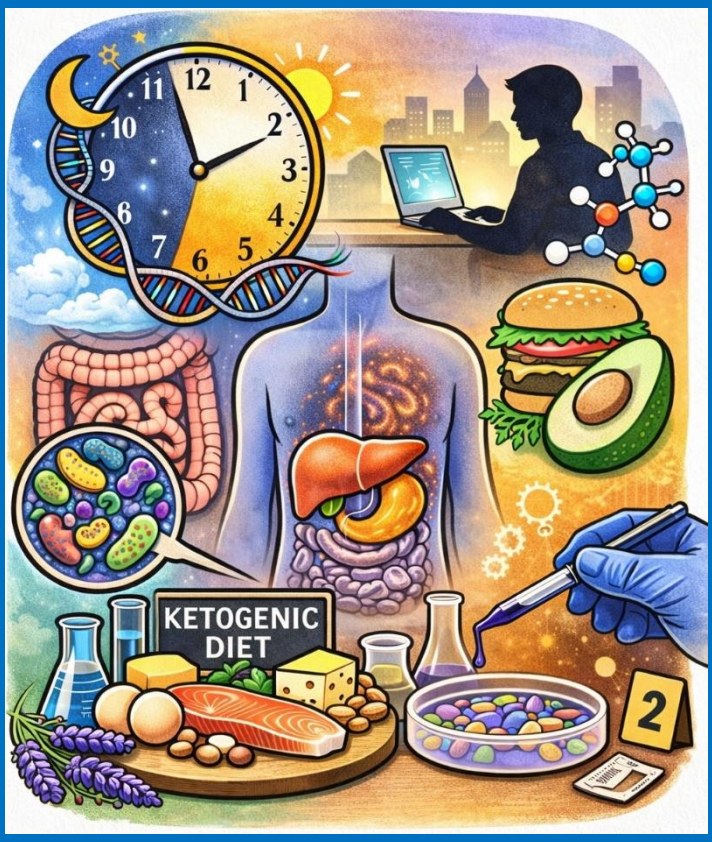
INTEGRATIVE BIOLOGY OF CIRCADIAN RHYTHMS, METABOLISM, AND CLINICAL APPLICATIONS

Prof. Dr. Bülent GÜNDÜZ



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PREFACE

This book chapter provides an integrated overview of contemporary biomedical research areas that intersect chronobiology, metabolism, nutrition, microbiology, experimental physiology, and therapeutic technologies. By synthesizing diverse but interconnected topics, the chapter highlights how biological timing, metabolic regulation, and targeted interventions collectively influence human health and disease.

Circadian rhythms play a fundamental role in regulating metabolic homeostasis, energy balance, and behavior. Disruption of the biological clock through irregular sleep patterns, shift work, or misaligned feeding times has been strongly associated with obesity and related metabolic disorders. Altered circadian signaling affects hormonal regulation, glucose metabolism, lipid storage, and appetite control, thereby increasing susceptibility to weight gain and insulin resistance. Beyond metabolic consequences, circadian rhythms also significantly influence job satisfaction and working life. Misalignment between internal biological clocks and external work schedules can impair cognitive performance, reduce productivity, increase fatigue, and negatively impact psychological well-being, emphasizing the importance of chronobiology-informed workplace practices.

Nutritional interventions represent a key strategy in managing metabolic dysfunction. The ketogenic diet, characterized by very low carbohydrate and high fat intake, has gained attention for its effects on metabolic syndrome. Evidence suggests that ketogenic dietary patterns can improve glycemic control, reduce body weight, lower triglyceride levels, and enhance insulin sensitivity. However, the metabolic benefits of this diet are closely linked to individual physiological responses and may interact with circadian timing of food intake, underlining the need for personalized and time-aware nutritional approaches.

Emerging research also underscores the importance of the human microbiota across multiple scientific disciplines. In forensic science, microbial communities associated with the human body and surrounding environment are increasingly used to estimate postmortem

intervals, determine cause of death, and provide associative evidence. The dynamic nature of microbiota reflects both biological and environmental factors, positioning it as a valuable tool in medico-legal investigations.

Experimental studies further contribute to understanding physiological modulation at the molecular level. Dose-dependent effects of lavender extract on liver proteins and antioxidant enzyme systems in pubertal Syrian hamsters illustrate how phytochemicals can influence oxidative stress and hepatic function during critical developmental periods. Such findings support the potential therapeutic and toxicological relevance of herbal compounds.

Collectively, this chapter emphasizes the interconnectedness of biological rhythms, metabolic regulation, microbiota dynamics, experimental therapeutics, and clinical interventions, offering a multidimensional perspective on modern biomedical science.

Sincerely,

25.12.2025

Prof. Dr. Bülent GÜNDÜZ

EDITOR

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CHAPTER 1

CIRCADIAN RHYTHM AND OBESITY: AN INTEGRATED REVIEW

Asst. Prof. Emine İnci BALKAN

Mehmet Serhat ÜZEL

INTRODUCTION

The rapid development of the global economy and the associated shift in lifestyle have led to a marked increase in the prevalence of obesity-related chronic diseases, particularly Type II diabetes, posing a significant threat to public health (Khan et al., 2020; Allocca et al., 2025). Obesity, defined by the World Health Organization (WHO) as excessive fat accumulation that may impair health, results from an excessive storage of body fat, particularly triglycerides. Global obesity prevalence has nearly tripled since 1975, with over 1.9 billion adults classified as overweight and 650 million as obese in 2016 (WHO, 2021). This increase constitutes a major public health and economic issue worldwide (Carr and Friedman, 2005; Puhl et al., 2010).

Circadian rhythms (from Latin *circa*: approximately, *dies*: a day) are endogenous rhythms that last about a day (Abbott and Zee, 2019; Begemann et al., 2025). They are cyclical, approximately 24-hour variations in physiological, biochemical, and behavioral processes that allow organisms to adapt to the Earth's rotation and the resulting light-dark, temperature, and humidity cycles (Yerushalmi and Green, 2009; DeCoursey et al., 2000; Dodd et al., 2005). The most evident human

circadian rhythm is the sleep-wake cycle. Other vital functions under circadian control include the hunger-satiety cycle, temperature regulation, immune system, respiratory system, and various key metabolic events (Zarrinpar et al., 2016; Sulli et al., 2018).

In modern life, factors such as shift work, nocturnal eating, and sleep disturbances disrupt this internal rhythm, leading to desynchronization. This disruption in synchronization, in turn, is linked to differences in energy balance, accelerating the development of obesity and related metabolic disorders. Mounting evidence suggests a strong, intricate link between disrupted circadian rhythms and the rising epidemic of obesity.

2. Regulation and Molecular Mechanism of the Circadian System

2.1.The Central Clock and Its Regulation

The circadian rhythm's primary control center is the Suprachiasmatic Nucleus (SCN), located in the anterior hypothalamus of the brain. The SCN is responsible for regulating the secretion of various hormones (such as melatonin and growth hormone), insulin secretion, and the sleep-wake cycle (Van Drunen and Eckel- Mahan, 2021; Cedernaes et al., 2019).

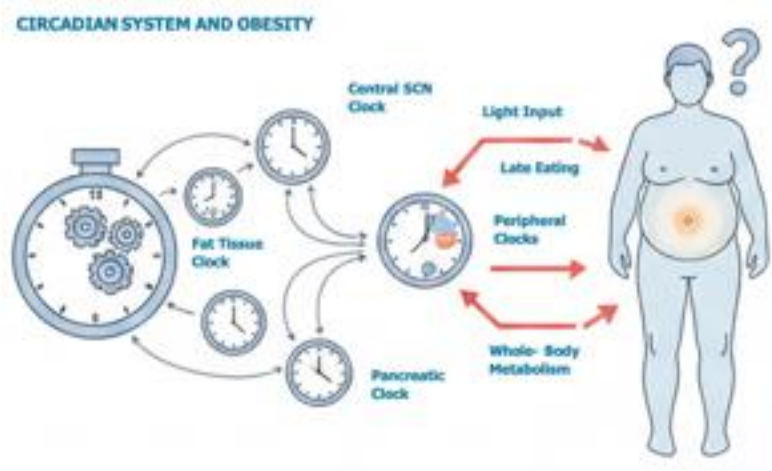

Figure 1. Circadian System and Obesity: The main diagram summarizing the effect of the biological clock on body metabolism, showing the central SCN clock, fat tissue, and pancreatic clocks.

The biological time is influenced by external factors (called *zeitgebers*), including light, temperature, and feeding time, which help synchronize the internal clock to the external 24-hour day. Among these, light is the most potent factor (la Fleur et al., 2024) (Figure 1). Exposure to daylight in the morning suppresses melatonin and promotes wakefulness, while darkness in the evening increases melatonin production, facilitating the transition to sleep (Gooley et al., 2011). The timing of nutrition is also a crucial factor affecting the biological clock; eating at irregular hours can weaken the circadian clock (Charlot et al., 2021; Alum 2025).

2.2.The Central Clock and Its Regulation

The circadian rhythm is governed by a molecular clock system involving a set of genes and mechanisms that contribute to protein

synthesis and translation. This system operates via an interlocked, self-regulating transcription-translation feedback loop.

Key components include:

- Positive Loop: The CLOCK (Circadian Locomotor Output Cycle Kaput) and BMAL1 (Brain and Muscle ARNT-like protein 1) proteins form a complex. This complex acts as a transcription factor, binding to the promoter regions of target genes to activate or suppress their transcription.
- Negative Loop: The PER (Period) and CRY (Cryptochrome) proteins are target genes activated by the CLOCK/BMAL1 complex. PER and CRY proteins then form complexes that inhibit the regulatory action of CLOCK/BMAL1, completing the feedback loop.

The expression of PER and CRY exhibits "peak and trough" cycles, which are influenced by external factors like the light cycle, temperature, food intake, and exercise. Disruption of these core clock genes has been shown to cause metabolic abnormalities in animal models, such as impaired glucose tolerance, decreased insulin secretion, increased susceptibility to high-fat diet, and ultimately, weight gain (Ren et al. 2025; Zhang et al., 2024) (Figure 2).

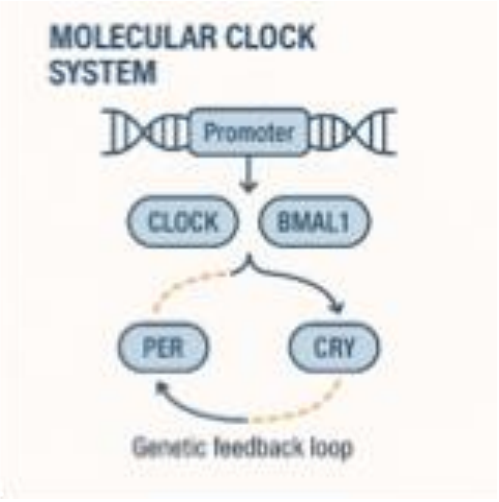

Figure 2. Molecular Clock System: The molecular-level diagram showing the genetic feedback loop that drives the clock mechanism, involving CLOCK, BMAL1, PER, and CRY proteins.

3. Factors Disrupting Circadian Rhythm and Contributing to Obesity

Several contemporary lifestyle factors can disrupt the delicate balance of the circadian system, subsequently contributing to metabolic dysregulation and obesity (Albrecht, 2017; Garaulet & Gómez-Abellán, 2014; Scheer et al., 2009).

3.1.Light and Melatonin Misalignment

Light is essential for regulating the biological clock, with daylight maintaining alertness and evening light delaying sleep (Cajochen et al., 2005; Foster & Kreitzman, 2014). Exposure to light, particularly blue light, at night suppresses the production of melatonin (Brainard et al., 2001; Chang et al., 2015). Melatonin is synthesized from tryptophan

and is secreted intensively by the pineal gland only at night, with peak levels typically reached between 02:00-03:00 (Arendt, 1995; Reiter, 1991). Melatonin is a reliable marker of the internal circadian phase and a powerful sleep-promoting and phase-regulating agent (Lewy & Sack, 1989; Pandi-Perumal et al., 2006). Disrupted sleep due to light exposure or other factors leads to a loss of concentration in melatonin secretion, predisposing individuals to cardiovascular disease and a weakened immune system (Stevens et al., 2013; Reiter et al., 2014) (Figure 3).

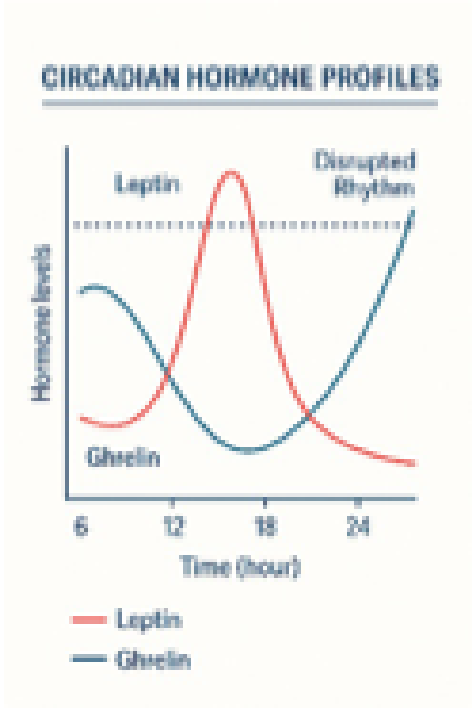

Figure 3. Circadian Hormone Profiles: The graph showing the change in hormone levels (usually including hormones like leptin and ghrelin) in a 24-hour cycle in individuals with disrupted rhythm and obesity.

3.2.Shift Work and Jet Lag

Shift work is a significant disruptor, as it forces individuals to work during the body's natural resting hours. Shift workers often exhibit irregular working patterns that conflict with their intrinsic daily rhythm. The misalignment between their sleep and work schedules is a major problem. This population is observed to have a higher incidence of obesity, diabetes, digestive issues, and cancer. Furthermore, a study suggested a link between shift work and hedonic hunger, finding that the longer the shift duration, the greater the hedonic hunger. Epidemiological studies have also confirmed that the prevalence of obesity and BMI are higher among night shift workers (Badar, 2018; Sever et al., 2024) (Figure 4).

Jet Lag is a condition caused by the body's inability to adapt to the abrupt time difference experienced during long-haul flights across multiple time zones. The individual's biological clock struggles to align with the local time of the destination country. This struggle can lead to symptoms such as fatigue, nausea, vomiting, headaches, and loss of appetite. Adapting the internal clock to a later time (traveling west) is generally easier than adapting to an earlier time (traveling east). To minimize jet lag, individuals should adjust meal times to the new time zone, ensure adequate water consumption to prevent dehydration, and avoid carbonated beverages, caffeine, and alcohol during the flight (Benardort, 2012) (Figure 4).

3.3.Nutrition and Sleep Misalignment

Inadequate sleep is a widespread public health issue in modern society, severely disrupting the regularity of the circadian clock. Short sleep duration is a stress factor for metabolic health and promotes the development of obesity. Analysis has shown that individuals who sleep for short periods have a 45% increased risk of obesity compared to those with normal sleep duration (Wu et al., 2014).

The timing of food intake is crucial. Eating at times when the body's metabolism is programmed for rest—such as late-night eating or snacking before bed—is associated with a higher risk of obesity. For instance, skipping breakfast increases the risk of obesity (Garaulet et al., 2013).

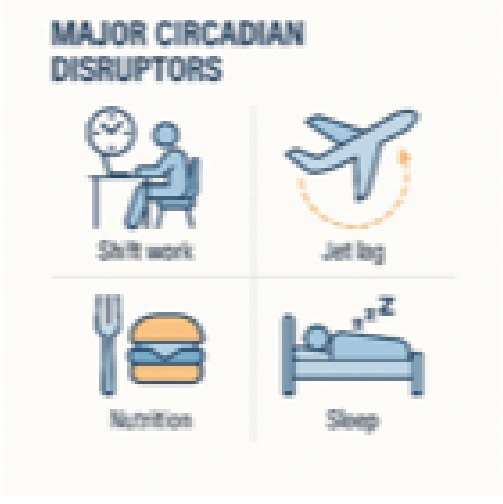

Figure 4. Major Circadian Disruptors: Items summarizing the key factors that disrupt the circadian rhythm, such as shift work/jet lag, and nutrition/sleep irregularities.

4. The Intricate Relationship Between Circadian Rhythm and Obesity

The link between a disrupted circadian system and obesity is multifaceted, primarily mediated by the dysregulation of key hormones and metabolic processes (Fonken & Workman, 2014).

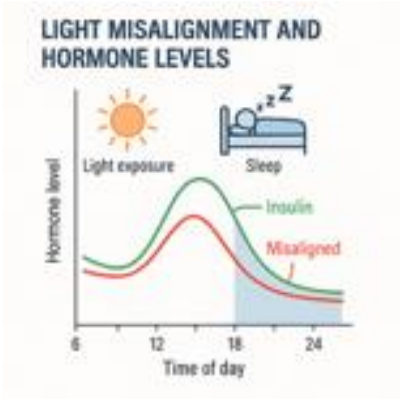

Figure 5. Light Misalignment and Hormone Levels (Light Misalignment Graph): A 24-hour graph showing the effect of light exposure and the sleep cycle on hormone levels, such as insulin.

4.1.Hormonal Dsyregulation

The levels of hormones critical for energy balance—including leptin, ghrelin, insulin, and glucocorticoids (cortisol)—are tightly controlled by the circadian clock, and their rhythmic secretion conveys information between the central nervous system and peripheral metabolic tissues (Kalsbeek et al., 2014).

- **Leptin (Satiety Hormone):** Leptin is released by white adipocytes and signals satiety to the brain, reducing food intake. Leptin secretion follows a circadian rhythm, with levels

typically low during the day and highest at night (24:00–03:00) (Sinha et al., 1996). Circadian disruption, such as from sleep deprivation or night shift work, lowers leptin secretion, which increases hunger, reduces the feeling of fullness, and promotes overeating. The amplitude (high-low difference) of the leptin circadian cycle is inversely proportional to BMI; the more obese an individual is, the flatter and less distinct the leptin rhythm becomes (Simon et al., 1998) (Figure 3).

- **Ghrelin (Hunger Hormone):** Ghrelin increases appetite. In a study involving 1024 individuals, reduced sleep duration was associated with decreased leptin and ghrelin levels, which paradoxically increased the individuals' appetite and subsequent BMI (Taheri et al., 2004).

- **Insulin:** Insulin, which regulates blood glucose and promotes the storage of excess glucose as fat, also has a circadian rhythm, with levels naturally highest in the morning and decreasing throughout the day (Morris et al., 2015). Circadian disruption impairs insulin production and sensitivity, leading to higher blood sugar, weight gain, and increased obesity risk. Irregular eating patterns, particularly eating late at night, disrupt insulin release, increasing hunger and predisposing to overeating (Scheer et al., 2009) (Figure 5)**.**

- **Glucocorticoids (Cortisol):** Cortisol, the primary glucocorticoid, has a diurnal rhythm, peaking in the early morning and falling during the day. Circadian misalignment

disrupts this rhythm, leading to high cortisol levels at night, which increases appetite for high-sugar/high-fat foods and promotes abdominal fat deposition (Adam et al. 2017). Chronic high cortisol exacerbates insulin resistance and facilitates weight gain by reducing the metabolic rate due to muscle protein breakdown (Björntorp, 2001).

4.2.Metabolic Dsyregulation

The circadian rhythm influences various metabolic processes, including glucose and lipid metabolism. Disruptions impair glucose tolerance and increase insulin resistance, raising the risk for weight gain and Type 2 diabetes (Bass & Takahashi, 2010).

- **Lipid Metabolism:** Adipose tissue has its own peripheral circadian clock that regulates adipocyte differentiation, lipolysis (fat breakdown), and adipokine secretion (Shostak et al., 2013). Disruption of this peripheral control leads to abnormal fat storage and adipokine imbalance, resulting in obesity-related metabolic disorders. In a rat model, a high-fat diet consumed during the normal resting phase (equivalent to night-eating in humans) disrupted both behavioral and molecular circadian rhythms and caused metabolic dysfunction (Arble et al., 2009).

5. Circadian Rhythm-Targeted Strategies for Obesity Treatment

Recognizing the central role of the circadian system suggests that targeted interventions can be effective for weight management and metabolic health.

5.1.Sleep Optimization and Light Management

Optimizing sleep is fundamental, as insufficient or poor-quality sleep impairs the normal secretion of appetite-regulating hormones like leptin. A consistent goal of 7–9 hours of uninterrupted sleep per night is recommended (Hirshkowitz et al., 2015). Key practices include maintaining a regular sleep and wake schedule and creating a conducive sleep environment (quiet, dark, cool). Limiting exposure to bright light and electronic screens before bed is crucial, as this can suppress melatonin production and disrupt the sleep-wake cycle (Roenneberg et al., 2013) (Figure 6).

Exposure to natural light during the day, particularly in the morning for at least 30 minutes, helps synchronize the internal clock, which, in turn, regulates the timing of various physiological and metabolic processes.

5.2.Chronutrition and Meal Timing

Chrononutrition is an approach that focuses on the timing and distribution of meals. Aligning meal schedules with the body's internal clock optimizes metabolism (Garaulet & Gómez-Abellán, 2014). The general advice is to consume the majority of daily calories during the active phase (daytime) and avoid late-night eating.

Time-Restricted Eating (TRE) is a specific chrononutrition method that limits food intake to a specific window each day, followed by a fasting period. TRE methods, such as 16:8 (16 hours fasting, 8 hours eating) or 14:10 (14 hours fasting, 10 hours eating), have shown positive effects

on weight loss, insulin sensitivity, and other metabolic parameters by synchronizing circadian rhythms (Panda, 2016; Sutton et al., 2018).

5.3.Physical Activity

Regular physical activity not only provides numerous health benefits but also plays a role in regulating the circadian rhythm (Mistlberger & Skene, 2004). Exercising during the active phase (daytime) is generally more efficient for endurance, strength, and fat burning. Conversely, intense exercise immediately before bedtime should be avoided, as it can disrupt the sleep-wake cycle (Stutz et al., 2019) (Figure 6).

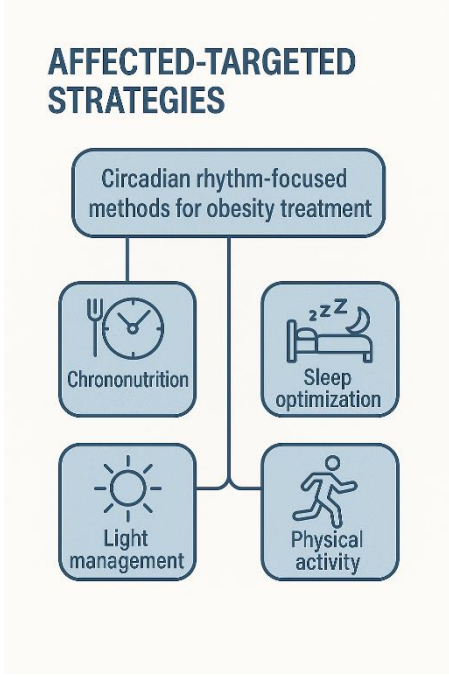

Figure 6. Affected-Targeted Strategies: The diagram showing circadian rhythm-focused methods for obesity treatment, such as chrononutrition, sleep optimization, light management, and physical activity.

6. Conclusion

The evidence from contemporary scientific studies confirms a strong and reciprocal relationship between circadian rhythm and obesity. Disruptions to the biological clock, caused by factors such as shift work, light exposure at night, and poor sleep or eating timing, lead to a misalignment of internal biological processes.

This misalignment results in the dysregulation of core circadian clock genes, as well as crucial metabolic hormones, including leptin, ghrelin, insulin, and cortisol. The consequence is a cascade of metabolic disturbances: increased appetite, insulin resistance, abnormal fat deposition, and reduced energy expenditure, all of which contribute to the development and progression of obesity.

Adopting a lifestyle that protects and reinforces the circadian rhythm—through sleep optimization, timed light exposure, chrononutrition strategies like TRE, and regular physical activity during the active phase—offers a powerful, holistic approach to combatting the obesity epidemic. A deeper understanding and implementation of these circadian-focused interventions can not only support weight loss but also significantly improve overall metabolic health.

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CHAPTER 2

CIRCADIAN RHYTHMS AND JOB SATISFACTION: EFFECTS OF THE BIOLOGICAL CLOCK ON WORKING LIFE

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INTRODUCTION

It has long been known that physiological processes in living organisms are temporally organized and exhibit a rhythmic structure. According to Reppert and Weaver (2002), circadian rhythms are biological clock mechanisms that operate in approximately 24-hour cycles and enable organisms to adapt to environmental conditions. Czeisler et al. (1999) emphasize that these rhythms play a decisive role in regulating many fundamental physiological functions, including the sleep–wake cycle, hormone secretion, body temperature, and metabolic processes.

The structure of modern working life is not always compatible with individuals' biological rhythms. Wright et al. (2013) state that shift work and night work can disrupt the synchronization of the circadian timing system with the environmental light–dark cycle. Boivin and Boudreau (2014) also report that irregular working hours and prolonged exposure to artificial light have negative effects on melatonin secretion

and the sleep–wake cycle. This situation can influence not only individuals' physiological balance but also their daily functioning and overall well-being.

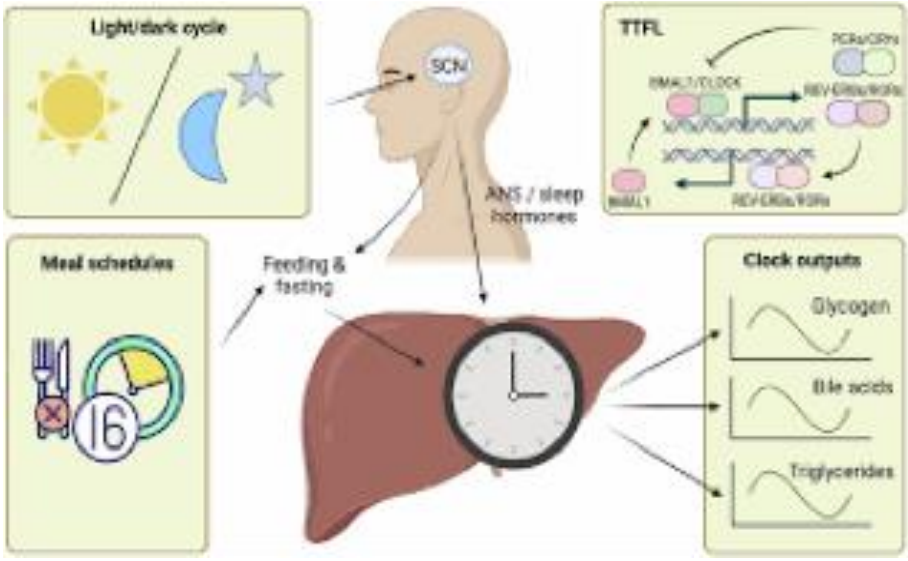
In working life, individuals' perceptions and experiences related to their jobs are addressed within the framework of the concept of job satisfaction. According to Locke (1976), job satisfaction is a subjective evaluation that emerges as a result of the congruence between an individual's expectations regarding their job and the opportunities provided by the job. High job satisfaction is associated with positive outcomes in terms of employee health, job performance, and organizational sustainability.

Recent studies indicate that the factors affecting job satisfaction are not limited to organizational and psychosocial elements, and that biological processes may also play an indirect role in this relationship. Åkerstedt et al. (2010) suggest that disruptions in circadian rhythms can affect employee well-being through sleep quality and fatigue levels, while Bambra et al. (2008) note that this condition may be related to job satisfaction. In this book chapter, the biological foundations of circadian rhythms are discussed, and the physiological consequences of

rhythm disruption and their possible reflections on working life are examined.

1. Biological Foundations of Circadian Rhythms

Circadian rhythms are biological processes regulated by internal timing systems and synchronized with the environmental light–dark cycle. According to Reppert and Weaver (2002), the main regulator of this system in humans is the suprachiasmatic nucleus (SCN) located in the hypothalamus. The SCN perceives light signals from the retina and ensures that the biological clock is aligned with environmental time.



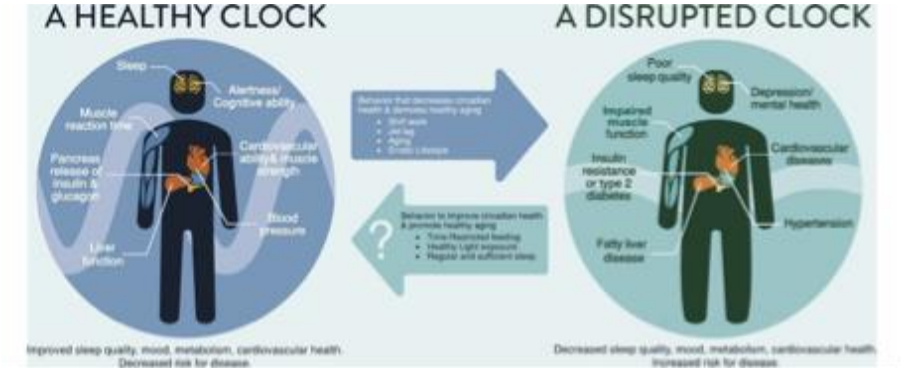
Signals originating from the SCN regulate peripheral clocks in tissues through neural and hormonal pathways. According to Boivin and Boudreau (2014), coordination between these central and peripheral clocks allows many physiological processes—such as metabolism, the endocrine system, and immune response—to function in temporal

harmony. Hormones such as melatonin and cortisol play a significant role in circadian regulation. While melatonin secretion increases in the evening to support sleep onset, cortisol levels rise in the morning, facilitating wakefulness and energy mobilization.

2. Physıologıcal Consequences Of Cırcadıan Rhythm Dısruptıons

Disruption of the alignment between circadian rhythms and environmental conditions can lead to various negative effects on physiological balance. According to Knutsson (2003), shift work and night work cause disturbances in melatonin secretion and the sleep–wake cycle. Wright et al. (2013) also demonstrated that these disruptions increase misalignment between the biological clock and environmental time.

One of the most prominent consequences of circadian disruption is a decline in sleep quality. Pilcher and Huffcutt (1996) report that shortened or fragmented sleep is associated with daytime fatigue, reduced attention, and decreased cognitive performance. Boivin and Boudreau (2014) further note that circadian misalignment can affect stress responses and lead to irregularities in cortisol rhythms.



According to Roenneberg et al. (2012), social jetlag caused by modern working arrangements refers to the misalignment between an individual's biological clock and socially imposed time schedules, and this condition can result in physiological and behavioral consequences.

3. The Concept of Job Satisfaction

According to Locke (1976), job satisfaction is a subjective evaluation that arises from the congruence between an individual's expectations regarding their job and the opportunities provided by the job. Job content, working conditions, organizational structure, managerial support, and individual characteristics are among the main factors influencing job satisfaction.

High job satisfaction enables employees to develop more positive attitudes toward their jobs and is associated with job performance, motivation, and organizational commitment. In contrast, low job satisfaction is linked to negative outcomes such as burnout, absenteeism, and intention to leave the job.

4. The Relationship Between Circadian Rhythms and Job Satisfaction

The relationship between circadian rhythms and job satisfaction is addressed through indirect mechanisms rather than direct causality. According to Åkerstedt et al. (2010), circadian misalignment among shift workers is associated with increased fatigue and reduced sleep quality. Saksvik et al. (2011) indicate that this situation may indirectly create negative effects on job satisfaction.

Pilcher and Huffcutt (1996) demonstrated that impairments in sleep quality negatively affect job performance and job-related satisfaction. Scott et al. (2007) reported that irregular sleep patterns among healthcare workers are associated with decreased job satisfaction and increased burnout levels. Bambra et al. (2008) similarly emphasized the negative effects of shift work on employee well-being and job satisfaction in their systematic reviews.

Individuals' chronotype characteristics also play an important role in this relationship. According to Adan et al. (2012), biological differences

between morning-type and evening-type chronotypes affect the level of adaptation to working hours. Hidalgo et al. (2009) and Vetter et al. (2015) reported that chronotype mismatch may lead to differences in perceived job stress and job satisfaction.

5. The Importance Of Bıologıcal Alıgnment In Workıng Lıfe

According to Folkard and Tucker (2003), organizing working hours in alignment with biological rhythms can contribute to the preservation of sleep quality and support employee well-being. Especially in fields such as healthcare, where shift work is common, considering biological alignment is important for both employee health and service quality.

It is suggested that working arrangements that do not take biological rhythms into account may have negative long-term effects on employee satisfaction and job satisfaction; therefore, considering biological alignment in work-life planning may be beneficial.

From an occupational health and safety (OHS) perspective, circadian rhythms are directly related to the health status of employees working shifts and night work, as well as to occupational accidents and diseases.

6. Effects of Shift and Night Work on Occupational Health and Safety

In the occupational health and safety literature, shift work is defined as one of the main organizational risk factors threatening employee health. Night work and frequent shift changes lead to circadian rhythm

disruptions, causing sleep deprivation, chronic fatigue, and reduced attention.

This situation increases the likelihood of occupational accidents. Studies show that accidents occurring during night shifts and early morning hours are both more frequent and more severe. Micro-sleep episodes, slowed reflexes, and perceptual errors pose vital risks in hazardous jobs such as machine operation, working at heights, and working with chemical substances.

In addition, shift work is known to be associated with cardiovascular diseases, gastrointestinal problems, and metabolic disorders in the long term. These conditions create a basis for the emergence of health problems that may be considered occupational diseases.

7. The Relationship Between Circadian Rhythm Disruptions And Occupational Accidents

From an OHS perspective, the human factor plays a significant role among the primary causes of occupational accidents. Fatigue, sleep deprivation, and lack of attention lead to an increase in unsafe behaviors that result in accidents. It has been reported that employees experiencing circadian rhythm disruptions exhibit weakened risk perception and reduced compliance with safe working practices.

In particular, long working hours, overtime, and insufficient rest periods further disrupt the biological clock. This situation makes it mandatory for employers to consider working hours and shift schedules when conducting risk assessments. Otherwise, preventable occupational accidents become inevitable.

8. Search for Solutions

The key to unlocking our full potential lies in aligning our work patterns with our natural rhythms. Companies that recognize and accommodate biological cycles can achieve significant gains in employee performance and well-being.

Some ways organizations can harness natural rhythms include:

- Flexible working hours: Allowing employees to work during times when they are naturally most alert and productive.

- Natural lighting and fresh air: Designing office spaces to maximize exposure to natural light and clean air, thereby supporting healthy circadian rhythms.
- Rest periods: Creating systems that encourage and incorporate short breaks throughout the day in alignment with ultradian rhythms of focus and rest.
- Seasonal adjustments: Adapting workloads or working hours in consideration of seasonal changes in energy levels.
- Nutrition timing: Providing healthy snacks and encouraging meal times that support natural blood sugar rhythms.

Benefits of Rhythm-Aligned Work

By creating an environment that respects and works in harmony with our natural rhythms, organizations can gain numerous benefits:

- Increased productivity: Employees working in sync with their natural cycles are likely to be more efficient and focused.
- Enhanced creativity: Allowing for natural fluctuations in energy can encourage more innovative thinking.
- Improved well-being: Aligning work with biological rhythms can reduce stress and improve overall health.
- Better teamwork: Understanding and respecting individual rhythms can contribute to more empathetic and effective collaboration.

9. Conclusion and Evaluation

Circadian rhythms are fundamental biological mechanisms that temporally regulate physiological processes in organisms. The structure of modern working life, particularly through shift and night work, interacts closely with these rhythms. According to Roenneberg et al. (2012), circadian misalignment can affect employee well-being through sleep quality and fatigue. Åkerstedt et al. (2010) also demonstrated that this condition may be associated with job satisfaction.

Nevertheless, it should be remembered that the relationship between circadian rhythms and job satisfaction is multidimensional and shaped by individual differences, chronotype characteristics, and working conditions. Therefore, future studies should address biological rhythms and work-related outcomes through more comprehensive and holistic approaches.

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CHAPTER 3

EFFECTS OF THE IMPLEMENTATION OF THE KETOGENIC DIET ON METABOLIC SYNDROME

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INTRODUCTION

With changes in dietary habits in recent years, there has been a marked increase in the prevalence of obesity, metabolic syndrome, and type 2 diabetes. This situation has increased scientific interest in different nutritional models and dietary approaches. The ketogenic diet (KD) has gained popularity in recent years not only for body weight control but also due to its potential beneficial effects on metabolic health.

The ketogenic diet was first developed in the 1920s to provide anticonvulsant effects in patients with pharmacoresistant epilepsy (Wheless, 2008). This dietary model is generally characterized by low carbohydrate and energy content, high fat intake, and adequate protein consumption. Due to its ability to increase satiety, support weight loss through carbohydrate restriction, and improve glycemic control, the ketogenic diet has been considered an alternative nutritional approach in the management of metabolic syndrome. Clinical and experimental studies conducted in recent years indicate that the ketogenic diet may exert significant effects on metabolic regulation, insulin sensitivity, lipid profile, and mitochondrial functions (Volek & Phinney, 2012).

1. Physiological Basis of the Ketogenic Diet

Under normal physiological conditions, the human body primarily uses glucose as its main energy source. However, when carbohydrate intake is severely restricted, ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) are synthesized in the liver from fatty acids via acetyl-CoA. This biochemical process is known as ketogenesis (Paoli et al., 2013).

During ketosis, the brain, muscle tissue, and other peripheral tissues can largely meet their energy requirements from ketone bodies. Reduced glucose metabolism leads to decreased insulin levels, which accelerates lipolysis; the released free fatty acids are directed toward energy production through mitochondrial β-oxidation. This metabolic adaptation is also observed during prolonged fasting and low-carbohydrate dietary patterns (source needed).

The macronutrient distribution in the ketogenic diet is generally as follows:

70–75%fat
20–25%protein
5–10% carbohydrates

Although these ratios may vary depending on individual energy requirements, maintaining daily carbohydrate intake below 50 grams is considered necessary to initiate ketosis (Westman et al., 2007).

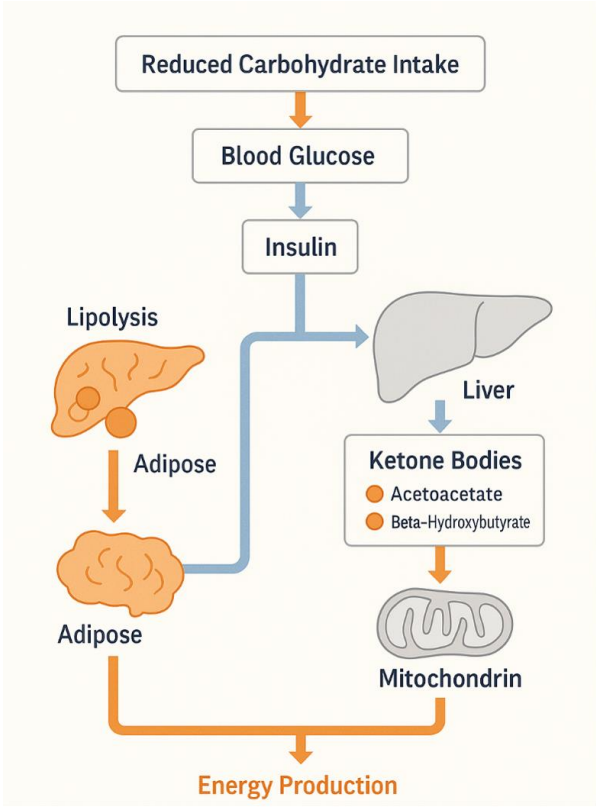

Figure 1. Metabolic adaptations and the ketosis process resulting from carbohydrate restriction in the ketogenic diet

2. Ketosis in the Ketogenic Diet

The primary goal of the ketogenic diet is to provide energy by promoting the production of ketone bodies from fatty acids during prolonged fasting or very low carbohydrate intake. When glucose availability is limited, the breakdown of adipose tissue releases free fatty acids and glycerol; these fatty acids are directed toward ketone production in the liver.

To achieve ketosis, daily net carbohydrate intake is generally recommended to be maintained between 25 and 50 grams (Filiz et al., 2022).

2.1. Types of Ketogenic Diets

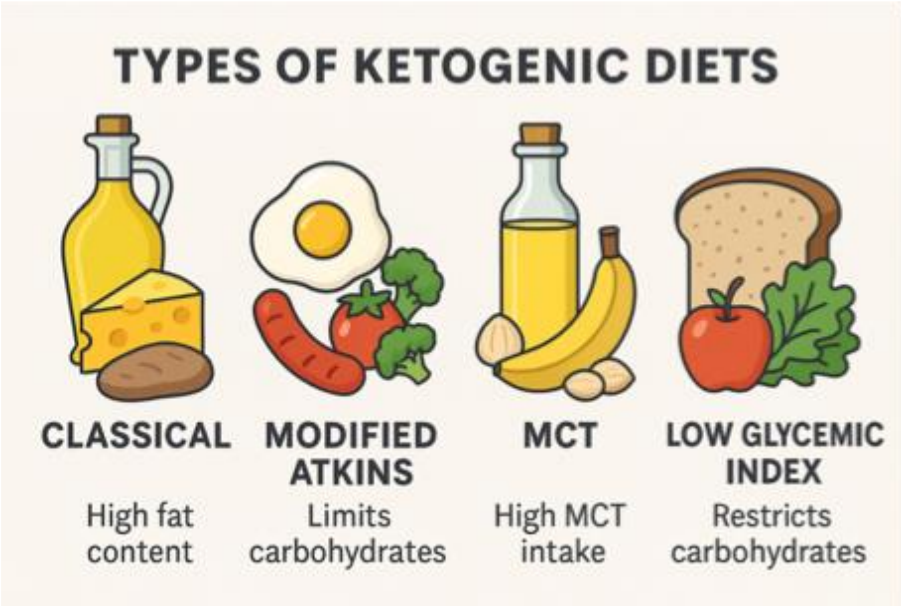

Figure 2. Main characteristics and example food groups of ketogenic diet types

2.1.1. Classical Ketogenic Diet (CKD)

The classical ketogenic diet is the strictest ketogenic dietary model, characterized by very high fat and very low carbohydrate content, resulting in strong induction of ketosis. It is primarily used in epilepsy treatment and, although it produces pronounced metabolic effects, its applicability is limited.

2.1.2. Modified Atkins Diet (MAD)

The Modified Atkins Diet (MAD) has a macronutrient distribution similar to the classical ketogenic diet but offers a more flexible implementation process. During the initial phase, daily carbohydrate intake is generally limited to 20 grams, and this restriction can be maintained indefinitely. Due to its greater ease of implementation and higher dietary adherence compared to the classical ketogenic diet, MAD is frequently preferred in clinical practice (Pietrzak et al., 2022).

In this dietary model, approximately 65% of total daily energy is derived from fats, 30% from proteins, and 5% from carbohydrates. MAD is considered an alternative ketogenic diet model, particularly in epilepsy treatment and weight control interventions.

2.1.3. Medium-Chain Triglyceride (MCT) Diet

The MCT diet is a ketogenic nutritional model in which a large proportion of energy is obtained from medium-chain triglycerides. Compared to long-chain fatty acids, medium-chain triglycerides are absorbed more rapidly and reach the liver faster, thereby enhancing ketone production. Due to these properties, the MCT diet is considered more effective than the classical ketogenic diet in inducing ketosis.

Furthermore, more efficient utilization of fat-derived energy reduces total fat requirements, allowing for higher carbohydrate and protein intake (Barzegar et al., 2021).

2.1.4. Low Glycemic Index Diet (LGID)

The low glycemic index diet (LGID) is characterized by the selection of low glycemic index (<50) carbohydrates and restricted total carbohydrate intake. Although this diet does not consistently induce ketosis, it demonstrates beneficial effects on carbohydrate metabolism and produces a lower rate of ketosis compared to the classical ketogenic diet.

Due to its ease of implementation, LGID facilitates patient care, particularly in younger patients, and may reduce hospitalization requirements in this population. It is preferred because it exhibits metabolic effects similar to the classical ketogenic diet while offering higher feasibility (Vega-López et al., 2018).

2.1.5. Cyclical Ketogenic Diet (CKD)

The cyclical ketogenic diet is a nutritional model in which periods of low-carbohydrate intake are periodically interrupted by higher carbohydrate consumption. Typically, carbohydrate intake is kept below 50 grams for several days, followed by days with 50–150 grams of carbohydrates.

This approach was developed primarily for individuals engaged in endurance sports. High-carbohydrate periods aim to replenish muscle glycogen stores. Studies indicate that this diet may support weight loss

in athletes, with losses largely attributable to changes in lean body mass and body water content (Noakes et al., 2017).

2.2. Adverse Effects of the Ketogenic Diet

The ketogenic diet may cause various side effects, particularly during the initial phase. These include gastrointestinal complaints, hypoglycemia, excessive ketosis, disturbances in acid–base balance, increased fatigue, and lethargy.

In older individuals, gastrointestinal issues may compromise the sustainability of this dietary model. Compared to other ketogenic diet types, the classical ketogenic diet requires greater caution due to biological risks and limited applicability. Although short- and long-term physiological effects have been examined in various studies, implementation should consider individual risks (Dhamija et al., 2013).

3. Metabolic Syndrome

In a nationwide study conducted in Türkiye in 2013, the prevalence of metabolic syndrome was found to be 36.6% according to Adult Treatment Panel III (ATP III) criteria and 44.0% according to International Diabetes Federation (IDF) criteria. According to both diagnostic criteria, metabolic syndrome prevalence was higher in women than in men. Additionally, the risk of developing metabolic syndrome was 2.75 times higher in overweight individuals and 7.80

times higher in obese individuals compared to those with normal body weight (Gündoğan et al., 2013).

Studies discuss whether the components of metabolic syndrome arise from different pathophysiological mechanisms or a common pathogenic process. Although no single definitive mechanism has been identified, decreased insulin sensitivity, chronic inflammation, and activation of neuroendocrine regulatory systems are considered contributing factors. Reduced sensitivity to insulin is characterized by impaired glucose utilization by peripheral tissues despite elevated circulating insulin levels, leading to hyperglycemia. Insulin regulates glucose uptake in liver and muscle cells as well as lipogenesis and gluconeogenesis. The development of insulin resistance disrupts these mechanisms, adversely affecting metabolic balance.

An increase in circulating free fatty acids contributes to dyslipidemia by enhancing hepatic triglyceride synthesis. Elevated free fatty acids also exert vasoconstrictive effects on vascular smooth muscle cells, creating a favorable environment for hypertension development. Leptin is an adipokine involved in energy balance regulation and immune system activation. In obesity, leptin levels increase and are associated with elevated cardiovascular morbidity risk (Rochlani et al., 2017).

Lifestyle modifications, including dietary regulation, increased physical activity, and reduction of visceral adipose tissue, are considered primary intervention strategies in managing metabolic syndrome. Metabolic syndrome is not regarded as a single disease but

rather as a cluster of metabolic disorders that increase the risk of cardiovascular disease and type 2 diabetes. Therefore, treatment approaches aim to address risk factors holistically (Samson & Garber, 2014).

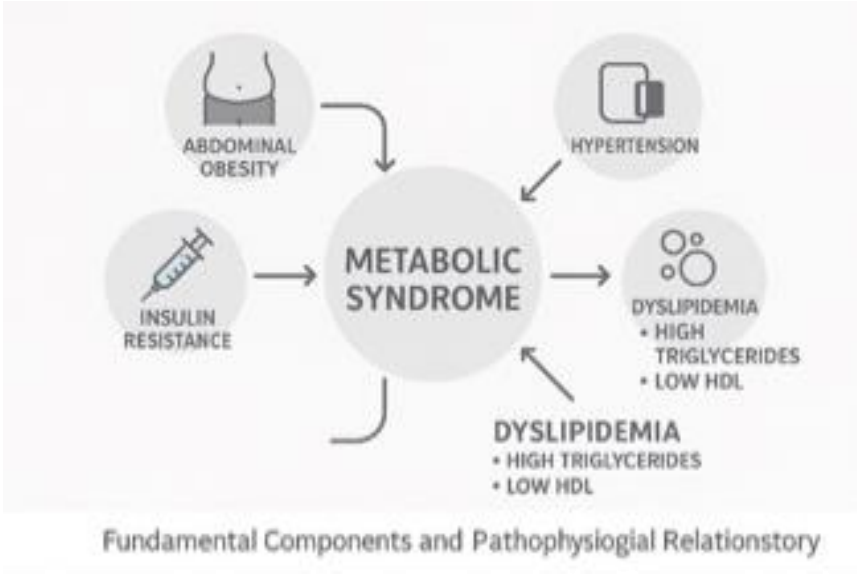

Figure 3. Core components and pathophysiological relationships of metabolic syndrome

4. Effects of Ketogenic Diet Implementation on Metabolic Syndrome

The effects of popular dietary models on disease progression and individual components are a focus of current research. In one study comparing the physiological effects of dietary models with varying carbohydrate levels applied for 28 days, a low-carbohydrate, high-fat

diet yielded more favorable outcomes on LDL cholesterol levels (Hyde et al., 2019).

Following ketogenic diet implementation, individuals enter ketosis, during which improvements are observed in blood glucose levels, fat metabolism, and various biomarkers associated with inflammatory response. These metabolic changes may contribute to increased insulin sensitivity, enhanced fat breakdown, and weight loss primarily from fat mass (Gershuni et al., 2018).

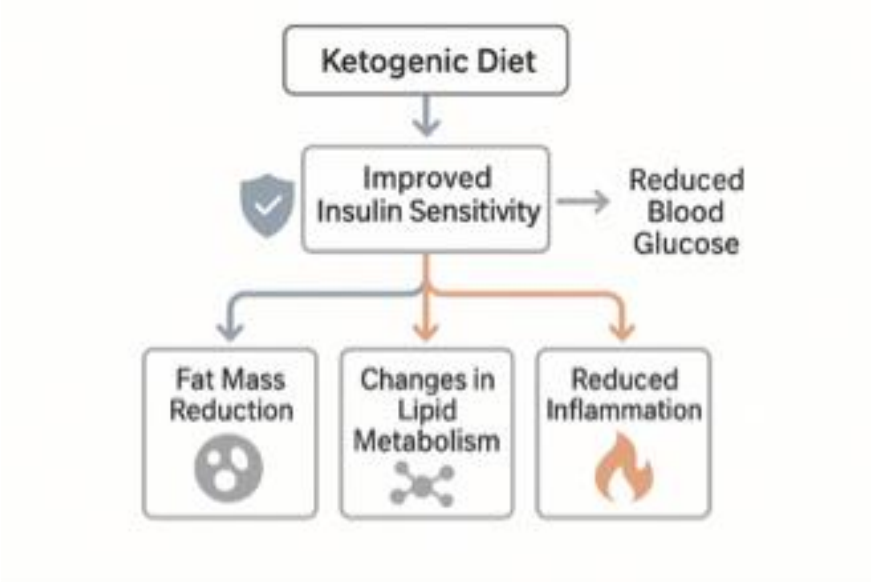

Figure 4. Effects of the ketogenic diet on components of metabolic syndrome

4.1. Effects on Obesity and Body Measurements

Increased fat accumulation in the abdominal region is considered a key component of metabolic syndrome. According to the Turkish Dietary Guidelines, waist circumference values above 80 cm for women and 94 cm for men indicate increased risk, while values above 88 cm for women and 102 cm for men indicate high risk (TUBER, 2022).

The weight loss effects of the ketogenic diet are explained by mechanisms such as reduced energy intake, increased gluconeogenesis due to carbohydrate restriction, appetite suppression during ketosis, and changes in leptin levels. The combined effects of these processes support reductions in body weight and fat mass (Kosinski & Jornayvaz, 2017).

4.2. Effects on Glycemic Control and Insulin Sensitivity

Reducing daily carbohydrate intake through the ketogenic diet decreases insulin secretion and prevents abrupt fluctuations in blood glucose levels. Low-carbohydrate dietary models are considered effective approaches in managing type 2 diabetes due to improvements in fasting insulin levels, HbA1c, and glucose parameters (Locatelli & Mulvihill, 2020).

Individuals following a ketogenic diet have demonstrated greater reductions in HbA1c levels and significant body weight loss compared to those consuming moderate-carbohydrate diets, suggesting a

supportive role for ketogenic diets in glycemic control (Michalczyk et al., 2020).

4.3. Lipid Metabolism and Cardiovascular Risks

Insulin plays a central role in regulating lipid metabolism. Dyslipidemia refers to abnormalities in lipoprotein levels and function. Triglyceride and LDL cholesterol levels are among the key parameters in defining metabolic syndrome.

According to the "lipid energy model" hypothesis, reducing carbohydrate intake increases the use of fatty acids as an energy source, resulting in decreased triglyceride levels. However, marked increases in LDL cholesterol should be considered a potential risk factor for atherosclerosis development (Norwitz et al., 2022). Current literature emphasizes that LDL particle size and ApoB levels may be more informative than traditional LDL measurements.

4.4. Mitochondrial Function and Oxidative Stress

The ketogenic diet may enhance mitochondrial efficiency in cellular energy production. Ketone bodies such as β-hydroxybutyrate activate signaling pathways that stimulate mitochondrial biogenesis and support antioxidant defense mechanisms. These effects may help control oxidative stress by reducing the accumulation of reactive oxygen species (Bough & Rho, 2007).

4.5. Neurological Disorders

The ketogenic diet has historically been used in the treatment of epilepsy. Ketone bodies are believed to exert neuroprotective effects by reducing neuronal excitability. Additionally, by regulating energy metabolism, the ketogenic diet may help preserve neuronal function in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Masino & Rho, 2012).

5. Current Findings and New Approaches

Studies published between 2023 and 2025 focus on the effects of the ketogenic diet on gut microbiota, inflammatory markers, and metabolic flexibility. In particular, adapting the ketogenic diet according to genetic background, microbiota profile, and metabolic phenotype in personalized nutrition approaches is suggested to yield safer and more effective outcomes.

6. Conclusion and Evaluation

Numerous studies have demonstrated that the ketogenic diet improves body measurements in individuals with obesity, supports glycemic control, and may contribute to reducing insulin resistance. Reductions in triglyceride levels and changes in lipid metabolism have also been reported. However, findings regarding the long-term effects of the ketogenic diet are inconsistent, and existing evidence remains limited.

Therefore, the ketogenic diet should be implemented under professional supervision, taking into account the individual's metabolic status, existing diseases, and clinical risks. Large-scale randomized controlled trials are needed to clarify its long-term effects on the components of metabolic syndrome.

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CHAPTER 4

MICROBIOTA IN FORENSIC SCIENCES

Prof. Dr. Hüseyin ÇAKAN

INTRODUCTION

Microorganisms are ubiquitous yet invisible to the naked eye. Since Hippocrates described oral candidiasis in 400 BC, scientists have sought to investigate the roles that fungal and microbial communities, whether commensal or pathogenic, play in human health and disease (Conlan et al., 2012; Kong et al., 2013) (Findley et al., 2013).

Research on the diversity of the human microbiome began in the early 1680s with Antonie van Leewenhoek, who compared the oral and fecal microbiota. In 2007, the US National Institutes of Health (NIH) launched the Human Microbiome Project (HMP), an initiative aimed at characterizing the nasal, oral, skin, gut, and genital microbiomes of 242 healthy volunteers (Mullard, 2008). The concept of the human microbiota was first introduced by Joshua Lederberg, who coined the term "microbiota" to symbolize the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space (Peterson et al., 2009).

Initial findings from the Human Microbiome Project (HMP) have revealed that three classes of bacteria reside within the human body: *Firmicutes, Proteobacteria,* and *Bacteroidetes.* Furthermore, the research indicates that an estimated 50% of this microflora is shared by individuals worldwide. The remaining portion varies due to external

factors such as diet, genetics, antibiotic use, environment, exposure to various food additives, and so on (Dekaboruah et al., 2020).

1.Physiological Basis of the Ketogenic Diet

Quantifying the microbiota is challenging. Recent studies estimate that the human body contains approximately 30 trillion human cells and 39 trillion microbial cells—a nearly equal ratio. Microbiota are so small that, despite their large numbers, their total weight does not exceed one or two kilograms (Savage, 2001; Müller et al., 2014).

The microbiota is as vital as any human organ, but it acts as a hidden organ composed of trillions of individual cells rather than a single, unified mass. Microbiota composition has been shown to vary across multivariate functions such as the environment, geographic distance, salinity, temperature, oxygen, nutrients, pH, day length, and biotic factors (Belkaid and Segre, 2014). The distribution of microbiota in and around us contributes to asthma, diabetes, obesity, infectious diseases, psychiatric illnesses, and other ailments (Toraman et al., 2025). The human microbiome is a target and source for new drugs. It is also an essential tool in precision medicine (Dorrestein et al., 2014).

Neuroscience researchers believe there's a close connection between the brain and gut microbiota (Cryan et al.,2019). The microbiota influences brain function, The factors contributing to gut–brain axis balance are summarized in Fig. 1. (Hou et al., 2022). which is why the gut is often called the "second brain." Links have been observed between gut microbiota and nervous system disorders like depression and autism spectrum disorder (ASD). For example, 70% of the neurochemical

serotonin, which promotes emotional well-being, self-confidence, and restful sleep, is produced in the gut (American Psychological Association, "That Gut Feeling," Carpenter, 2012). If your microbiome is in good shape, your levels of serotonin and

Figure 1. Bidirectional gut-brain axis interactions and the common factors contributing to the gut–brain activity (Hou et al., 2022).

many other neurochemicals are more likely to be normal. Neurochemical balance means we feel calmer, more balanced, more optimistic, more confident, and sleep better. However, if your microbiome is out of balance, levels of neurochemicals like serotonin decrease in the gut.

The microbiota may have the capacity to influence our behavior and mood. Through neural signals sent along nerves from the gut, they can influence our brains by altering sensory receptors, producing toxins that make us feel bad, or releasing chemical reward molecules that make us

feel good. Because the microorganisms living in the gut have a profound impact on our thoughts, emotions, and mood, some researchers have dubbed them "mind-affecting microorganisms" (Scientific American, Hadhazy, 2010).

The gut-brain axis, or the connection between our gut and our brain, has recently been a topic of intense interest for researchers (Cryan et al.,2019). For example, a correlation has been found between anxiety and depression and low levels of *Lactobacillus* helveticus and *Bifidobacterium longum* strains (Öğdür and Çakan, 2022). A balanced balance of microorganisms that live in the mouth and form the microbiome in that area prevents harmful bacteria, which emerge from the food we consume lodged between our teeth, from negatively impacting our oral and dental health (Bozaslan and Çakan, 2021).

The personal microbiota is composed primarily of bacteria, along with viruses, fungi, and eukaryotic microorganisms. Contemporary microbiota studies have abandoned the notion that microorganisms are hostile and harm some cells, and instead embraced a new perspective: the belief that our personal microbiota creates a healthy microbial environment within our bodies. When we examine the microbial communities within our bodies, we see a constantly present microbiota that exists throughout life and, in many cases, exists as commensals, and a transient microorganism community that exists in the body for hours or days, can induce chemical or physical changes, and does not allow the establishment of surrounding microorganisms. The composition of microbiota varies from site to site (depicted in Fig. 2).In

healthy individuals, the intestinal microbiota consists of a complex and diverse community of microorganisms. There are six types of bacterial microbiota: *Firmicutes* (Gram-positive such as *Clostridium sp*., *Eubacterium sp*.), *Bacteroidetes* (Gram-negative such as *Porphyromonas sp., Prevotella sp.), Proteobacteria* (Gram-negative such as *Enterobacteriaceae*), *Actinobacteria* (Gram-positive *Bifidobacterium*), *Fusobacteria* and *Verrucomicrobia* (including genera such as *Akkermansia*) (Hou et al., 2022).

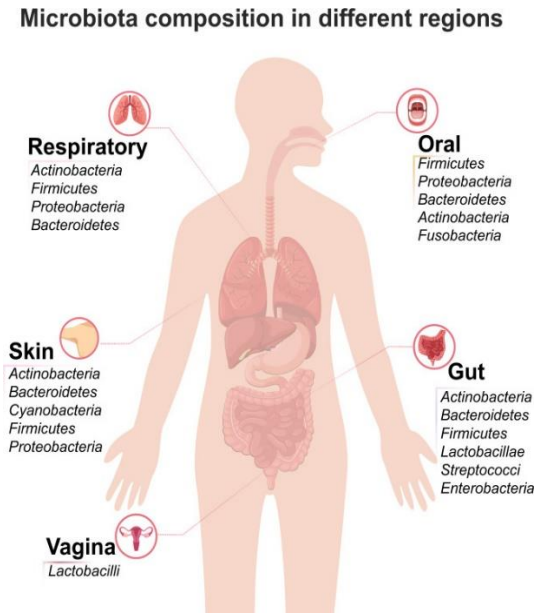

Figure. 2 Human microbiota composition in different locations (Hou et al., 2022).

Before the human microbiome can be used as a study, further investigation is necessary to explore the benefits and potential limitations surrounding microbial profiling. This includes the

detectability of microbial transfer between individuals or items, associated risks (such as contamination events), and the applicability of microbial profiling for forensic purposes (Neckovic et al., 2020).

Forensic microbiology is a sub-branch of forensic sciences and is crucial for identifying microscopic organisms to identify perpetrators. Furthermore, because microscopic organisms present in different parts of a person's body differ among themselves, further examination can be crucial to determine the tissue origin of legal biological traces (Brezee et al., 2011). In reality, it involves uncovering microbial traces on various surfaces, hidden within the traces left on objects by human contact and invisible from the outside. Therefore, individual microflora is a valuable resource for identifying individuals involved in criminal activity today (Çakan, 2019; Çakan and Öğdür, 2024).

The microbiome and microbiota are the populations of pathogenic, symbiotic, and commensal microflora that often interchangeably exist within an individual's body (Knights et al., 2011).As we grow, we acquire numerous bacteria from our food, drink, our environment, and those we come into contact with. These microorganisms can be friendly, hostile, or a combination of the two. In a healthy human body, friendly bacteria predominate. The friendly/hostile ratio is approximately 80/20.Each person carries a collection of microbes that are likely both unique to that individual and dynamic as a result of significant flux with the surrounding environment. It is the interaction between the human microbiome (i.e., the microbes in direct contact with a person in locations such as the gut, mouth, and skin) and the microbiome of

accessory objects (e.g., shoes, clothing, phones, jewelry) that is of potential interest for both epidemiology and the burgeoning field of microbial forensics. Therefore, the microbiome of personal accessories is of interest as it serves as both a microbial source and reservoir for an individual, can provide information about microbial exposure experienced by an individual, and can be sampled non-invasively (Coil et al., 2020).

The personal microbiome, defined as the collection of microbes associated with an individual's personal influences (i.e., the items regularly worn or carried on a person), likely varies from person to person (Meadow et al., 2014a). Research has shown that traces of human microbiota remain in the rooms we occupy and on the surfaces we touch (Meadow et al., 2014a). In some cases, these microbial signatures can be attributed to individual individuals (Fierer et al., 2010). Therefore, mobile phones likely carry a strong signal of their owner's human microbiome and may be identifiable by that owner (Meadow et al., 2014b; Relay, 2024).

2. The Microbiome's Relevance to Forensic Science

Research on microbial interactions between individuals and their environments has revealed the microbiome's forensic capacity. Under certain circumstances, human microbial signatures have been applied to match individuals with the objects they interact with, including computer keyboards (Fierer et al., 2010).Studies of the microbiological makeup of multiple living spaces have shown that a household's microbiological signature can largely predict its microbiome and has

characteristics that distinguish individuals within a household (Lax et al., 2014).

In forensic science, microbiology has focused on research using microbes or their products (e.g., toxins) as weapons and/or biological threats. However, technological advances over the last 15 years have rapidly revealed the richness and abundance of the microbial world, allowing the field to expand rapidly into areas where microbes and their products can assist in human identification, time-of-death determination (Castello et al., 2009), and other forensic investigations (Fierer et al., 2010). Microbes are present at every crime scene and have been used as physical evidence for over a century. Advances in DNA sequencing and computational approaches have led to recent breakthroughs in the use of microbiome approaches for forensic science, particularly in estimating postmortem intervals (PMIs), locating clandestine graves, and recovering soil and skin impression evidence (Çevik and Çakan 2020; Jun et al.,2022; Fig.3)

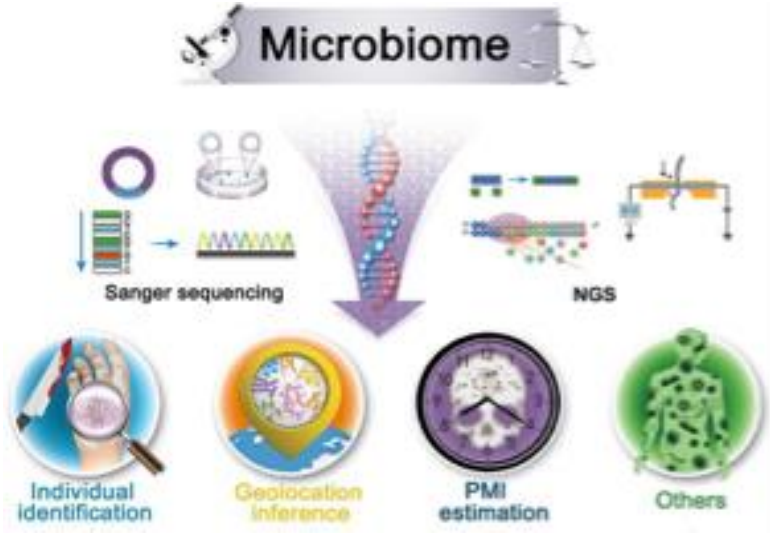

Figure 3. Analysis of microbiome data in forensic science (Jun et al.,2022).

Microbial interactions between human-associated objects and the environments we inhabit can have forensic implications, and the degree to which microbes are shared among individuals in the same area may also be relevant to human health and disease transmission ((Lax et al., 2014). Human DNA-based identification testing allows for the analysis of stable heritable markers in the human genome related to individual ancestry, relatedness, ancestry, and phenotype. In contrast, forensic microbiome testing allows for the analysis of both stable and fluctuating changes in microbial communities within the body related to individuals, diet, health, recent location, and postmortem time intervals. Microorganisms carried by humans and their accompanying nucleic acids are routinely shed, stored, and altered, similar to human DNA used to identify individuals who commit sexual assault, homicide, and theft. (Metcalf et al.,2017).

Additionally, because microorganisms found in different parts of the human body differ from one another, additional research value can be gained from determining the tissue source of forensic biological evidence. Therefore, the human microbiome could be another target for identifying (or excluding) individuals involved in crimes (Castello et al., 2009).

We humans release bacterial products into the air around us. But we also shed bacteria themselves. We are all constantly sowing our microbes into the world. Every time we touch an object, we leave our

microbial trail. Every time we walk, talk, scratch or sneeze, we release our own unique cloud of microbes into the air (Turnbaugh et al., 2007). We spray approximately 37 million bacteria per person per hour. Researchers continue to study the microbiomes of cities, buildings, hospitals, aquariums, gyms, and school dormitories. The "Home Microbiome Project," one of these studies, has revealed that people can be traced to some extent by the microbes they leave behind (Adams et al., 2015).

Studies conducted in recent years have shown that microorganisms occupy an estimated 1-3% of an individual's body weight, and that on a cell-by-cell basis, they outnumber other cells by 10 times. Furthermore, these microbial cells possess 150 times more genes than a person's genetic code, and this genome they create is called the microorganism flora. This raises the question: "Do our bodies belong to our microflora, or do we?" (Aslan and Altındiş, 2017).

Fierer and colleagues investigated whether the microbial characteristics of human finger residues left on inanimate objects could provide a sufficient pattern that could be used to generate a useful microbial fingerprint for forensic purposes (Fierer et al., 2010). Future advances in this area could include greater sequencing depth, microbial genes beyond 16S rRNA, or inanimate objects such as glass, ceramics, or even clothing. Just as a detective can detect a unique print, we can track a microbial print using nucleic acid technologies and information technologies (Blaser, 2010). Identifying the tissue source of forensic biological samples can be critical in some investigations to reconstruct

crime scenes and events, but current techniques are limited. Possible tests for certain body fluids are used as screening tests and tend to have specificity limitations.

3. Evaluation

Based on the extensive scientific literature we've compiled, we must evaluate whether we can demonstrate that these tiny, invisible organisms can exist on objects, just as the Dutchman Antonie Van Leewenhoek demonstrated many years ago by examining them with a simple microscope, and thus, we can infer something in the criminal field.

Research on the microbiota has shed light on many issues related to human life (Yong, 2018). Today, we need to identify and thoroughly understand the bacteria present in various locations where many criminal incidents occur, perhaps even on objects used, in the trace data they leave behind, linking them to the crime scene, the suspect, and the victim. In other words, if a microbial trace unique to an individual, such as a suspect within a group we're investigating, should be evaluated from a different perspective than the others. In fact, as mentioned, if a single bacteria found is found only on that individual, it requires a more cautious approach and detailed consideration.

In reality, because these microorganisms vary temporally among individuals, potential forensic applications of the microbiome complicate human identification. These data demonstrate that the differences between bacterial communities are substantial. Future criminal studies using these microbial communities, which inhabit the

human body and can contaminate objects with which individuals come into contact, will contribute to forensic science by demonstrating a clear distinction between individuals and contributing to identification. In forensic science, it is possible that traces of microorganisms present on the hands at the time of contact with objects will be found on the objects and can be matched by similarity or exclusion. Microbiota profiles can be used to confirm or refute individuals' claims regarding their whereabouts and contacts (Turnbaugh et al., 2008). Recent studies of microbial communities inhabiting the human body have revealed strong differences in community membership among individuals. Some of this variation is stable over time, and individuals may possess unique microbial "fingerprints" that distinguish them from the general population. Microbiota research provides us with a new perspective on who we are, our place in the world, and our responsibilities to each other.

4.Conclusion

Our microbiota provides small traces of evidence that can sometimes be useful in forensic investigations that are often inconclusive in the face of rapidly advancing technological innovations. It should not be forgotten that human DNA, the most important biological evidence in personification, may play a decisive role in challenging cases. Of course, all available evidence must be analyzed in detail when solving a forensic case. However, we anticipate that bacteria, the living witnesses within our microbiota, are as important as visible evidence and require more comprehensive and diverse study plans. In fact, we

must reiterate the fundamental principle of the renowned French criminal investigator Edmond Locard, the "Principle of Change." We believe that future studies will enable researchers to uncover traces of various objects and interpret them.

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CHAPTER 5

DOSE- DEPENDENT MODULATION OF LIVER PROTEIN AND ANTIOXIDANT ENZYME LEVELS BY LAVENDER EXTRACT IN PUBERTAL SYRIAN HAMSTERS

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Asst. Prof. Neslihan GÜLEÇ

Prof. Dr. Bülent GÜNDÜZ

INTRODUCTION

Gestation and lactation are physiologically demanding periods characterized by significant metabolic hormonal, and biochemical alterations within the organism. The elevated energy demands during these stages lead to increased mitochondrial activity, which subsequently results in an overproduction of reactive oxygen species (ROS). A disruption in the equilibrium between ROS production and antioxidant defense mechanisms leads to oxidative stress; this condition can cause damage to cellular macromolecules and negatively impact tissue functions (Agarwal et al., 2005). It has been reported that oxidative stress occurring during critical developmental stage, such as gestation and the early postnatal period, can affect not only the maternal organism but also the long-term physiological and biochemical characteristics of the offspring (Barker, 2007).

One of the most significant consequences of oxidative stress is lipid peroxidation. Polyunsaturated fatty acids, which are essential structural

components of cell membranes, undergo peroxidation under the influence of free radicals, leading to the disruption of membrane integrity and the impairment of cellular functions (Halliwell & Gutteridge, 2015). To counteract these deleterious effects, the organism has evolved enzymatic antioxidant defense systems. Superoxide dismutase (SOD) acts at the first stage of oxidative damage by converting superoxide anions into hydrogen peroxide; meanwhile, catalase (CAT) protects cells from oxidative injury by facilitating the decomposition of hydrogen peroxide into water and oxygen. The activity levels of these enzymes are among the most widely used biochemical indicators for assessing the oxidative status of tissues (Birben et al., 2012). Additionally, total protein levels serve as a cricial parameter providing general information regarding the metabolic state and structural integrity of tissues.

Melatonin is a hormone primarily secreted by the pineal gland that plays a pivotal role in regulating circadian rhythms and is known to interact with antioxidant defense mechanisms. Its regulatory effects on oxidative stress contribute significantly to maintaining redox balance, particularly in metabolic tissues (Reiter et al., 2014; Hardeland, 2019).However, in the present study, melatonin levels were not directly measured; rather, the melatonin-oxidative stress relationship was addressed within theoretical framwork of the research.

In recent years, plant-derived natural compounds have gained considerable attention as potential prodective agents in the modulation of oxidative stress. Lavender (*Lavandula angustifolia*) is an aromatic

plant long utilized in traditional medicine, with its biological effects being extensively investigated in modern pharmacological studies. Various experimental studies have reported that linalool and linalyl acetate, the primary active components of lavender, exhibit potent antioxidant and anti-inflammatory properties (Cavanagh & Wilkinson, 2002; Hancianu et al., 2013). Nevertheless, data regarding the long-term effects of lavender extract exposure during gestation and lactation on the oxidative stress responses of offspring in the postnatal period remain limited.

It is well established that exposure to biological agents during gestation and the early postnatal period can lead to permanent physiological alterations in later life through developmental programming mechanisms (Gluckman et al., 2007). These effects can be particularly pronounced in the liver, which plays a central role in metabolic and detoxification functions.

The aim of this study was to evaluate the levels of total protein, superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) in the liver tissue of 60-day-old male hamster offspring whose mothers were treated with lavender extract througout gestation and lactation.

MATERIAL AND METHODS

Preparation of *Lavandula angustifolia* Extract

The *Lavandula angustifolia* used in this research was obtained from a local herbalist in Çanakkale, Türkiye. The dried flowers were

pulverized into a fine powder using a laboratory-scale mechanical grinder to ensure a homogeneous particle size. A 50 g prortion of the ground palnt material was subjected to continuous extraction in an soxhlet apparatus with 400 mLof 70% ethanol for 8 hours.

Following the extraction process, the solvent phase was removed using a rotary evaporator at 40 °C. The resulting concentrated extract was stored at +4°C under dark conditions. For expermental applications, extract solutions were prepared at the desired concentration using physiological saline.

Gas Chromatography-MAss Spectrometry (GC-MS) Analysis of the Extract

Since the composition of essential oils and other bioactive compounds in *Lavandula angustifolia* can vary depending on environmental factors such as habitat, climatic conditions, and altitude, GC-MS analysis was performed to determine the chemical profile of the extract.

Experimental Design

This study was conducted with the approval of the Çanakkale Onsekiz Mart University Animal Care and Use Ethics Committee (Protocol No: 2021/06-01). A total of 8 breeder and 40 male offspring Syrian hamsters (*Mesocricetus auratus*), housed under long photoperiod conditions (16h light/8 h dark), were used in the study. The male offspring were randomly divided into four groups (n=10 per group): control group and three expermental groups treated with 50, 100, and 200 mg/kg of *L. angustifolia* extract, respectively. Each group was further divided into

two subgroups to evaluate the effects of circadian rhythm: midday (12:00, n=5) and midnight (00:00, n=5).

During the final week of gestation and the first 15 days of lactation, dams in the experimental groups received daily, intraperitoneal (i.p.) injections of the respective doses of lavender extract at 12:00. Control dams were injected with 0.9% sterile physiological saline at the same time.

Starting from postnatal day 20 (PND20), male hamsters were administered the designated doses of lavender extract through i.p. injection for 40 days. Control hamsters received 0.9% saline by the same route and duration. At the end of the experimental period, blood and liver tissue samples were collected by decapitation without the use of anesthetic agents.

Biochemical Analyses

Serum total protein levels were determined using the Sigma-Aldrich Protein Quantification Kit–Rapid (Cat. No: 51254-1KT). Superoxide dismutase (SOD) and catalase (CAT) activities in liver tissues were measured using SunRed Rat SOD (Cat. No: 201-11-0169) and Rat CAT (Cat. No: 201-11-5106) ELISA kits, respectively. Serum lipid peroxidation (LPO) levels were evaluated with a BT-LAB Rat LPO ELISA kit (Cat. No: E0285Ra).

All absorbance measurements were performed using a Chromate 4300 microplate reader (Awareness Technology, Inc., USA). For the data evaluation of total protein and LPO analyses, a four-parameter logistic

(4PL) regression model was employed, whereas SOD and CAT activities were calculated using linear regression analysis. The results were expressed in ng/mL for total protein and antioxidant enzymes and in nmol/mL for lipid peroxidation.

RESULT

GC-MS Analysis of *Lavandula angustifolia* Extract

The chemical composition of the ethanolic extract obtained from *Lavandula angustifolia* flowers was determined via gas chromatography-mass spectrometry (GC-MS) analysis. The total ion chromatogram (TIC) revealed that the extract contains a large number of volatile and semi-volatile components.

A total of 49 compounds were identified through GC-MS analysis. With the primary constituents characterized as monoterpenes and oxygenated monoterpene derivatives. The most abundant compounds were identified as linalool (14.02%), coumarin (10.38%), linalyl acetate (1.86%), camphor (4.09%), borneol (3.81%), linalool oxide isomers, and α-bisabolol (1.24%) (Table 1).

Table 1. Adjustable particles and relative area percentages in ethanolic extract of *Lavandula angustifolia* by GC-MS analysis.

Pik No	RT	Component	%
1	13.46	Eucalyptol	1.60
2	13.90	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	0.90
3	15.25	LINALOOL OXIDE CIS	2.84
4	15.88	Linalool oxide <cis->	2.42

5	16.55	Linalool	14.02
6	16.66	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	0.41
7	18.02	Camphor	4.09
8	18.54	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.41
9	18.81	Acetic acid, 3-methyl-6-oxo-hex-2-enyl ester	0.53
10	19.05	BORNEOL L	3.81
11	19.17	Epoxylinalol	0.62
12	19.40	Epoxylinalol	0.36
13	19.89	Isobutyrate <hexyl->	0.25
14	20.29	3,7-dimethyl-1,5-octadien-3,7-diol	3.01
15	22.34	Linalyl acetate	18.56
16	23.36	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	1.25
17	23.54	Lavandulyl acetate	0.76
18	24.61	2-Methoxy-4-vinylphenol	0.59
19	24.97	Cyclobutanecarboxylic acid, octyl ester	0.77
20	25.44	8-ACETOXYLINALOOL	4.91
21	25.74	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	1.28
22	26.51	Bicyclo[2.2.1]heptan-2-ol, 7,7-dimethyl-, acetate (CAS)	0.42
23	26.76	Geranyl acetate	0.24
24	26.89	Coumarin <3,4-dihydro->	0.42
25	27.30	3,7-Nonadien-2-ol, 4,8-dimethyl-	0.45
26	27.43	LINALOOL OXIDE CIS	0.61
27	27.93	8-ACETOXYLINALOOL	1.05
28	28.05	Caryophyllene	0.39
29	28.88	Coumarin	10.38
30	29.22	Farnesene <(E)-, beta->	0.77
31	30.32	6,7-Dioxabicyclo[3.2.1]octane, 1-methyl-	0.46

32	30.78	8-ACETOXYLINALOOL	0.55
33	30.91	Neryl (S)-2-methylbutanoate	0.28
34	31.18	(+)-Aromadendrene	0.49
35	31.32	trans-Linalool oxide	1.50
36	31.47	Linalool oxide <trans->	1.47
37	31.87	Octanal, 7-methoxy-3,7-dimethyl-	0.79
38	32.09	Octanal, 7-methoxy-3,7-dimethyl-	0.55
39	33.37	Caryophyllene oxide	0.72
40	35.10	3-(2'-Hydroxy-4'-methoxyphenyl) propanoic acid	0.37
41	36.39	Bisabolol <alpha->	1.24
42	37.74	2H-1-Benzopyran-2-one,7-methoxy-	5.53
43	43.27	Ascaridole	0.72
44	44.04	Palmitic acid	1.79
45	44.24	LIMONENE DIOXIDE 1	0.55
46	48.33	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	1.73
47	48.84	Octadecanoic acid	1.10
48	50.90	Tributyl acetylcitrate	0.74
49	53.38	Palmitate <isopropyl->	0.28
			100.00

Furthermore, lower concentrations of caryophyllene, farnesene, geranyl acetate, lavandulyl acetate, and various oxygenated terpenoid compounds were detected within the extract. A significant portion of the identified components are known to be volatile compounds that exhibit antioxidant, anti-inflammatory, and other biological activities in the literature.

In this study, the levels of total protein, superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) were evaluated in the liver

tissues of male hamster offspring exposed to lavender extract throughout the gestation and lactation periods until postnatal day 60. The obtained mean values are presented in Table 2.

Liver Total Protein Levels

When the liver total protein levels were analyzed, significant variations were observed in the groups treated with lavender extract compared to the control group. The highest total protein level was recorded in the 50 mg/kg lavender extract group (8475.35 µg/mL), followed by the 100 mg/kg (8096.65 µg/mL). In the control group, the total protein level was measured at 6602.65 µg/mL. Conversely, the lowest total protein value was detected in the 200 mg/kg lavender extract group (5648.45 µg/mL) (Table 2).

Liver Antioxidant Enzyme Activities

The evaluation of liver SOD activity, highest mean value was observed in the control group (21.584 ng/mL). Among the lavender extract experimental groups, SOD activity levels were determined as 15.341 ng/mL, 14.669 ng/mL, and 20.556 ng/mL for the 50, 100, and 200 mg/kg groups, respectively (Table 2). Regarding CAT activity, the value measured at 74.422 ng/mL in the control group was found to be 53.424 and 47.677 ng/mL in the groups treated with 50 and 100 mg/kg of lavender extract, respectively. In contrast, CAT activity in the group administered with 200 mg/kg of lavender extract was determined to be 72.875 ng/mL (Table 2).

Liver Lipid Peroxidation (LPO) Levels

The LPO level in the control group was recorded as 18.08 nmol/mL. Among the groups treated with lavender extract, the lowest LPO value was identified in the 50 mg/kg group (14.851 nmol/mL). While the LPO level in the 100 mg/kg was measured at 16.231 nmol/mL, it was detected at 18.607 nmol/mL in the 200 mg/kg group (Table 2).

Table 2. Total protein, SOD, CAT, and LPO levels in liver tissue of male offspring obtained from hamsters exposed to lavender extract during pregnancy and lactation were measured at day 60.

Group	Total Protein (µg/mL)	SOD (ng/mL)	CAT (ng/mL)	LPO (nmol/mL)
Control	6602.65	21.584	74.422	18.08
50 mg/kg	8475.35	15.341	53.424	14.851
100 mg/kg	8096.65	14.669	47.677	16.231
200 mg/kg	5648.45	20.556	72.875	18.607

DISCUSSION

The present study investigated the long-term effects of maternal and postnatal exposure to *Lavandula angustifolia* extract on hepatic

oxidative stress and antioxidant defense parameters in 60-day-old male Syrian hamster offspring. By evaluating total protein content, antioxidant enzyme activities (SOD and CAT), and lipid peroxidation levels in liver tissue, this research provides insight into how exposure to a commonly used medicinal plant during critical developmental windows—gestation and lactation—may influence redox homeostasis later in life. The findings demonstrate that lavender extract exerts dose-dependent effects on hepatic biochemical parameters, supporting the concept that early-life exposure to bioactive plant compounds can induce persistent physiological adaptations.

Chemical composition and biological relevance of the extract

GC-MS analysis revealed that the ethanolic extract of *L. angustifolia* flowers was rich in monoterpenes and oxygenated monoterpene derivatives, with linalool, coumarin, camphor, borneol, and linalyl acetate being the predominant constituents. This chemical profile aligns closely with previously reported compositions of lavender extracts and essential oils, although the relative abundance of individual compounds may vary depending on extraction method, solvent polarity, geographical origin, and environmental conditions (Cavanagh & Wilkinson, 2002; Lis-Balchin, 2002). Many of the identified compounds have been shown to possess antioxidant, anti-inflammatory, and cytoprotective properties, which provides a plausible biochemical basis for the observed effects on hepatic oxidative stress parameters.

Linalool, in particular, has been reported to scavenge free radicals and modulate oxidative stress pathways by reducing lipid peroxidation and altering antioxidant enzyme activity in experimental models (Peana et al., 2002; Hancianu et al., 2013). Similarly, borneol and camphor have been shown to influence cellular redox status and membrane stability, while coumarin derivatives are known for their capacity to modulate oxidative and inflammatory signaling pathways. The presence of these compounds suggests that the lavender extract used in this study possesses a multifaceted biological activity rather than acting through a single mechanism.

Effects on hepatic total protein levels

One of the notable findings of this study was the increase in hepatic total protein levels in offspring exposed to low (50 mg/kg) and medium (100 mg/kg) doses of lavender extract, whereas a reduction was observed at the highest dose (200 mg/kg). Total protein content in liver tissue is often regarded as a general indicator of metabolic activity, protein synthesis capacity, and cellular integrity. The elevated protein levels observed at lower doses may reflect enhanced hepatic metabolic function or improved cellular preservation, potentially due to the antioxidant and cytoprotective effects of lavender-derived compounds.

These findings are consistent with previous studies reporting that low doses of plant-derived antioxidants can support protein synthesis and stabilize cellular structures by reducing oxidative damage to proteins and nucleic acids (Jaeschke et al., 2012). Conversely, the reduction in

total protein observed at the highest dose suggests the possibility of a threshold beyond which beneficial effects diminish or reverse. High concentrations of certain phytochemicals may exert cytotoxic or metabolic stress effects, leading to altered protein turnover or impaired synthesis. This biphasic, dose-dependent response—often referred to as hormesis—is a well-recognized phenomenon in phytotherapy and toxicology and underscores the importance of dose optimization when considering herbal interventions, particularly during sensitive developmental periods.

Antioxidant enzyme activities and redox adaptation

The activities of SOD and CAT exhibited a complex, dose-dependent pattern in lavender-treated groups. Both enzymes showed lower activity levels in the low- and medium-dose groups compared to the control group, while values in the high-dose group approached those of controls. At first glance, a reduction in antioxidant enzyme activity might appear contradictory to the presumed antioxidant effects of lavender extract. However, this observation can be interpreted within the framework of redox homeostasis rather than simple antioxidant upregulation.

Antioxidant enzymes such as SOD and CAT are inducible systems that respond dynamically to the level of oxidative challenge. When oxidative stress is reduced due to the presence of effective radical scavengers, the cellular demand for enzymatic antioxidant activity may decrease, resulting in lower measured enzyme levels (Halliwell &

Gutteridge, 2015). In this context, the reduced SOD and CAT activities observed in the 50 and 100 mg/kg groups may reflect a decreased oxidative burden rather than impaired antioxidant defense.

This interpretation is further supported by the concomitant reduction in lipid peroxidation levels, particularly in the low-dose group. Similar patterns have been reported in studies investigating natural antioxidants, where decreases in lipid peroxidation are accompanied by unchanged or reduced antioxidant enzyme activities (Sebai et al., 2013). In contrast, the near-normalization of SOD and CAT activities in the high-dose group may indicate that higher concentrations of lavender extract do not further suppress oxidative stress or may even elicit compensatory responses to maintain redox balance.

Lipid peroxidation and membrane integrity

Lipid peroxidation is widely regarded as one of the most reliable markers of oxidative stress, as it directly reflects oxidative damage to cell membranes and lipid-containing structures. In the present study, the lowest LPO levels were observed in the 50 mg/kg lavender-treated group, indicating a protective effect against lipid peroxidation at low doses. This finding is in agreement with previous reports demonstrating that lavender extract and its major constituents reduce malondialdehyde and other lipid peroxidation products in various tissues (Chen et al., 2023).

The moderate reduction in LPO observed in the 100 mg/kg group suggests that the antioxidant effect persists but may be less pronounced at higher doses. Notably, LPO levels in the 200 mg/kg group were comparable to those of the control group, indicating a loss of protective efficacy. This pattern supports the hypothesis that the antioxidant potential of lavender extract may reach a saturation point or that certain constituents may exert pro-oxidant effects at elevated concentrations. Such dual behavior has been described for several phenolic and terpenoid compounds, which can act as antioxidants at low concentrations and pro-oxidants under specific conditions (Halliwell, 2008).

Developmental programming and long-term effects

A key strength of this study lies in its focus on exposure during gestation and lactation, periods characterized by heightened vulnerability to environmental and biochemical influences. According to the developmental origins of health and disease (DOHaD) hypothesis, early-life exposures can induce long-lasting changes in organ structure and function through epigenetic and metabolic programming mechanisms (Gluckman et al., 2007; Barker, 2007). The liver, as a central organ in metabolism and detoxification, is particularly susceptible to such programming effects.

The persistence of altered oxidative stress parameters at postnatal day 60 suggests that lavender extract exposure during early development may induce lasting adaptations in hepatic redox regulation. These

adaptations may be beneficial at appropriate doses, as evidenced by reduced lipid peroxidation and maintained protein levels, but could potentially be neutral or adverse at higher doses. This highlights the importance of carefully evaluating not only the immediate but also the long-term consequences of herbal compound exposure during critical developmental windows.

Potential involvement of melatonin-related mechanisms

Although melatonin levels were not directly measured in this study, the potential interaction between lavender extract and the melatonergic system warrants consideration. Melatonin is a potent endogenous antioxidant that directly scavenges free radicals and modulates antioxidant enzyme expression (Reiter et al., 2013; Hardeland, 2018). Lavender has been widely reported to influence circadian rhythms, sleep quality, and neuroendocrine regulation, suggesting a possible indirect modulation of melatonin secretion or signaling pathways.

Given that the experimental design included circadian considerations and exposure during pregnancy and lactation—periods critical for circadian and endocrine system development—it is plausible that some of the observed effects on oxidative stress parameters may be mediated, at least in part, through melatonin-related mechanisms. Future studies incorporating direct measurements of melatonin and clock gene expression would be valuable in clarifying this potential interaction.

Limitations and future directions

Despite its contributions, this study has several limitations that should be acknowledged. The exclusive focus on male offspring limits the generalizability of the findings, as sex-specific differences in oxidative stress regulation are well documented. Additionally, the use of ELISA-based measurements provides valuable quantitative data but does not capture gene expression or enzyme activity at the transcriptional and post-translational levels. Expanding the panel of oxidative stress markers, including glutathione-related parameters and inflammatory mediators, would offer a more comprehensive understanding of the underlying mechanisms.

CONCLUSION

In summary, the findings of this study demonstrate that *Lavandula angustifolia* extract administered during gestation and lactation exerts dose-dependent effects on hepatic oxidative stress and antioxidant defense parameters in male hamster offspring. Low-dose exposure appears to support redox balance by reducing lipid peroxidation and maintaining protein integrity, whereas higher doses do not confer additional benefits. These results underscore the importance of dosage considerations and highlight the potential long-term biochemical consequences of early-life exposure to herbal agents. The study contributes to the growing body of literature on developmental programming and natural antioxidants and provides a foundation for future mechanistic and translational research.

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