diet supports cellular changes that regulate energy metabolism and affect oxidative damage and inflammation, thereby enhancing cellular protection. The approach of restricting the energy and nutrient content of the diet is applied either at regular intervals in the form of intermittent fasting (IF) or as prolonged fasting cycles lasting several days (PF) (Longo & Mattson, 2014).

In fasting-based nutritional practices, prolonged fasting states involving the consumption of only water pose practical difficulties for individuals. Particularly in elderly and underweight individuals, such practices may exacerbate malnutrition and lead to the emergence of existing functional impairments. It is reported that alternative dietary approaches providing similar benefits are needed to minimize these adverse effects (Fanti, Mishra, Longo, & Brandhorst, 2021). In this context, the fasting-mimicking diet (FMD) was developed by Longo and his team (2019) as a novel dietary intervention that allows food intake. This diet aims to provide fasting-associated health benefits, such as cellular renewal, improved metabolic health, and potential longevity benefits, without requiring complete abstinence from food (Brandhorst & Longo, 2019).

The implementation of the fasting-mimicking diet allows the consumption of light meals during the fasting period, thereby minimizing the burden of restriction. The fasting-mimicking diet represents a medically designed fasting-like state and allows for periodic consumption patterns. The human fasting-mimicking diet program is generally plant-based, low in protein, low in carbohydrates, and high in healthy fats. At the same time, it also aims to provide essential micronutrients (vitamins and minerals) in order to reduce the burden of fasting (Nuanchankong, Jaroennon, & Manakla, 2024).

Nutritional Composition of the Fasting-Mimicking Diet

The fasting-mimicking diet is formulated as a plant-based, very low-calorie, low-protein, low-carbohydrate, and high-healthy-fat diet (Fanti, Mishra, Longo, & Brandhorst, 2021; Rangan, et al., 2022). The structure of the diet particularly emphasizes foods rich in fiber, vitamins, and minerals, such as vegetables, nuts, seeds, and plant-based oils. In this regard, the fasting-mimicking diet aims to support gastrointestinal functions and induce favorable changes in metabolic processes due to its high fiber content (Ostfeld, 2017).

The protocol excludes all animal-derived foods such as red meat, poultry, fish, eggs, and dairy products, providing an entirely plant-based composition. The removal of animal products helps maintain the diet's low protein content, contributing to the emergence of fasting-related cellular adaptations. Foods used in the FMD generally include vegetable soups, plant-based broth-like liquids, low-sugar nutrient bars, healthy fat sources such as olives, low-carbohydrate crackers, and various herbal teas. These foods can be formulated to provide sufficient micronutrients to the body while restricting energy intake (Nuanchankong, Jaroennon, & Manakla, 2024).

Table 1 Energy and Nutrient Composition Ratios of the Fasting-Mimicking Diet

Day	Approximate Calorie Intake	Normal Consumption Rate	Protein	Carbohydrate	Fate
1st Day	1100 kcal	30-50% of normal Daily intake	11%	43%	46%
2nd – 5th Days	700 kcal	10-20% of normal Daily intake	9%	47%	44%

Source: (Fanti, Mishra, Longo, & Brandhorst, 2021; Nuanchankong, Jaroennon, & Manakla, 2024; Rangan, et al., 2022)

In the fasting-mimicking diet (FMD) protocol, approximately 1100 kcal of energy intake is provided on the first day, corresponding to 30–50% of an individual's normal daily energy requirement. On the first day, 11% of the energy comes from protein, 43% from carbohydrates, and 46% from fats. During the following 2nd–5th days, energy intake is restricted to approximately 700 kcal, corresponding to 10–20% of normal daily intake. On these days, the protein proportion is further reduced to 9%, while energy from carbohydrates and fats is adjusted to 47% and 44%, respectively (Fanti, Mishra, Longo, & Brandhorst, 2021; Nuanchankong, Jaroennon, & Manakla, 2024; Rangan, et al., 2022).

- **Protein Restriction**

The diet is implemented with a low-protein approach in order to reduce the activity of IGF-1 and mTOR, which play key roles in growth and aging. Protein intake is set at approximately 30 g/day on Day 1 (11% of 1,100 kcal) and about 16 g/day on Days 2–5 (9% of 700 kcal). The majority of the consumed protein is derived from plant-based sources (Brandhorst, et al., 2015)

Protein Sources: The protein sources consumed should be plant-based. Examples include legumes, lentils, soybeans, cereals or pseudocereals, chia seeds, peanuts, and other seeds such as sunflower seeds, pumpkin seeds, almonds, and cashews (Nuanchankong, Jaroennon, & Manakla, 2024)

- **Carbohydrate Restriction**

OTD programs are formulated as low-carbohydrate diets. In addition, sugar (simple carbohydrates) is restricted, and the diet should be free of added sugars. The carbohydrates consumed should generally be low–glycemic index (GI) and low–glycemic load (GL) foods (Nuanchankong, Jaroennon, & Manakla, 2024). As carbohydrate sources, whole grains, oats, and legumes are recommended (Augusti, et al., 2015)

- **Fat Restriction**

Although the Fasting-Mimicking Diet (FMD) is a protocol in which energy intake is significantly restricted, it is defined as a diet with a high proportion of healthy fats in terms of macronutrient distribution. On the first day of the diet, approximately 46% of total energy intake is derived from fats, while from the second to the fifth day, an average of 44% of energy is provided by fats. Healthy fats, particularly essential fatty acids (EFAs), play a critical role in the diet. Omega-3 and omega-6 fatty acids, which cannot be synthesized by the human body and must be obtained from external sources, play a crucial role in the development of the metabolic effects of the diet (Kaur, Chugh, & Gupta, 2014).

Flaxseed, hemp seed, canola, walnuts, pumpkin seeds, and soybeans are among the foods frequently used in the Fasting-Mimicking Diet (FMD) due to their rich essential fatty acid content. Nuts (such as almonds, walnuts, and hazelnuts) and dark green leafy vegetables (such as kale, Swiss chard, and parsley) also contribute to the diet's nutritional density by providing both healthy fats and additional micronutrients (Nuanchankong, Jaroennon, & Manakla, 2024).

- **Supplements**

During Fasting-Mimicking Diet (FMD) protocols, the use of supplements is an important supportive component to prevent potential deficiencies that may arise from restricted caloric intake and limited dietary variety. Due to the low-energy and plant-based nature of the diet, micronutrient intake may be reduced; therefore, the integration of vitamin and mineral supplementation into the program is recommended. In this context, the use of high-potency multivitamin–mineral supplements is frequently advised to support overall health and prevent deficiencies (Zhang, Chen, Huo, Huang, & Zhao, 2024).

According to protocol recommendations, taking a multivitamin supplement every three days is generally considered sufficient. Since the FMD is entirely plant-based, it may pose a risk for deficiencies in certain micronutrients predominantly found in animal-derived foods, particularly iron and vitamin B12 (Nuanchankong, Jaroennon, & Manakla, 2024).

- **Hydration**

In the Fasting-Mimicking Diet (FMD) protocol, while caloric intake and macronutrients—particularly protein and carbohydrates—are restricted, maintaining adequate hydration is of critical importance. To help manage potential mild side effects associated with the low-calorie nature of the diet, such as dehydration, no restrictions are placed on water intake, and adequate fluid consumption is strongly encouraged (Nuanchankong, Jaroennon, & Manakla, 2024).

Protocol and Implementation of the Fasting-Mimicking Diet

FMD cycles are periodic and short-term nutritional protocols developed to mimic the physiological effects of traditional fasting without completely eliminating food intake. In this approach, fasting periods are applied in regular cycles followed by periods of normal eating (Lin & Gao, 2024).

FMD protocols generally last for 4–5 consecutive days, with the most commonly used duration in humans being a 5-day intervention (Fanti, Mishra, Longo, & Brandhorst, 2021). This cycle is typically repeated no more than once per month. It is reported that the FMD is designed with sufficient flexibility to be implemented, for example, every two weeks or once every few months. To achieve clear clinical benefits, three consecutive FMD cycles are generally recommended. In fasting-mimicking diets, the primary aim of determining duration and frequency is to reduce the challenges associated with long-term restriction while sustainably providing

the metabolic and physiological benefits associated with fasting (Nuanchankong, Jaroennon, & Manakla, 2024).

A five-day FMD cycle is typically followed by an approximately 25-day period of normal eating. During the refeeding phase, individuals return to their usual diet; however, adherence to certain dietary principles is recommended to sustain metabolic benefits. In this phase, the consumption of animal-based foods such as fish, lean meat, and semi-skimmed dairy products is kept to a minimum. Instead, the preference for carbohydrate sources with low glycemic index (GI) and low glycemic load (GL) is emphasized. Additionally, it is recommended that a dietary pattern rich in essential fatty acids be maintained during the refeeding period (Rangan, et al., 2022).

Although FMD programs are generally considered safe, commonly reported side effects include mild to moderate fatigue, headache, dizziness, weakness, nausea, and dehydration (Rangan, et al., 2022; Nuanchankong, Jaroennon, & Manakla, 2024; Fanti, Mishra, Longo, & Brandhorst, 2021).

Comparison of Intermittent Fasting, Time-Restricted Eating, and the Fasting-Mimicking Diet Applications

The Fasting-Mimicking Diet (FMD), Intermittent Fasting (IF), and Time-Restricted Eating (TRE) are three popular dietary interventions that aim to induce fasting physiology in order to promote weight loss and trigger metabolic benefits in overweight or obese individuals. However, they differ in terms of implementation methods, duration, and degree of caloric restriction (Fanti, Mishra, Longo, & Brandhorst, 2021).

In the table below, the Fasting-Mimicking Diet (FMD), Intermittent Fasting (IF), and Time-Restricted Eating (TRE) approaches are compared based on the characteristics reported in the literature.

Table 2 Comparison of the Fasting-Mimicking Diet, Intermittent Fasting, and Time-Restricted Eating Approaches

	Fasting-Mimicking Diet (FMD)	Intermittent Fasting (IF)	Time-Restricted Eating (TRE)
Definition	A periodic, very-low-calorie dietary protocol developed to mimic the physiological effects of fasting while allowing limited food intake; typically plant-based, low in protein and carbohydrates, and relatively high in healthy fats.	A dietary approach characterized by alternating periods of fasting and eating, in which calorie intake is either significantly reduced or eliminated during fasting periods.	A dietary intervention that aims to maintain a consistent daily eating–fasting cycle by restricting food intake to a specific time window during the day.
Duration and Frequency	Applied in periodic cycles. Typically administered once every few weeks; most human studies use a 5-day protocol.	Common protocols include the 5:2 diet (2 fasting days per week, 5 normal eating days) and alternate-day fasting (ADF), where fasting is performed every other day.	Practiced daily. Food intake is usually limited to a 4–12-hour eating window (e.g., 16:8, consisting of 8 hours of eating and 16 hours of fasting).
Caloric Restriction	Energy intake is markedly reduced: approximately 50–55% of daily energy needs on day 1 and around 30–35% on days 2–5.	Varies by protocol. In the 5:2 diet, energy intake on fasting days is reduced to about 25% of daily requirements (approximately 500–600 kcal). In ADF, fasting days involve 0–25% of normal caloric intake.	Does not primarily focus on caloric restriction; benefits arise mainly from meal timing, circadian alignment, and prolonged daily fasting duration rather than explicit calorie reduction.
Weight Loss and Outcomes	After three cycles, a 3.1–3.4% reduction in body weight has been observed in healthy individuals, with greater reductions reported in obese participants. Additionally, fat-adjusted lean body mass has been shown to increase.	When compared with continuous daily calorie restriction (CR), similar weight loss outcomes have been reported, typically ranging between 3% and 7%	Short-term studies have reported approximately 3–5% reductions in body weight, along with decreases in waist circumference and visceral fat.
Adherence and Challenges	Considered relatively safe and feasible when properly applied. Reported adverse effects are	Dropout rates may be higher than continuous calorie restriction. Common complaints include	Long-term adherence may be challenging and may conflict with social life or work schedules.

	generally mild to moderate, including fatigue and weakness. Compared with complete fasting, it reduces the risks of rapid weight loss and malnutrition.	hunger, cold sensation, and low energy levels. In individuals with type 2 diabetes, medical supervision is required due to the risk of hypoglycemia.	Prolongation of the fasting window may increase the risk of skipping breakfast, potentially negatively affecting cardiometabolic health.

Source: (Panda, 2016; Anton , et al., 2018; Welton, et al., 2020)

The primary mechanism underlying these dietary approaches is the restriction of food intake through caloric limitation, exclusion of specific nutrients, or prolonged fasting, thereby eliciting beneficial health effects. Although studies indicate that these diets are effective in promoting weight loss in the short term, long-term success is considered to depend largely on the individual's ability to adhere to the selected dietary plan (Panda, 2016; Anton , et al., 2018; Welton, et al., 2020).

Relationship Between the Fasting-Mimicking Diet and Diseases

Findings from clinical studies conducted in humans indicate that this dietary intervention exerts beneficial effects on health-related parameters in both healthy individuals and those with various pathological conditions. Moreover, adopting the Fasting-Mimicking Diet as a preventive approach may contribute to the reduction and delay of age-related comorbidities (Panebianco, Potenza, & Pazienza, 2017) and support the process commonly referred to as "healthy aging" (Nardon, Venturelli, Ruzzante, Longo, & Bertucco, 2022).

Cycles of the Fasting-Mimicking Diet (FMD) have been suggested to be a safe, practical, and effective strategy for lowering risk factors associated with aging and major age-related conditions, including metabolic syndrome, cancer, diabetes, and cardiovascular diseases (CVD) (Wei, et al., 2017).

Relationship Between Alzheimer's Disease and the Fasting-Mimicking Diet

The aging population poses significant social, medical, and economic challenges on a global scale due to the high prevalence of chronic diseases in older age groups. Among these chronic conditions, Alzheimer's disease (AD) stands out as both the most common form of dementia and the most prevalent neurodegenerative disorder. AD is a degenerative central nervous system (CNS) disease characterized by progressive impairments in memory, learning, and executive functions, substantially affecting individuals' abilities to perform daily living activities. According to the World Alzheimer Report 2021, AD affects more than 50 million individuals worldwide, and its prevalence is rapidly increasing in parallel with population aging. In addition to reducing quality of life, AD imposes a substantial economic burden on families and healthcare systems (Śliwińska & Jeziorek, 2021).

It is reported that approximately two-fifths of all Alzheimer's disease cases worldwide are potentially associated with modifiable risk factors. This indicates that targeting the underlying risk factors that predispose individuals to cognitive impairment may reduce the incidence of Alzheimer's disease and offer a novel approach to disease prevention. According to the Lancet Commission report, modifying a total of 12 lifestyle and environmental risk factors—including improvements in dietary patterns—could prevent or delay approximately 40% of Alzheimer's disease cases (Livingston, et al., 2020). These findings suggest that preventive interventions focusing particularly on lifestyle modifications could substantially reduce the societal burden of the disease (Wen, et al., 2025).

Nutrition is considered one of the most important modifiable environmental factors in relation to Alzheimer's disease, and a growing body of evidence indicates that dietary patterns may modulate neurodegenerative processes through effects on the gut microbiota, microglial activation, and peripheral inflammatory pathways (Madore, Yin, Leibowitz, & Butovsky, 2020).

It has been reported that the Mediterranean diet (MD), which is rich in antioxidants, dietary fiber, and omega-3 polyunsaturated fatty acids, as well as specific components such as glutathione, polyphenols, curcumin, coenzyme Q10, vitamins B6 and B12, folic acid, unsaturated fatty acids, lecithin, urolithin A (UA), caffeine, and certain probiotics, may exert beneficial effects on the course of Alzheimer's disease. In contrast, diets rich in saturated fatty acids and branched-chain amino acids have been shown to accelerate cognitive decline. Therefore, nutritional interventions aimed at reducing the risk of dementia are recommended to be initiated as early as possible. Recent studies indicate that high-quality dietary patterns such as the Mediterranean diet, DASH diet, and particularly the MIND diet may provide protective effects against Alzheimer's disease; it is suggested that the neuroprotective components of these diets act synergistically to support cognitive health (Wen, et al., 2025; van den Brink, Brouwer-Brolsma, Berendsen, & van de Rest, 2019).

Dietary restriction (DR) is proposed as a reliable and effective intervention that has been demonstrated to extend lifespan and improve health status across various model organisms. In recent years, Periodic Dietary Restriction—particularly the Fasting-Mimicking Diet (FMD)—has attracted increasing attention and has emerged among DR regimens due to its potential preventive and therapeutic effects. The FMD offers a structured dietary plan involving specific meals and caloric restriction, thereby providing a more feasible and sustainable approach compared with prolonged fasting protocols. Considering these characteristics, the Fasting-Mimicking Diet is regarded as a potential preventive and therapeutic strategy for Alzheimer's disease (Boccardi, Pigliautile, Guazzarini, & Mecocci, 2023).

In one study, the effectiveness of periodic dietary restriction as a potential treatment for Alzheimer's disease was investigated,

and the effects of Fasting-Mimicking Diet (FMD) cycles on Alzheimer's disease were examined in both preclinical mouse models and in patients diagnosed with amnestic mild cognitive impairment (aMCI) or mild Alzheimer's disease. The researchers applied FMD cycles, a low-calorie dietary regimen, in two different transgenic mouse models. The FMD was observed to significantly improve cognitive function in mice and to attenuate hallmark Alzheimer's disease pathologies, including amyloid-β and tau pathology. Mechanistically, the diet was found to reduce neuroinflammation and levels of NADPH oxidase (Nox2), which are closely associated with neurotoxicity. These findings were further confirmed in mouse models with Nox2 gene knockout, demonstrating that Nox2 inhibition plays a key role in reversing memory impairments (Rangan, et al., 2022).

Relationship Between Cardiovascular Diseases and the Fasting-Mimicking Diet

According to the World Health Organization, cardiovascular diseases (CVD) are recognized as the leading cause of death worldwide. Four out of five deaths related to CVD occur as a result of heart attacks and strokes. Moreover, one-third of CVD-related deaths occur in individuals under the age of 70 (Virani, et al., 2020).

Early identification and management of cardiovascular risk factors are of critical importance for the prevention of CVD-related mortality. Risk factors associated with cardiovascular diseases include hypertension, diabetes and hyperglycemia, dyslipidemia, overweight, and obesity (Crupi, Haase, Brandhorst, & Longo, 2020).

Nutrition is considered to play a central role in the prevention of cardiovascular diseases (CVD). Dietary patterns influence CVD risk by affecting aging, adiposity, glycemic control, blood pressure, cholesterol levels, and inflammation (Crupi et al., 2020). To reduce CVD risk, plant-based dietary patterns—particularly those rich in

minimally processed foods, vegetables, and fruits—are recommended. Diets such as the Mediterranean, DASH, and vegetarian diets have been shown to reduce CVD risk, whereas dietary habits rich in ultra-processed foods, meat, salt, sugar, and saturated fats are advised against. General dietary guidelines emphasize maintaining a healthy body weight, preferring whole grains over refined carbohydrates, selecting healthy protein sources (especially plant-based proteins, fish, seafood, and low-fat or fat-free dairy products), and minimizing the intake of added sugars and salt (Marques-Vidal, et al., 2025).

In addition to total caloric intake and macronutrient composition, meal timing and meal frequency have also been shown to influence CVD risk. Fasting-based dietary approaches—including intermittent fasting (IF), time-restricted eating (TRE), prolonged fasting, and the fasting-mimicking diet (FMD)—have demonstrated promising results in reducing CVD risk factors. These fasting regimens are generally associated with reductions in cardiovascular risk, often independent of weight loss, although weight loss frequently accompanies these interventions. However, difficulties in long-term adherence may limit the overall effectiveness of these approaches (Crupi, Haase, Brandhorst, & Longo, 2020; Marques-Vidal, et al., 2025).

Nutrition is stated to play a central role in the prevention of cardiovascular diseases (CVD). Nutrition influences CVD risk by affecting aging, adiposity, glycemic control, blood pressure, cholesterol levels, and inflammation (Crupi et al., 2020). To reduce CVD risk, plant-based dietary patterns—particularly those rich in minimally processed foods, vegetables, and fruits—are recommended. Dietary models such as the Mediterranean, DASH, and vegetarian diets have been shown to reduce CVD risk, whereas dietary habits high in ultra-processed foods, meat, salt, sugar, and saturated fat should be avoided. General dietary guidelines

emphasize maintaining a healthy body weight, choosing whole grains instead of refined carbohydrates, selecting healthy protein sources (especially plant-based proteins, fish, seafood, and low-fat or fat-free dairy products), and minimizing added sugars and salt intake (Marques-Vidal, et al., 2025).

It has also been observed that nutrition affects CVD risk not only through caloric intake and macronutrient composition but also through meal timing and eating intervals. Fasting-related approaches such as intermittent fasting (IF), time-restricted eating (TRE), prolonged fasting, and fasting-mimicking diets (FMD) have shown promising results in reducing CVD risk factors. These fasting regimens are generally associated with reductions in cardiovascular risk independent of weight loss, as well as with weight loss itself. However, difficulties in long-term adherence may limit the effectiveness of these approaches (Crupi, Haase, Brandhorst, & Longo, 2020; Marques-Vidal, et al., 2025).

A study was conducted to evaluate the effects of the fasting-mimicking diet (FMD) on risk factors associated with aging, diabetes, cancer, and cardiovascular diseases. This randomized controlled trial was carried out in the United States with 100 participants aged between 20 and 70 years. The study was conducted between April 2013 and July 2015. Participants were instructed to continue their usual diets for a period of three months in order to estimate changes not attributable to dietary intervention. Measurements were taken at the end of this three-month period. Following baseline assessments, 43 participants from the control group were enrolled in the FMD program. The FMD intervention was implemented for five consecutive days per month over a three-month period. Participants in the intervention group were randomized to undergo five-day FMD cycles each month for three months. The FMD consisted of a low-calorie (~3000–4600 kJ/day), low-sugar, and low-protein, but high–unsaturated fat, plant-based

diet. All foods to be consumed during the FMD were individually packaged and provided to participants to ensure compliance. After completing each five-day FMD cycle, participants were instructed to return to their usual diets. The study demonstrated that monthly five-day FMD cycles were safe, feasible, and effective in reducing risk factors associated with aging and age-related diseases, including body fat, blood pressure, and insulin-like growth factor 1 (IGF-1). These effects were more pronounced in individuals who already exhibited elevated risk factors. However, the authors emphasized that larger-scale studies are needed to confirm the long-term efficacy of FMD in individuals with diagnosed diseases such as cardiovascular disease and diabetes (Wei, et al., 2017).

Another clinical study aimed to compare the effectiveness of a four-month continuous Mediterranean Diet (MD) with four cycles of a Fasting-Mimicking Diet (FMD) applied for only five days per month in individuals with obesity/overweight and hypertension, and to evaluate changes in biochemical markers and body composition from baseline to the end of the study. The study was conducted between September 2018 and May 2019. Participants included men and women aged 35–75 years with a body mass index (BMI) ≥ 28, who had been diagnosed with hypertension, endothelial dysfunction, and reduced small resistance artery compliance. In this randomized study, 44 participants were assigned to the FMD group and 40 participants to the Mediterranean diet group. The intervention lasted a total of four months. The FMD group followed the FMD protocol for five consecutive days per month throughout the four-month period. The Mediterranean diet group was instructed to adhere daily for four months to a Mediterranean dietary pattern rich in foods such as grains, legumes, vegetables, fruits, fish, and fats derived from olive oil. At the end of the four-month intervention, both diets resulted in similar reductions in body weight, waist circumference, BMI, body fat mass, total cholesterol, and leptin levels. While the

FMD reduced HbA1c and insulin-like growth factor 1 (IGF-1) levels, the Mediterranean diet decreased glucose, HOMA-IR, and HDL-C levels. In terms of the primary outcome of endothelial function, only the FMD led to a reduction in the Reactive Hyperemia Index (RHI). Both dietary interventions improved the PULS cardiac test score, which assesses the risk of myocardial infarction and stroke. Differences between the two dietary approaches became apparent at the three-month follow-up: no loss of fat-free mass (FFM) was observed in the FMD group, whereas a significant loss of FFM and leg muscle mass was detected in the Mediterranean diet group. Additionally, reductions in insulin, HbA1c, and HOMA-IR levels persisted throughout the follow-up period in participants following the FMD, while this effect was not observed in the Mediterranean diet group. In conclusion, both the FMD and the Mediterranean diet were effective in reducing cardiometabolic risk; however, the periodic implementation of the FMD appeared to offer a potential advantage in preserving fat-free body mass and providing more sustained improvements in insulin and glucose metabolism (Mishra, et al., 2023).

In a study conducted in Switzerland, the aim was to compare the physiological, metabolic, and molecular effects of fasting-mimicking diets (FMDs) according to their macronutrient composition. This was a randomized, three-arm clinical trial. Participants consisted of 46 generally healthy men and women aged 18–65 years with a body mass index (BMI) < 35 kg/m². The control group continued their weight-maintaining habitual diet for seven days. Participants in the low-protein fasting-mimicking diet (LP-FMD) group followed a low-protein, high-fat dietary pattern, in which 10% of daily energy intake was derived from protein, 45% from fat, and 45% from carbohydrates. The third arm of the study, the high-protein fasting-mimicking diet (HP-FMD) group, followed a plant-based FMD with a high-protein, low-fat composition,

providing 30% of energy from protein, 25% from fat, and 45% from carbohydrates. Evaluation of the study outcomes demonstrated that a seven-day fasting-mimicking diet provided cardiometabolic health benefits regardless of macronutrient composition (LP-FMD or HP-FMD); however, the HP-FMD conferred additional advantages. Both FMD interventions resulted in significant reductions in body weight and fat mass compared with the control group. Additionally, both FMDs reduced fasting plasma glucose by approximately 10% and decreased insulin-like growth factor 1 (IGF-1), a growth factor associated with aging and disease, by approximately 35%. Only the HP-FMD led to a reduction in visceral fat mass, which is closely associated with metabolic risk, compared with the control group. Furthermore, only the HP-FMD increased heart rate variability, a dynamic marker of cardiovascular health, showed a trend toward reduced diastolic blood pressure, and decreased circulating triglycerides and saturated fatty acids. Serum HDL concentrations decreased similarly in both FMD groups. These findings suggest that caloric restriction is a key determinant of the cardiometabolic benefits and autophagy induction associated with the fasting-mimicking diet; however, macronutrient composition—particularly a high-protein, low-fat formulation—may provide additional benefits for specific health targets such as body composition, lipid profiles, heart rate variability, and gut microbiota (Burns, et al., 2025).

A study conducted in mice aimed to investigate whether fasting-mimicking diet (FMD) cycles enhance the efficacy of immune checkpoint inhibitors (ICIs) used in cancer therapy, while simultaneously reducing ICI-associated cardiovascular effects. Immune checkpoint inhibitors are immunotherapeutic agents that reactivate the immune system’s capacity to recognize and eliminate tumor cells. In this study, the fasting-mimicking diet (FMD) cycle was designed as four consecutive days of FMD followed by three

days of refeeding with a standard diet. FMD cycles were shown to prevent or reverse cardiovascular adverse effects induced by immune checkpoint inhibitors, including cardiac fibrosis, necrosis, and hypertrophy. The cardioprotective effects of FMD were associated with a significant reduction in CD3+ and CD8+ T-cell infiltration in cardiac tissue, as well as marked decreases in oxidative stress and inflammatory markers at both systemic and myocardial levels. These findings suggest that FMD cycles may be incorporated into clinical strategies to enhance the anticancer efficacy of immunotherapy while reducing life-threatening cardiovascular side effects (Cortellino, et al., 2023).

In conclusion, fasting-mimicking diet cycles demonstrate substantial potential in reducing risk factors for cardiovascular disease (CVD), which remains the leading cause of mortality worldwide. Through mechanisms based on macronutrient composition and periodic dietary restriction, FMD may improve metabolic risk factors and contribute to the preservation of cardiovascular health.

The Relationship Between Obesity, Metabolic Syndrome, and the Fasting-Mimicking Diet

Obesity is defined as a multifactorial and complex disease characterized by an excess accumulation of body fat resulting from a long-term imbalance in energy homeostasis (Bouchard, 2021).

Fundamentally, obesity is triggered by the interaction between genetic predisposition and environmental factors. According to the literature, obesity arises from a disruption in the central regulation of body weight. Beginning in early childhood, it becomes a lifelong burden. According to 2020 data from the World Health Organization (WHO), it is estimated that 12% of children aged 7–9 years in 33 countries within the European Region are classified as obese. Furthermore, the WHO estimates that the

number of overweight or obese children under the age of five worldwide is approximately 39 million (Faccioli, Poitou, Clément, & Dubern, 2023).

Obesity is a disease that affects multiple systems in the human body, including the cardiovascular, respiratory, endocrine, and digestive systems, and predisposes individuals to numerous serious health conditions. Cardiovascular diseases, hypertension, diabetes mellitus, hypercholesterolemia, respiratory disorders, joint diseases, menstrual irregularities, infertility, erectile dysfunction, gallbladder diseases, gallstone formation, and certain types of cancer are among the conditions directly associated with obesity (Green, Arora, & Prakash, 2020; Keirns & Hawkins, 2019).

In the nutritional management of obesity, current approaches focus not solely on calorie counting or individual self-control but rather on the effects of food quality on physiological processes. Studies indicate that the consumption of fruits, non-starchy vegetables, nuts, yogurt, and fish has a protective effect against weight gain, whereas refined grains, starchy foods such as potatoes, sugar-sweetened beverages, and processed meats directly contribute to the development of obesity. Therefore, the most effective strategy in combating obesity is not to focus exclusively on total fat or calorie intake, but to sustainably adopt a dietary pattern rich in nutrient-dense healthy foods while restricting refined carbohydrates and added sugars (Mozaffarian, 2016).

Small adjustments in diet or lifestyle generally lead to mild metabolic changes that can be detected after a few months. These metabolic alterations largely depend on lifestyle modifications. However, in dietary interventions such as the fasting-mimicking diet (FMD), variations in human metabolism occur much more rapidly, and measurable changes may be observed within a few days (Hof & Wall, 2024).

In a study conducted to explore the direct effects of the fasting-mimicking diet on human physiology, the effects of the FMD on human salivary metabolic profiles and body weight were investigated. By monitoring key metabolites associated with changes in energy metabolism in response to the FMD—such as acetone and tricarboxylic acid (TCA) cycle–related metabolites including L-glutamine, L-glutamate, and succinic acid—insights into human energy metabolism were obtained, and evaluations were conducted to estimate fat loss as a component of sustained weight loss. The study was carried out with six adult participants with normal or elevated body mass index (BMI). Participants followed the reSET diet, an FMD designed to mimic fasting effects through low-calorie, plant-based meals, for five days. Compared with a regular diet, this dietary regimen was relatively low in carbohydrates and protein but higher in healthy fats. The findings indicated that the FMD resulted in weight loss and influenced multiple metabolic changes in the body. The FMD increased acetone levels, a by-product of ketogenesis, indicating a metabolic shift from glucose utilization to fat oxidation (ketosis) as the primary energy source. In addition to reductions in body weight, the FMD was also associated with decreases in body fat and blood pressure. These findings suggest that the FMD may represent an effective approach with the potential to promote weight and fat loss (Hof & Wall, 2024).

Obesity is also associated with metabolic syndrome (MetS), which is characterized by the clustering of multiple risk factors related to obesity. With the global increase in waist circumference and body mass index values, modern societies are considered to be at significant risk for obesity-related metabolic disorders. The diagnostic components of metabolic syndrome include:

- Abdominal obesity
- Hypertension

- Hypertriglyceridemia
- Low HDL cholesterol
- Hyperglycemia

According to the definition of the World Health Organization (WHO), the diagnosis of metabolic syndrome requires the presence of insulin resistance in addition to at least two of the aforementioned criteria. Current epidemiological data indicate that metabolic syndrome affects approximately one-quarter of the global adult population, highlighting the substantial burden it imposes on both individual health and healthcare systems (Green, Arora, & Prakash, 2020).

A study was conducted among patients with prostate cancer who also exhibited features of metabolic syndrome, aiming to investigate the effects of the fasting-mimicking diet on metabolic health factors. These patients followed a periodic FMD protocol consisting of four-day cycles administered once per month for three months. Baseline measurements of body weight, waist circumference, blood pressure, and selected laboratory parameters were compared with data obtained three months after the intervention. The results demonstrated that the FMD cycles were safely implemented without observed toxicity and achieved a high adherence rate of 83%. Additionally, the intervention resulted in weight loss, reductions in waist circumference, and significant decreases in both systolic and diastolic blood pressure. Furthermore, subgroup analyses indicated that the FMD exerted more favorable effects particularly in patients classified as “at risk” compared with those who had normal metabolic risk factor values (Fay-Watt, et al., 2023).

Another study aimed to determine the effects of the fasting-mimicking diet compared with continuous energy restriction on anthropometric measurements, body composition, glucose

metabolism, and appetite-regulating hormones (leptin, neuropeptide Y, and total ghrelin). The study was conducted as a randomized controlled parallel trial comparing the effects of the fasting-mimicking diet and continuous energy restriction in metabolically healthy women aged 18–55 years with a BMI between 30 and 35 kg/m² who had been diagnosed with obesity. The study was carried out between August 2019 and March 2020. In this two-group study, the first group followed a continuous energy restriction diet with an average daily energy deficit of 500 kcal. Participants in the second group followed the fasting-mimicking diet in two cycles over a two-month period, with each cycle implemented approximately every 25 days. The results showed that although there was no significant difference between the FMD and continuous energy restriction in terms of weight loss, the FMD was more effective in preserving muscle mass and basal metabolic rate. In contrast, continuous energy restriction was associated with significant increases in total ghrelin and neuropeptide Y (NPY), hormones known to stimulate appetite. These findings suggest that the FMD may have the potential to better regulate appetite-related hormones and minimize muscle loss compared with continuous energy restriction (Sadeghian, Hosseini, Javid, Angali, & Mashkournia, 2021).

The Relationship Between Cancer Diseases and the Fasting-Mimicking Diet

Cancer is a large and heterogeneous group of malignant tumors characterized by abnormal cell growth and the potential to spread from their tissue of origin to other parts of the body. In 2020, cancer caused approximately 600,000 deaths in the United States (USA) alone, highlighting its significant impact on public health. It is the second leading cause of death after cardiovascular diseases (Hanahan & Weinberg, 2011).

A large proportion of cancers arise from factors that can be controlled by individuals and governments. The most important causes of cancer include tobacco use, excessive alcohol consumption, obesity, physical inactivity, infectious agents, and sun exposure. Diets low in fruits and vegetables are also considered among the causes of cancer. Most of the factors associated with increased cancer risk are therefore modifiable (Schwartz, 2024).

In patients with cancer, nutrition is regarded as a critical area of management due to the negative effects of both the disease itself and treatment modalities such as surgery, chemotherapy, radiotherapy, or immunotherapy on metabolism, appetite, digestion, and nutrient absorption (Pio, Senent, Tavira, & Ajona, 2025). For this reason, nutritional assessment and appropriate medical nutrition support should not be considered merely a complementary element of cancer care, but rather an integral component of a multidisciplinary treatment approach (Muscaritoli, et al., 2021).

Low-energy dietary approaches such as calorie restriction and fasting, which are used as nutritional therapy strategies in cancer, have been shown to exert beneficial effects by preventing the development of malignant tumors and enhancing the efficacy of cancer treatments. However, long-term calorie restriction may lead to both beneficial and potentially harmful outcomes and is associated with significant adherence challenges in terms of feasibility. In contrast, periodic fasting, fasting-mimicking diets, and dietary restriction approaches that do not require a marked reduction in caloric intake stand out as more sustainable and widely applicable interventions for the prevention and treatment of cancer (Brandhorst & Longo, 2016).

A study was conducted to evaluate the effects of the Fasting-Mimicking Diet (FMD) on tumor-associated macrophages (TAMs) within the tumor microenvironment in oral cancer treated with gefitinib (EGFR-TKI), and to enhance the therapeutic efficacy of

gefitinib. The research was carried out using in-vitro cell cultures, animal models, and computer-based data analyses. EGFR-TKIs (Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors) are molecularly targeted therapies used in cancer treatment. This study demonstrated that the Fasting-Mimicking Diet (FMD) enhances the effectiveness of gefitinib, a targeted agent used in oral cancer therapy, by directly weakening cancer cells. Under normal conditions, gefitinib treatment induces STAT3 activation in cancer cells, leading to the secretion of CCL2, which promotes the recruitment of drug-resistant macrophages into the tumor microenvironment. The application of FMD was shown to interrupt this process at its earliest stage, reducing macrophage infiltration into the tumor site by approximately 65% and suppressing cancer cell proliferation by about 74%. As a result, FMD was found to disrupt the defense mechanisms developed by cancer cells against the drug, thereby significantly improving treatment success (Wang, et al., 2025).

Studies conducted in laboratory animals and humans have demonstrated that fasting—whether continuous (calorie restriction) or intermittent (via various fasting cycles)—improves health and extends lifespan. In recent years, fasting and fasting-like conditions, including Fasting-Mimicking Diets (FMDs), have increasingly become a focus of cancer research. Preclinical and clinical evidence indicates that fasting or fasting-mimicking conditions have the potential to enhance the efficacy of antitumor therapies and are being considered as a novel strategy in cancer treatment (Pio, Senent, Tavira, & Ajona, 2025).

Nutrient deprivation sensitizes cancer cells to the antitumor activity of standard therapies, while simultaneously protecting normal cells against stress and promoting tissue regeneration, thereby mitigating toxic side effects associated with cancer treatments. Fasting-like dietary interventions not only modulate

tumor metabolism but also enhance antitumor immune responses (Liu, et al., 2023; Pio, Senent, Tavira, & Ajona, 2025).

A study was conducted to investigate the effects of the Fasting-Mimicking Diet (FMD) during neoadjuvant chemotherapy in patients with breast cancer. Based on preclinical evidence suggesting that FMD may reduce chemotherapy-related side effects and toxicity while increasing cancer cell sensitivity to chemotherapy, a randomized controlled trial was designed to evaluate the effects of FMD on toxicity and metabolic changes. A total of 44 newly diagnosed HER2-negative breast cancer patients aged 18 years and older were enrolled from Taleghani Hospital in Tehran between February 2022 and January 2024, with 22 patients assigned to each group. Patients were allocated either to an FMD group, in which the diet was applied for three days prior to each chemotherapy cycle and on the day of chemotherapy, or to a control group that continued their usual diet. This regimen was repeated every three weeks for a total of eight cycles. The effects of FMD were evaluated through metabolic parameters (insulin, glucose, and IGF-1), hematological parameters (erythrocyte, platelet, and leukocyte counts), and inflammatory markers (high-sensitivity C-reactive protein, hs-CRP). The results indicated that cyclic FMD was better tolerated in breast cancer patients receiving neoadjuvant chemotherapy, allowing patients to complete their treatment protocols. Compared with the control group, the incidence of vomiting and neutropenia was lower, while erythrocyte and neutrophil counts were higher in the FMD group. These findings suggest that cyclic FMD is safe and feasible, may enhance the effectiveness of chemotherapy depending on tumor response, and can reduce gastrointestinal and hematological toxicities (Bahrami, Haghighi, Moghani, Khodakarim, & Hejazi, 2024).

Another study investigated the safety, feasibility, and biological effects of cyclic FMD when combined with standard

antitumor therapies in cancer patients, focusing on systemic metabolism and antitumor immunity. The study aimed to determine whether FMD could maintain the incidence of severe (Grade 3/4) adverse events below a predefined threshold of 20%, and whether the metabolic and immunomodulatory changes observed in preclinical models could be reproduced in humans. Conducted in a total of 101 patients, the study demonstrated that FMD was safe and feasible, with FMD-associated Grade 3/4 adverse events remaining below the predefined threshold. Biologically, FMD consistently reduced blood glucose and growth factor levels, particularly IGF-1, confirming metabolic changes that mediate preclinical anticancer effects and indicating that FMD reshapes antitumor immunity (Zhong, et al., 2023; Vernieri, et al., 2022).

Addressing treatment resistance and cancer stem cell (CSC)-mediated tumor regrowth—key contributors to the lethality of metastatic tumors—another study examined the effects of the Fasting-Mimicking Diet (FMD) on Triple-Negative Breast Cancer (TNBC) cells. The study investigated whether FMD reduces glucose-dependent Protein Kinase A (PKA) signaling to decrease TNBC stem cells, and whether it activates "fasting escape pathways" in differentiated cancer cells that could be pharmacologically targeted. The findings showed that FMD lowers glucose levels, downregulates PKA signaling, and thereby reduces TNBC stem cells, stemness markers, and stem cell numbers. Additionally, in differentiated cancer cells, FMD activated survival and growth pathways such as PI3K/AKT, mTOR, and CDK4/6. When these fasting escape pathways were targeted with agents such as Pictilisib, Ipatasertib, and Rapamycin in combination with FMD, tumor regression with low toxicity and prolonged progression-free survival were observed in mice. Clinical data were consistent with these findings, revealing that metastatic TNBC patients with lower

baseline blood glucose levels exhibited longer survival (Salvadori, et al., 2021).

In conclusion, the Fasting-Mimicking Diet represents a feasible and low-cost strategy that provides a strong foundation for advanced clinical studies, as it enhances the efficacy of anticancer therapies while protecting normal cells from treatment-related toxicity. However, the studies also emphasize that fasting or FMD should be applied with caution in clinical practice to avoid adverse effects, particularly in cancer patients who are at risk of malnutrition.

The Relationship Between Diabetic Diseases and Fasting-Mimicking Diets

Diabetes is defined as a heterogeneous group of metabolic disorders characterized by elevated blood glucose concentrations (hyperglycemia) (Harreiter & Roden, 2023). This disease and its associated complications are high-morbidity metabolic conditions that significantly impair health and quality of life. The pathophysiology of diabetes involves β-cell destruction, impaired insulin secretion, and loss of insulin sensitivity in peripheral tissues, driven by factors such as genetic predisposition, viral infections, unhealthy lifestyle, and other physical or chemical insults; these processes ultimately result in elevated blood glucose levels (Sun, et al., 2021). Severe hyperglycemia manifests with classical symptoms, including polyuria, polydipsia, fatigue, unexplained weight loss, visual disturbances, and increased susceptibility to infections. Chronic hyperglycemia, through impaired insulin secretion and/or action, is associated with long-term damage and dysfunction in various tissues and organs, including the eyes, kidneys, nerves, heart, and blood vessels (Harreiter & Roden, 2023).

Diabetes is classified into four main categories: Type 1 Diabetes (T1D), Type 2 Diabetes (T2D), specific diabetes types, and gestational diabetes. While treatments such as oral hypoglycemic

agents and insulin injections provide temporary glycemic control, they do not fully reverse the disease or its complications. Therefore, effective diabetes management strategies include smoking cessation, regular physical activity, maintenance of a healthy body weight, and notably, healthy nutrition. Nutritional interventions include calorie restriction, consumption of low glycemic index diets, regular intake of minimally processed whole grains, nuts, legumes, vegetables, and other nutrient-dense foods, as well as increased dietary fiber intake (Sun, et al., 2021; Ojo, 2021; Reynolds & Mann, 2022).

In addition to diet composition, various types of low-energy diets are considered appropriate for weight loss, provided they are sustainable and feasible for patients (Reynolds & Mann, 2022). Among energy restriction methods, fasting has been applied in individuals with diabetes. According to the literature, fasting can provide both beneficial metabolic effects and potential risks. Fasting interventions aimed at supporting diabetes treatment have the potential to increase insulin sensitivity, reduce endogenous glucose production, and lower body weight (Herz, et al., 2023). Fasting periods are characterized by elevated circulating ketone bodies, while fatty acid, amino acid, glucose, and insulin levels remain low. This metabolic state improves lipid metabolism. Additionally, fasting triggers adaptive cellular responses that activate AMP-activated protein kinase (AMPK), enhancing mitochondrial function and promoting autophagy. Moreover, fasting exhibits systemic anti-inflammatory effects (Hardie, 2015).

Fasting reduces body weight and body mass index (BMI) and improves quality of life in individuals with diabetes. It is considered a particularly acceptable approach for the prevention and treatment of T2D and prediabetes. Studies have shown that fasting lowers fasting insulin levels, improves insulin sensitivity, and enhances glucose tolerance in prediabetic and obese individuals. Furthermore, intermittent or continuous caloric restriction has been

shown to contribute to reductions in HbA1c levels in T2D patients. However, in T1D, uncontrolled fasting may lead to hyperglycemia and diabetic ketoacidosis (DKA) due to prolonged glycogen depletion, increased gluconeogenesis, and ketogenesis. Therefore, safe fasting in T1D requires individualized insulin adjustments under medical supervision. Consequently, while fasting can be considered a supportive approach to improve metabolic markers and body composition in both T1D and T2D, it must be performed under medical supervision due to the risks of hypoglycemia and DKA, especially in T1D (Herz, et al., 2023).

Since fasting-mimicking diets (FMDs) designed to replicate the physiological effects of water-only fasting had not previously been applied in primary care for T2D treatment, a study was conducted to address this question. Focusing on lifestyle modification, the first-line treatment for T2D, the study aimed to evaluate the effects of a 5-day periodic fasting-mimicking diet on metabolic control. Conducted at Leiden University Medical Center in the Netherlands, the study included 100 T2D individuals aged 18–75 years with BMI ≥27 kg/m². Participants were randomized into control and FMD groups. The FMD group received 12 cycles of a 5-day fasting-mimicking diet in addition to standard care provided by general practitioners, while the control group received only standard care. Standard care included clinical and biochemical assessments, lifestyle counseling, and medication adjustments every three months. The FMD group demonstrated a significant reduction in the need for glucose-lowering medications. Correspondingly, HbA1c levels improved compared to the control group. Additionally, the FMD program led to significant reductions in body weight, BMI, waist circumference, and body fat percentage, while improving insulin resistance. These results suggest that FMD could be a safe and effective therapeutic option in routine clinical practice. Further research in T2D patients examined lifestyle changes initiated

by participants during the 5-day FMD program, particularly regarding diet quality and physical activity. Quantitative analyses indicated that FMD did not affect diet quality between fasting periods but increased total weekly physical activity. Qualitative analyses revealed that participants perceived FMD as a motivator for small lifestyle changes, reporting spontaneous improvements in both diet quality and physical activity (Burg, et al., 2024; Burg, et al., 2024).

Cheng et al. (2017) investigated whether periodic FMD cycles could regulate β-cell regeneration and promote insulin secretion and glucose homeostasis in both T1D and T2D. This multi-stage study, conducted using diabetic mouse models and human pancreatic islets, aimed to explore β-cell regeneration with the potential to reverse diabetes. Results showed that periodic FMD cycles reversed β-cell deficiency in both T1D and T2D mouse models, restoring insulin secretion and glucose homeostasis. In T2D mice, FMD cycles significantly reduced blood glucose levels to near-normal values and reversed the decline in insulin secretion. These findings suggest that FMD may act as a potential intervention to promote β-cell regeneration in T1D and T2D and reprogram pancreatic cells to restore insulin production (Cheng, et al., 2017).

The effects of FMD on myocardial triglyceride content (MTGC) in T2D patients have also been investigated. The primary hypothesis was that FMD could reduce MTGC levels. Patients aged 18–75 years with BMI ≥27 kg/m² were randomized to receive either 12 months of FMD in addition to standard care or standard care alone. Results demonstrated a significant reduction in MTGC after 12 months in the FMD group, indicating a beneficial effect on cardiac metabolism and suggesting FMD as a strategy to reduce cardiovascular risk in T2D patients (Roos, et al., 2025).

Tang and Lin (2020) conducted a 4-month randomized controlled trial to examine the effects of FMD and specific meal

replacements on glycemic control in T2D patients with BMI ≥28 kg/m². Participants were randomly assigned to a test group consuming low-energy FMD meal replacements or a control group consuming standard adult calorie meals. At the end of 4 months, the test group demonstrated significant improvements in metabolic parameters, including fasting plasma glucose, 2-hour postprandial glucose, and HbA1c, compared to the control group. Additionally, the test group showed reductions in BMI, waist circumference, blood pressure, and lipid levels (triglycerides, cholesterol, LDL), while HDL levels were increased. The study concluded that FMD with specific meal replacements is a safe and effective intervention for weight loss, improvement of metabolic syndrome, and stable glycemic control in T2D patients (Tang & Lin, 2020).

Clinically, periodic FMD improves glycemic control, enhances biochemical and physiological well-being, and reduces cardiovascular risk; therefore, it should be implemented under medical supervision in patients with diabetes.

Conlusion

Changes in contemporary living conditions; excessive nutrition, increased total caloric intake, and irregularities in meal timing lead to the widespread prevalence of obesity and metabolic health problems. This situation directs individuals toward alternative dietary approaches that may provide health benefits. Therefore, popular diet models aimed at combating excessive nutrition have come to the forefront. Popular diet models are increasing day by day, and strategies are being developed in the form of calorie restriction and fasting. Among the newly developed methods, the FMD practice stands out as a metabolic intervention that provides “repair and cleansing” for the body without requiring complete abstinence from food. Studies have shown that FMD improves metabolic health by causing reductions in fasting blood glucose and

insulin resistance levels and increases in ketone levels. In addition, reductions occur in body weight, waist circumference, and visceral fat mass, and positive health effects are observed in total cholesterol, LDL, and inflammatory markers. As a result, the literature review shows that FMD cycles are a safe and effective approach in reducing disease-related risk factors. However, among the common side effects of FMD programs are mild to moderate fatigue, headache, dizziness, weakness, nausea, and dehydration, and there are no findings regarding long-term health outcomes.

Kaynakça

Alirezaei, M., Kemball, C. C., Flynn, C. T., Wood, M. R., Whitton, J. L., & Kiosses, W. B. (2010). Short-term fasting induces profound neuronal autophagy. *Autophagy, 6(6)*, 702-710. doi:https://doi.org/10.4161/auto.6.6.12376

Anic, K., Schmidt, M. W., Furtado, L., Weidenbach, L., Battista, M. J., Schmidt, M., . . . Hasenburg, A. (2022). Intermittent Fasting-Short- and Long-Term Quality of Life, Fatigue, and Safety in Healthy Volunteers: A Prospective, Clinical Trial. *Nutrients*, 14(19). doi:https://doi.org/10.3390/nu14194216

Anton , S., Moehl, K., Donahoo, W., Marosi, K., Lee, S., 3rd, A., . . . Mattson, M. (2018). Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity (Silver Spring), 26(2)*, 254-268. doi:https://doi.org/10.1002/oby.22065

Augusti, L., Kendall, C., Jenkins, D., Willett, W., Astrup, A., Barcla, A., . . . Poli, B. (2015). Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). *Nutrition, metabolism, and cardiovascular diseases, 25(9)*, 795-815. doi:https://doi.org/10.1016/j.numecd.2015.05.005

Bahrami, A., Haghighi, S., Moghani, M. M., Khodakarim, N., & Hejazi, E. (2024). Fasting mimicking diet during neo-

adjuvant chemotherapy in breast cancer patients: a randomized controlled trial study. *Frontiers in nutrition*, 11. doi:https://doi.org/10.3389/fnut.2024.1483707

Bekar, C., & Gerboğa, R. (2023). Aralıklı açlığın ağırlık kaybı ve kardiyometabolik sağlık üzerine etkileri. *Sağlık, 4*, 1-9. doi:https://doi.org/10.58208/cphs.1218650

Boccardi, V., Pigliautile, M., Guazzarini, A. G., & Mecocci, P. (2023). The Potential of Fasting-Mimicking Diet as a Preventive and Curative Strategy for Alzheimer's Disease. *Biomolecules, 13*(7), 1133. doi:https://doi.org/10.3390/biom13071133

Bouchard, C. (2021). Genetics of Obesity: What We Have Learned Over Decades of Research. *Obesity (Silver Spring, Md.), 29*(5), 802-820. doi:https://doi.org/10.1002/oby.23116

Brandhorst, S. (2021). Fasting and fasting-mimicking diets for chemotherapy augmentation. *GeroScience, 29*(5), 1201-1216. doi:https://doi.org/10.1002/oby.23116

Brandhorst, S., & Longo, V. D. (2016). Fasting and Caloric Restriction in Cancer Prevention and Treatment. *Recent results in cancer research, 207*, 241–266. doi:https://doi.org/10.1007/978-3-319-42118-6_12

Brandhorst, S., & Longo, V. D. (2019). Protein Quantity and Source, Fasting-Mimicking Diets, and Longevity. *Advances in nutrition, 10*, S340–S350. doi:https://doi.org/10.1093/advances/nmz079

Brandhorst, S., Choi'de, Y., Wei, M., Cheng, C. W., Sedrakyan, S., Navarrete, G., . . . Longo , V. (2015). A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. *Cell*

metabolism, 22(1), 86-99. doi:https://doi.org/10.1016/j.cmet.2015.05.012

Burg, E. L., Schoonakker, M. P., Korpershoek, B., Sommeling, L. E., Sturm, C. A., Lamb, H. J., . . . Peet, P. G. (2024). Self-initiated lifestyle changes during a fasting-mimicking diet programme in patients with type 2 diabetes: a mixed-methods study. *BMC primary care, 67*(7), 148. doi:https://doi.org/10.1007/s00125-024-06137-0

Burg, E. L., Schoonakker, M. P., Peet, P. G., Marle, E. M.-v., Lamb, H. J., Longo, V. D., . . . Pijl, H. (2024). Integration of a fasting-mimicking diet programme in primary care for type 2 diabetes reduces the need for medication and improves glycaemic control: a 12-month randomised controlled trial. *Diabetologia, 25*(1), 1245-1259. doi:https://doi.org/10.1186/s12875-024-02405-5

Burns, L., Cooper, S., Sarmad, S., Funke, G., Mauro, A. D., Gaitanos, G. C., & Tsintzas, K. (2025). Effects of fasting-mimicking diets with low and high protein content on cardiometabolic health and autophagy: A randomized, parallel group study. *Clinical nutrition, 52*, 299-312. doi:https://doi.org/10.1016/j.clnu.2025.08.004

Chaix, A., Manoogian, E. N., Melkani, G. C., & Panda, S. (2019). Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. *Annual review of nutrition, 39*, 291–315. doi:https://doi.org/10.1146/annurev-nutr-082018-124320

Cheng, C.-W., Villani, V., Buono, R., Wei, M., Kumar, S., Yilmaz, O. H., . . . Longo, V. D. (2017). Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. *Cell, 168*(5), 775-788.

doi:https://doi.org/10.1016/j.cell.2017.01.040

Conde-Pipó, J., A. M.-F., Martinez-Bebia, M., Gimenez-Blasi, N., Lopez-Moro, A., Latorre, J. A., . . . Mariscal-Arcas, M. (2024). Intermittent Fasting: Does It Affect Sports Performance? A Systematic Review. *Nutrients, 16*(1), 168. doi:https://doi.org/10.3390/nu16010168

Cortellino, S., Quagliariello, V., Delfanti, G., Blaževitš, O., Chiodoni, C., Maurea, N., . . . Longo, V. D. (2023). Fasting mimicking diet in mice delays cancer growth and reduces immunotherapy-associated cardiovascular and systemic side effects. *Nature communications, 14*(1), 5529. doi:https://doi.org/10.1038/s41467-023-41066-3

Crupi, A. N., Haase, J., Brandhorst, S., & Longo, V. D. (2020). Periodic and Intermittent Fasting in Diabetes and Cardiovascular Disease. *Current diabetes reports, 20*(12), 83. doi:https://doi.org/10.1007/s11892-020-01362-4

Di Francesco, A., Di Germanio, C., Bernier , M., & de Cabo, R. (2018). A time to fast. *Science (New York, N.Y.), 362*(6416), 770-775. doi:https://doi.org/10.1126/science.aau2095

Faccioli, N., Poitou, C., Clément, K., & Dubern, B. (2023). Current Treatments for Patients with Genetic Obesity. *Journal of clinical research in pediatric endocrinology, 15*(2), 108-119. doi:https://doi.org/10.4274/jcrpe.galenos.2023.2023-3-2

Fanti, M., Mishra, A., Longo, V., & Brandhorst, S. (2021). Time-Restricted Eating, Intermittent Fasting, and Fasting-Mimicking Diets in Weight Loss. *Curr Obes Rep, 10*(2), 70-80. doi:https://doi.org/10.1007/s13679-021-00424-2

Fay-Watt, V., O'Connor, S., D. R., A. C., Longo, V. D., & Sullivan,

F. J. (2023). The impact of a fasting mimicking diet on the metabolic health of a prospective cohort of patients with prostate cancer: a pilot implementation study. *Prostate cancer and prostatic diseases, 26*(2), 317-322. doi:https://doi.org/10.1038/s41391-022-00528-3

Finnell, J. S., Saul, B. C., A. C., & Myers, T. R. (2018). Is fasting safe? A chart review of adverse events during medically supervised, water-only fasting. *BMC complementary and alternative medicine, 18*(1), 67. doi:https://doi.org/10.1186/s12906-018-2136-6

Gabriel, S., Ncube, M., Zeiler, E., Thompson, N., Karlsen, M. C., Goldman, D. M., . . . Myers, T. R. (2022). A Six-Week Follow-Up Study on the Sustained Effects of Prolonged Water-Only Fasting and Refeeding on Markers of Cardiometabolic Risk. *Nutrients, 14*(20), 4313. doi:https://doi.org/10.3390/nu14204313

Green, M., Arora, K., & Prakash, S. (2020). Microbial Medicine: Prebiotic and Probiotic Functional Foods to Target Obesity and Metabolic Syndrome. *International Journal of Molecular Sciences, 21*(8), 2890. doi:https://doi.org/10.3390/ijms21082890

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646-674. doi:https://doi.org/10.1016/j.cell.2011.02.013

Hardie, D. G. (2015). AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Current opinion in cell biology, 33*, 1–7. doi:https://doi.org/10.1016/j.ceb.2014.09.004

Harreiter, J., & Roden, M. (2023). Diabetes mellitus: definition, classification, diagnosis, screening and prevention (Update

2023). *Wiener klinische Wochenschrift*, 7-17. doi:https://doi.org/10.1007/s00508-022-02122-y

Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E. A., Gill, Ş., . . . Panda, S. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism, 15*(6), 848 – 860. doi:https://doi.org/10.1016/j.cmet.2012.04.019

Herz, D., Haupt, S., Zimmer, R. T., Wachsmuth, N. B., Schierbauer, J., Zimmermann, P., . . . Moser, O. (2023). Efficacy of Fasting in Type 1 and Type 2 Diabetes Mellitus: A Narrative Review. *Nutrients, 15*(16), 3525. doi:https://doi.org/10.3390/nu15163525

Hof, K. S., & Wall, C. T. (2024). Comprehensive Assessment of a Fasting-Mimicking Diet: Salivary Metabolite Profiles, Weight Loss, and Biomarker Analysis. *bioRxiv*. doi:https://doi.org/10.1101/2024.12.29.630652

Kaur, N., Chugh, V., & Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. *Journal of food science and technology, 51*(10), 2289-2303. doi:2303. https://doi.org/10.1007/s13197-012-0677-0

Keirns, N. G., & Hawkins, M. A. (2019). Intuitive eating, objective weight status and physical indicators of health. *Obesity science & practice, 5*(5), 408-415. doi:https://doi.org/10.1002/osp4.359

Lin, X., & Gao, Y. (2024). A bibliometric analysis of the Fasting-Mimicking Diet. *Frontiers in nutrition, 11*, 1328450. doi:https://doi.org/10.3389/fnut.2024.1328450

Liu, X., Peng, S., Tang, G., Xu, G., Y. X., Shen, D., . . . Luo, Y. (2023). Fasting-mimicking diet synergizes with ferroptosis

against quiescent, chemotherapy-resistant cells. *EBioMedicine, 30*, 104496. doi:https://doi.org/10.1016/j.ebiom.2023.104496

Livingston, G., Huntley, J., Liu, K. Y., Costafreda, S. G., Selbæk, G., Alladi, S., . . . Mukadam, N. (2020). Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. *Lancet, 390*, 572–628. doi:https://doi.org/10.1016/S0140-6736(17)31363-6

Longo, V. D., & Mattson, M. P. (2015). Fasting: Molecular Mechanisms and Clinical Applications. *Cell Metabolism, 19*(2), 181-192. doi:10.1016/j.cmet.2013.12.008.

Longo, V., & Mattson, M. (2014). Fasting: Molecular Mechanisms and Clinical Applications. *Cell Metab, 19*(2), 181-192. doi:https://doi.org/10.1016/j.cmet.2013.12.008

Madore, C., Yin, Z., Leibowitz, J., & Butovsky, O. (2020). Microglia, Lifestyle Stress, and Neurodegeneration. *Immunity, 52*(2), 222-240. doi:https://doi.org/10.1016/j.immuni.2019.12.003

Marques-Vidal, P., Tsampasian, V., Cassidy, A., Biondi-Zoccai, G., Chrysohoou, C., Koskinas, K., . . . Panagiotakos, D. (2025). Diet and nutrition in cardiovascular disease prevention: a scientific statement of the European Association of Preventive Cardiology and the Association of Cardiovascular Nursing & Allied Professions of the European Society of Cardiology. *European journal of preventive cardiology, 32*(16), 1540-1552. doi:doi: 10.1093/eurjpc/zwaf310

Mehrdad Alirezaei, C. C. (2010). Short-term fasting induces profound neuronal autophagy. *Autophagy, 6*(6), 702-710. doi:10.4161/auto.6.6.12376

Michalsen, A. (2010). Prolonged fasting as a method of mood enhancement in chronic pain syndromes: a review of clinical evidence and mechanisms. *Curr Pain Headache Rep, 14*(2), 80-87. doi:10.1007/s11916-010-0104-z

Mishra, A., Fanti, M., Ge, X., Vaughn, D., Brandhorst, S., Wei, M., . . . Longo, V. D. (2023). Fasting mimicking diet cycles versus a Mediterranean diet and cardiometabolic risk in overweight and obese hypertensive subjects: a randomized clinical trial. *npj metabolic health and disease, 1*(1), 1. doi:10.1038/s44324-025-00084-z

Moon, S., Kang, J., Kim, S. H., Chung, H. S., Kim, Y. J., Yu, J. M., . . . Kim, T. (2020). Beneficial Effects of Time-Restricted Eating on Metabolic Diseases: A Systemic Review and Meta-Analysis. *Nutrients, 12*(5), 1267. doi:10.3390/nu12051267

Mozaffarian, D. (2016). Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. *Circulation, 133*(2), 187-225. doi:10.1161/CIRCULATIONAHA.115.018585

Muscaritoli, M., Arends, J., Bachmann, P., Baracos, V., Barthelemy, N., Bertz, H., . . . Bischoff, S. (2021). ESPEN practical guideline: Clinical Nutrition in cancer. *Clinical nutrition, 40*(5), 2898-2913. doi:10.1016/j.clnu.2021.02.005

Nardon, M., Venturelli, M., Ruzzante, F., Longo, V. D., & Bertucco, M. (2022). Fasting-Mimicking-Diet does not reduce skeletal muscle function in healthy young adults: a randomized control trial. *European journal of applied physiology, 122*(3), 651–661. doi:10.1007/s00421-021-04867-2

Nuanchankong, J., Jaroennon, P., & Manakla, S. (2024). Fasting-mimicking diets. *Journal of Food Health and*

Bioenvironmental Science, 17(2), 77-84. doi:https://li01.tci-thaijo.org/index.php/sdust/article/view/264049

Ojo, O. (2021). Recent Advances in Nutrition and Diabetes. *Nutrients, 13*(5). doi:10.3390/nu13051573

Ostfeld, R. J. (2017). Definition of a plant-based diet and overview of this special issue. *J Geriatr Cardiol, 14*(5), 315. doi:10.11909/j.issn.1671-5411.2017.05.008

Panda, S. (2016). Circadian physiology of metabolism. *Science, 354*(6315), 1008-1015. doi:10.1126/science.aah4967

Panebianco, C., Potenza, A., & Pazienza, V. (2017). Fasting and engineered diets as powerful tool in the medical practice: an old approach in the new era. *Ann Transl Med, 5*(21), 429. doi:10.21037/atm.2017.08.34

Paoli, A., Tinsley, G., Bianco, A., & Moro, T. (2019). The Influence of Meal Frequency and Timing on Health in Humans: The Role of Fasting. *Nutrients, 11*(4), 719. doi:10.3390/nu11040719

Patterson, R. E., & Sears, D. D. (2017). Metabolic Effects of Intermittent Fasting. *Annual review of nutrition*, 371–393. doi:10.1146/annurev-nutr-071816-064634

Patterson, R. E., Laughlin, G. A., LaCroix, A. Z., Hartman, S. J., Natarajan, L., Senger, C. M., . . . Gallo, L. C. (2015). Intermittent Fasting and Human Metabolic Health. *Journal of the Academy of Nutrition and Dietetics, 115*(8), 1203–1212. doi:10.1016/j.jand.2015.02.018

Pio, R., Senent, Y., Tavira, B., & Ajona, D. (2025). Fasting and fasting-mimicking conditions in the cancer immunotherapy era. *Journal of physiology and biochemistry, 81*(2), 383-389. doi:10.1007/s13105-024-01020-3

Rangan, P., Lobo, F., Parrella, E., Rochette, N., Morselli, M., Stephen, T.-L., . . . Longo, V. (2022). Fasting-mimicking diet cycles reduce neuroinflammation to attenuate cognitive decline in Alzheimer's models. *Cell reports, 40*(13), 111417. doi:10.1016/j.celrep.2022.111417

Reynolds, A., & Mann, J. (2022). Update on Nutrition in Diabetes Management. *The Medical clinics of North America, 106*(5), 865-879. doi:10.1016/j.mcna.2022.03.003

Roos, P. R., Burg, E. L., Schoonakker, M. P., Peet, P. G., Numans, M. E., Pijl, H., . . . Lamb, H. J. (2025). Fasting-mimicking diet in type 2 diabetes reduces myocardial triglyceride content: A 12-month randomised controlled trial. *Nutrition, metabolism, and cardiovascular diseases, 35*(7). doi:10.1016/j.numecd.2025.103860

Rynders, C. A., Thomas, E. A., Zaman, A., Tavası, Z., Catenacci, V. A., & Melanson, E. L. (2019). Effectiveness of Intermittent Fasting and Time-Restricted Feeding Compared to Continuous Energy Restriction for Weight Loss. *Nutrients, 11*(10), 2442. doi:10.3390/nu11102442

Sadeghian, M., Hosseini, S. A., Javid, A. Z., Angali, K. A., & Mashkournia, A. (2021). Effect of Fasting-Mimicking Diet or Continuous Energy Restriction on Weight Loss, Body Composition, and Appetite-Regulating Hormones Among Metabolically Healthy Women with Obesity: a Randomized Controlled, Parallel Trial. *Obesity surgery, 31*(5), 2030-2039. doi:10.1007/s11695-020-05202-y

Salvadori, G., Zanardi, F., Iannelli, F., Lobefaro, R., Vernieri, C., & Longo, V. D. (2021). Fasting-mimicking diet blocks triple-negative breast cancer and cancer stem cell escape. *Cell metabolism, 33*(11), 2247-2259.

doi:10.1016/j.cmet.2021.10.008

Schwartz, S. M. (2024). Epidemiology of Cancer. *Clinical chemistry, 70*(1), 140-149. doi:10.1093/clinchem/hvad202

Śliwińska, S., & Jeziorek, M. (2021). The role of nutrition in Alzheimer's disease. *Roczniki Panstwowego Zakladu Higieny, 72*(1), 29-39. doi:10.32394/rpzh.2021.0154

Sun, Y., Tao, Q., Wu, X., Zhang, L., Liu, Q., & Wang, L. (2021). The Utility of Exosomes in Diagnosis and Therapy of Diabetes Mellitus and Associated Complications. *Frontiers in endocrinology*. doi:10.3389/fendo.2021.756581

Sutton, E. F., Beyl, R., Early, K. S., Cefalu, W. T., Ravussin, E., & Peterson, C. M. (2018). Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. *Cell metabolism, 27*(6), 1212-1221. doi:10.1016/j.cmet.2018.04.010

Tang, D., Tang, Q., Huang, W., Zhang, Y., Tian, Y., & Fu, X. (2023). Fasting: From Physiology to Pathology. *Advanced science (Weinheim, Baden-Wurttemberg, Germany), 10*(9). doi:10.1002/advs.202204487

Tang, F., & Lin, X. (2020). Effects of Fasting-Mimicking Diet and Specific Meal Replacement Foods on Blood Glucose Control in Patients with Type 2 Diabetes: A Randomized Controlled Trial. *Oxidative medicine and cellular longevity*. doi:10.1155/2020/6615295

Templeman, I., Gonzalez, J. T., Thompson, D., & Betts, J. A. (2019). The role of intermittent fasting and meal timing in weight management and metabolic health. *The Proceedings of the Nutrition Society, 79*(1), 76–87.

doi:10.1017/S0029665119000636

Upadhyay, A., Anjum, B., Godbole, N. M., Rajak, S., Shukla, P., Tiwari, S., . . . Godbole, M. M. (2019). Time-restricted feeding reduces high-fat diet associated placental inflammation and limits adverse effects on fetal organ development. *Biochemical and biophysical research communications, 514*(2), 415–421. doi:10.1016/j.bbrc.2019.04.154

van den Brink, A., Brouwer-Brolsma, E., Berendsen, A., & van de Rest, O. (2019). The Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) Diets Are Associated with Less Cognitive Decline and a Lower Risk of Alzheimer's Disease-A Review. *Advances in nutrition, 10*(6), 1040-1065. doi:10.1093/advances/nmz054

Varady, K. A., Cienfuegos, S., Ezpeleta, M., & Gabel, K. (2022). Clinical application of intermittent fasting for weight loss: progress and future directions. Nature reviews. *Endocrinology, 18*(2), 309-321. doi:10.1038/s41574-022-00638-x

Vernieri, C., Fucà, G., Ligorio, F., Huber, V., Vingiani, A., Iannelli, F., . . . de Braud, F. (2022). Fasting-Mimicking Diet Is Safe and Reshapes Metabolism and Antitumor Immunity in Patients with Cancer. *Cancer discovery, 12*(1), 90-107. doi:10.1158/2159-8290.CD-21-0030

Virani, S. S., Alonso, A., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., Carson, A. P., . . . Tsao, C. (2020). Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. *141*(9). doi:10.1161/CIR.0000000000000757

Visioli, F., Mucignat-Caretta, C., Anile, F., & Panaite, S.-A. (2022). Traditional and Medical Applications of Fasting. *Nutrients, 14*(3), 433. doi:10.3390/nu14030433

Wang, L., Wang, Y.-J., Wang, R., Gong, F.-L., Chen, J.-Y., Yu, Y.-T., . . . Yuan, Y.-F. (2025). Fasting-mimicking diet enhances EGFR-TKI efficacy in oral cancer through dual mechanisms: direct cancer cell sensitization and tumor-associated macrophage crosstalk. *Frontiers in pharmacology*. doi:10.3389/fphar.2025.1641024

Wang, Y., & Wu, R. (2022). The Effect of Fasting on Human Metabolism and Psychological Health. *Dis Markers*. doi:10.1155/2022/5653739

Wei, M., Brandhorst, S., Shelehc, M., Mirzaei, H., Cheng, C. W., Budniak, J., . . . Longo, D. V. (2017). Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. *Science translational medicine, 9*(377). doi:10.1126/scitranslmed.aai8700

Welton, S., Minty, R., O'Driscoll, T., Willms, H., Poirier, D., Madden, S., & Kelly, L. (2020). Intermittent fasting and weight loss: Systematic review. *Canadian family physician Medecin de famille canadien, 66*(2), 117-125.

Wen, B., Han, X., Gong, J., Wang, P., Sun, W., Xu, C., . . . Wei, C. (2025). Nutrition: A non-negligible factor in the pathogenesis and treatment of Alzheimer's disease. *Alzheimers Dement, 21*(2). doi:10.1002/alz.14547

Wilhelmi de Toledo, F., Grundler, F., Bergouignan, A., Drinada, S., & Michalsen, A. (2019). Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects. *PLoS One, 14*(1), 1. doi:10.1371/journal.pone.0209353

Zeiler, E., Gabriel, S., Ncube, M., Thompson, N., Newmire, D., Scharf, E. L., . . . Myers, T. R. (2024). Prolonged Water-Only Fasting Followed by a Whole-Plant-Food Diet Is a Potential Long-Term Management Strategy for Hypertension and Obesity. *Nutrients, 16*(22), 3959. doi:10.3390/nu16223959

Zhang, W., Chen, P., Huo, S., Huang, X., & Zhao, Y. (2024). Requirements for essential micronutrients during caloric restriction and fasting. *Frontiers in nutrition*. doi:10.3389/fnut.2024.1363181

Zhong, Z., Zhang, H., Nan, K., Zhong, J., Wu, Q., Lu, L., . . . Miao, C. (2023). Fasting-Mimicking Diet Drives Antitumor Immunity against Colorectal Cancer by Reducing IgA-Producing Cells. *Cancer research, 83*(21), 3529–3543. doi:10.1158/0008-5472.CAN-23-0323


BİDGE