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Impact of Infectious Exposure on Baseline Gut Microbiota in Obesity and Type 2 Diabetes Mellitus

(SIDERALE Project)

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Abstract

Obesity and type 2 diabetes mellitus (T2DM) are chronic metabolic disorders characterized by systemic inflammation, immune dysregulation, and alterations in host–microbiota interactions. These metabolic conditions may also influence susceptibility to persistent infections and potentially modulate the composition of the gut microbiota. The aim of this study was to investigate whether individuals with obesity and T2DM exhibit differences in baseline gut microbiota composition according to the presence or absence of chronic bacterial and/or viral infections.

This observational, non-interventional study included 41 patients (14 females and 27 males) with obesity and T2DM, stratified according to the presence or absence of chronic infections and further categorized by sex. Baseline fecal samples were collected for gut microbiota analysis and microbial profiling was performed using 16S rDNA gene sequencing. Microbial diversity was evaluated using alpha diversity indices (Observed, Shannon, and Simpson), while differential abundance analysis was performed using Linear Discriminant Analysis Effect Size (LEfSe).

No statistically significant differences in alpha diversity were observed between patients with and without chronic infections. However, consistent directional trends toward reduced microbial richness and diversity were observed among infected individuals. LEfSe analysis identified two bacterial taxa, *Blautia intestinalis* and *Dorea* sp., with elevated LDA scores, showing higher relative abundance in non-infectious subgroups compared with infectious subgroups. Although these differences did not remain statistically significant after false discovery rate correction, both taxa are known to be associated with short-chain fatty acid production and metabolic homeostasis.

Overall, the findings suggest the presence of subtle directional trends in gut microbiota composition associated with chronic infectious exposure in individuals with obesity and T2DM. While statistical significance was not achieved, likely due to the limited sample size, these observations highlight potentially relevant microbial signals that warrant further investigation in larger and more statistically robust populations.

Keywords: obesity; type 2 diabetes mellitus; gut microbiota; chronic infection; microbial diversity; 16S rDNA sequencing.

1. Definition and Classification of Obesity

Obesity has now become one of the most important global public health problems, affecting individuals of all ages, socioeconomic backgrounds, and geographic regions worldwide. It is a major risk factor for several chronic diseases, including type 2 diabetes, cardiovascular disease, certain types of cancer, respiratory disorders, and musculoskeletal conditions (Ahmed & Mohammed, 2025). Obesity is seen as a complex condition influenced by genetic, environmental, and behavioral factors, as evidenced by the low success rates of sustainable weight control and prevention despite advancements in nutrition research and public health programs (Kong *et al*., 2025). The World Obesity Federation has called this a global epidemic and emphasizes the need for swift action to control it. It also argues that obesity is a chronic, relapsing, and progressive disease process (Bray *et al*., 2017).

Obesity is a chronic complex disease defined by excessive fat deposits that can impair health. Body mass index (BMI) is a basic anthropometric measure that relates height and weight and is frequently used to determine whether or not humans have extra body fat. It is calculated by dividing weight (kg) by the square of height (m) (kg/m2). According to the World Health Organization (WHO), individuals with a BMI of 25.0–29.9 kg/m² are classified as overweight, while those with a BMI of 30 kg/m² or higher are classified as obese. Obesity is further categorized into three classes: Class I (30.0–34.9 kg/m²), Class II (35.0–39.9 kg/m²), and Class III (≥40 kg/m²), the latter of which is typically associated with severe metabolic and clinical complications. Body Mass Index (BMI) is widely used in clinical and epidemiological studies because it is easy to apply and provides rapid results. However, it has significant limitations when it comes to assessing obesity at the individual level. BMI cannot distinguish between body fat and lean tissues such as muscle, water, and bone. Therefore, it can lead to misclassifications in individuals with high muscle mass, the elderly, children and adolescents, athletes, or those undergoing weight loss (Sweatt *et al*., 2024). While precision medicine is evolving today, BMI alone is not sufficient to understand the complex nature of obesity or create a personalized treatment plan. More detailed assessment of factors such as metabolic status, how fat is distributed in the body, visceral fat, muscle mass, and obesity-related health problems is necessary. Therefore, the use of phenotyping methods that include more advanced techniques such as body composition measurements, functional assessments, genetic data, and metabolic markers is recommended (Salmón-Gómez et al., 2023).

All these data demonstrate that obesity is a multifaceted disease that requires comprehensive assessment techniques, not just BMI, to be accurately classified. All things considered, obesity

cannot be considered solely as a personal choice or behavior. It should also be viewed as a multifaceted disease resulting from complex interactions among environmental, genetic, metabolic, and social factors (Ahmed & Mohammed, 2025).

1.1 Epidemiology

Obesity has become one of the fastest growing public health problems worldwide, with its prevalence increasing across all age groups and regions. The World Health Organization (WHO) reports that throughout the past four decades, obesity rates have almost tripled worldwide. Significant changes in food habits, environmental exposures, and lifestyle are reflected in this increase. Recent analyses emphasize that obesity is a multifactorial condition shaped by complex interactions between biological, behavioral, socioeconomic and environmental determinants (Chang Chusan et al., 2025). The escalating burden of obesity has led to substantial increases in noncommunicable diseases, including type 2 diabetes, cardiovascular disorders, chronic respiratory conditions and several cancer types, placing a significant strain on healthcare systems globally (Ahmed & Mohammed, 2025).

The worldwide prevalence of overweight and obesity has doubled since 1980 to an extent that nearly a third of the world's population is now classified as overweight or obese. Globally, a total of 1.9 billion and 609 million adults were estimated to be overweight and obese in 2015, respectively, representing approximately 39% of the world's population. Obesity rates have increased in all ages and both sexes irrespective of geographical locality, ethnicity or socioeconomic status, although the prevalence of obesity is generally greater in older persons and women. The rates of both overweight and obesity increase with age from young adulthood onwards, reach their peak between the ages of 50 and 65 years, and decline slightly thereafter, with obesity being more prevalent in women than men across most age groups. Although absolute prevalence rates of overweight and obesity vary widely, similar increasing trends have been observed across regions and countries (Chooi et al., 2019).

Once regarded as a problem only in high-income countries, overweight and obesity are also rising in low- and middle-income countries facing a "double burden" of disease. While these countries continue to deal with infectious diseases and undernutrition, they are simultaneously experiencing a rapid upsurge in noncommunicable disease risk factors such as obesity and overweight, particularly in urban settings (Kitzinger & Karle, 2013).

Body mass index (BMI) is typically used to define overweight and obesity in epidemiological studies; however, BMI has low sensitivity and there is a large inter-individual variability in percent

body fat for any given BMI value, partly attributed to age, sex, and ethnicity. Furthermore, greater cardiometabolic risk has been associated with the localization of excess fat in visceral adipose tissue and ectopic depots, suggesting that obesity may be far more common than what large epidemiological studies relying solely on BMI estimates suggest (Chooi et al., 2019).

1.2 Biological Basis of Obesity

Obesity considered as a chronic relapsing disease process in the biological systems that regulate appetite, energy expenditure, metabolism, and immune responses. A substantial body of evidence supports the view of obesity as a chronic, relapsing disease with physiological adaptations that defend increased body weight and promote weight regain. Food is the primary agent, particularly foods that are high in energy density such as fat, or in sugar-sweetened beverages. An abundance of food, low physical activity and several other environmental factors interact with the genetic susceptibility of the host to produce positive energy balance. Most of the excess energy consumed is stored as fat in enlarged and numerous fat cells; however, some fat can also leak into other organs, such as the liver (ectopic fat). Enlarged fat cells and ectopic fat accumulation can produce and secrete numerous metabolic, hormonal, and inflammatory substances, causing damage to organs such as the arteries, heart, liver, muscle tissue, and pancreas. (Bray et al., 2017).

Energy homeostasis is largely dependent on neurobiological mechanisms. The hypothalamus integrates hormonal and nutritional signals such as leptin, insulin, ghrelin, and glucose to control hunger, satiety, and energy expenditure. Dysregulation of these pathways can promote overeating and weight gain, which can increase appetite, reduce satiety, and increase the perceived reward associated with food. Consequently, neuronal imbalance is thought to be a crucial factor in the physiological and behavioral aspects of obesity. (Morton et al., 2014).

Adipokines are hormones produced by fat tissue that help control the balance between inflammation and insulin. This balance is disrupted, particularly in individuals with high levels of visceral fat, such as those with obesity. The system works as follows: adiponectin levels decrease, while leptin, TNF-α, and IL-6 levels increase. Inflammation makes it difficult for the body to maintain metabolic adaptability and facilitates the development of insulin resistance. As a result, the immune system is weakened, and the risk of metabolic and cardiovascular disorders increases due to the inflammatory environment created by excess fat tissue (Sam et al., 2014).

Genetic factors also influence obesity risk. Individual differences in susceptibility to obesity stem from differences in genes controlling hunger, energy expenditure, and adiposity. Studies have

identified numerous genetic loci that influence body weight regulation. This supports the idea that obesity is a physiologically regulated disorder with significant genetic components. Understanding a person's genetic vulnerability would make it easier to anticipate who is at risk of gaining weight and provide a chance to take action earlier to more successfully prevent obesity. Also knowing a patient’s genotype may enable a more precise diagnosis of the type of obesity, which in turn allows the prescription of personalized treatment or prevention strategies. Furthermore, early detection of a person's genetic vulnerability to obesity may assist identify people who are most likely to gain weight in the future (Loos & Yeo, 2022).

Neurobiological, endocrine, genetic, and inflammatory pathways together illustrate the complexity of obesity and the need for integrated approaches to research, clinical assessment, and treatment.

1.3 Obesity and Immune Dysfunction

The immune system is essential for maintaining physiological homeostasis. In the ancient world, food was not as abundant as in many other parts of the world. Consequently, with the changing times, these biological characteristics, which evolved in the past to regulate food intake and store fat, are now overactive in today's environment, becoming a maladaptation leading to disease and dysfunction in various bodily systems. Obesity now impacts all aspects of immune function, including microbial defense, inflammatory disease progression, antitumor immunity, and homeostatic tissue function. It is now more than just an altered metabolic state characterized by excess body weight. It causes a variety of changes in systemic and tissue metabolism and inflammatory responses that extend beyond adipose tissue. (Jiang et al., 2025).

1.3.1 Innate Immune Alterations

The relationship between nutrient excess and activation of the innate immune response in many organs involved in energy homeostasis has been central to studies over the past 20 years aimed at understanding the pathophysiology of obesity, insulin resistance, and diabetes. Chronic low-grade inflammation, a hallmark of obesity, alters innate immune function in several ways. During this process, adipose tissue transitions from an anti-inflammatory M2 macrophage-dominated environment to one dominated by a pro-inflammatory M1 phenotype, leading to increased cytokines such as TNF-α and IL-6 (Saltiel et al., 2017). However, structural and metabolic perturbations in the intestinal barrier, such as increased permeability and microbial translocation, contribute to the deepening of systemic inflammation and the weakening of interstitial microbial defense (Jiang et al.,

2025). All of these changes can be considered part of a broader innate immune dysregulation that accompanies the metabolic imbalance that occurs in obesity.

1.3.2 Adaptive Immune Dysregulation

Obesity is associated with alterations in T cell homeostasis, including patterns of chronic activation and features consistent with reduced functional capacity in tissue-resident lymphocytes (Jiang et al., 2025). Proinflammatory cytokines originating from adipose tissue contribute to persistent inflammation and metabolic dysfunction by affecting both T and B cell regulation (Saltiel et al., 2017). Overall, inflammatory signals originating from adipose tissue alter adaptive immunity, impairing coordinated immune responses in obesity (Jiang et al., 2025).

1.3.3 Impact of Adipokines on Immunity

Adipose tissue is the site of synthesis and release of a number of adipokines that are important regulators of appetite and body weight as well as insulin sensitivity and inflammation. Adiponectin is one such adipokine whose levels decrease with obesity especially central adiposity and increasing visceral fat.Therefore, adipokines play a central role in linking excess adipose tissue to immune dysregulation. Studies have shown that TNF-α expression is significantly increased in hypertrophic adipose tissue, contributing to systemic inflammation and impaired insulin signaling. Therfore, adipose tissue becomes an important source of proinflammatory adipokines in obesity. TNF-α blocks insulin receptor phosphorylation, leading to the release of more inflammatory cytokines. This, in turn, alters macrophage activity, disrupting immunological and metabolic homeostasis. Similarly, obesity elevates IL-6 levels, particularly in visceral fat tissue. Here, fat tissue macrophages promote liver CRP production, perpetuating chronic low-grade inflammation. Adiponectin, on the other hand, has potent anti-inflammatory and insulin-sensitizing properties, but its immunomodulatory and protective effects on endothelial function diminish with increasing visceral fat. In humans, higher adiponectin levels have been associated with higher insulin sensitivity and a lower risk of type 2 diabetes across all racial and ethnic groups. Leptin, the first identified adipokine, is a key regulator of food intake and body weight and also has antilipotoxic effects, preventing lipid accumulation in the wrong tissues. Other adipokines, such as resistin, retinol-binding protein 4, visfatin, and omentin, have also been reported to be involved in insulin sensitivity and the regulation of metabolic dysfunction (Sam et al., 2014).

Leptin is a critical mediator of the immune response to changes in overall nutrition. Leptin is produced by adipocytes in proportion to adipose tissue mass and is therefore increased in obesity.

Leptin activates neutrophils, exerts proliferative and anti-apoptotic activities on T lymphocytes, and affects cytokine production and the activation of monocytes/macrophages. Overall, leptin plays a critical role in obesity-associated inflammation by promoting pro-inflammatory immune phenotypes (Kiernan & MacIver, 2021).

1.3.4 Systemic Effects Contributing to Immune Dysfunction

High levels of free fatty acids, ceramides, low oxygen levels in tissues, extracellular matrix stress, and the leaking of bacterial components from the gut are just a few of the metabolic stresses that obesity exposes the body through. These elements enhance the generation of inflammatory signals and trigger crucial inflammatory pathways within immune cells (Saltiel et al., 2017).

Widespread disruptions in immunological and metabolic balance result from this activation, which also interferes with normal communication between immune cells. Additionally, immune cells are under metabolic pressure from variations in glucose and lipid availability, which limits their capacity to react efficiently in various organs (Jiang et al., 2025).

Additionally, lipotoxicity, a dangerous process that harms the mitochondria of metabolically active organs including the liver, skeletal muscle, and pancreas, is brought on by an excessive release of free fatty acids from increased fat tissue. Systemic inflammation is exacerbated by this mitochondrial stress, which also increases oxidative damage and interferes with organ communication (Cavaliere et al., 2023).

Overall, these processes show that metabolic stress and immunological dysfunction interact continuously and reinforce each other when obesity is present.

1.4 Obesity-Related Comorbidities Relevant to Infection

Obesity is tightly linked to diseases that greatly increase susceptibility to infectious diseases, and this link can be metabolic or physiological. Visceral fat plays an important and often central role in immune dysregulation, chronic inflammation, and metabolic dysfunction. Type 2 diabetes, nonalcoholic fatty liver disease, cardiovascular disease, respiratory disorders, and alterations in the gut microbiota are directly associated with visceral fat accumulation.These factors combined weaken host defenses and worsen viral outcomes. Obesity-associated comorbidities, when combined, form a complex biological network that significantly increases susceptibility to infectious diseases. Understanding these interactions is important for interpreting infection risk in obesity and

contextualizing the role of gut dysbiosis, immune dysfunction, and dietary interventions in shaping clinical outcomes (Zhang et al., 2023).

1.4.1 Type 2 Diabetes Mellitus

One of the most common metabolic conditions linked to obesity is type 2 diabetes mellitus (T2DM), which is a significant contributor to increased vulnerability to infection. In T2DM, glucose homeostasis is disrupted, and individuals who are overweight or obese are more prone to develop the condition. Visceral obesity leads to metabolic stress, which increases insulin resistance and systemic inflammation, and thereby inhibits neutrophil migration, phagocytosis, and microbial killing. Hyperglycemia disrupts cytokine signaling and antigen presentation, further weakening both innate and adaptive immunity (Zhang et al., 2023). People with these abnormalities are more susceptible to recurrent bacterial infections and more severe viral infections. The co-occurrence of diabetes and obesity during COVID-19 has been linked to increased mortality, hospitalization rates, and the need for critical care (Popkin et al., 2020).

1.4.2 Non-Alcoholic Fatty Liver Disease (NAFLD)

One of the most common hepatic disorders linked to obesity is non-alcoholic fatty liver disease (NAFLD), which is closely associated with visceral fat accumulation. Overweight causes oxidative stress, metabolic inflammation, and hepatic fat deposition, all of which impair innate immunity and decrease liver function. Hepatic inflammation is made worse by disruptions in immune cell activity and adipokine communication, which also impair the liver's capacity to eliminate microbiological products. Additionally, gut microbial changes and increased intestinal permeability, which allow bacterial products like lipopolysaccharide (LPS) to enter the systemic circulation, are characteristics of obesity-related NAFLD. This metabolic endotoxemia increases the risk of bacterial and viral infections by increasing systemic inflammation and reducing the liver's ability to regulate the immune system. Because of these combined immunologic and metabolic abnormalities, NAFLD is a significant risk factor for infection in obese individuals (Zhang et al., 2023).

1.4.3 Cardiovascular Disease and Systemic Inflammation

Cardiovascular disease (CVD) is the leading cause of death worldwide, and obesity significantly contributes to its development through multiple metabolic and inflammatory pathways. Obesity and its related comorbidities, including insulin resistance and dyslipidemia, collectively promote cardiovascular dysfunction by triggering endothelial injury and accelerating atherosclerosis. Excess

adiposity also affects the diagnosis and treatment of CVD, worsening complications such as heart failure and arrhythmias. Importantly, the risk of obesity-related cardiovascular complications is influenced not only by total body fat mass but also by the distribution of regional fat depots and the capacity of adipose tissue to expand and store lipids safely. Visceral adipose tissue, in particular, drives chronic inflammation, oxidative stress, and alterations in vascular homeostasis that undermine immune regulation and reduce the body's ability to respond effectively to infectious challenges (Zhang et al., 2023).

1.4.4 Respiratory Dysfunction and Infection Severity

A common and clinically significant comorbidity of obesity is respiratory dysfunction, which has been clearly shown in studies to significantly increase the risk of respiratory tract infections. The excess adipose tissue around the chest and abdomen limits lung expansion, diaphragmatic movement, and lung dimensions, including expiratory reserve volume and functional residual capacity. These mechanical restrictions impair ventilation and oxygenation, which slows the removal of mucus and inhaled microorganisms and makes the lower respiratory tract more prone to infection (Littleton et al., 2012).

Apart from these mechanical effects, visceral adipose tissue contributes significantly to the chronic low-grade inflammation associated with obesity. It is challenging to effectively fight respiratory infections because of this inflammatory state, which weakens local airway defenses and modifies immunological signaling pathways. The systemic inflammatory burden associated with excess adiposity further increases the risk of severe respiratory disease, leading to organ-level dysfunction, including weakened lung immune responses (Zhang et al., 2023).

More serious consequences during lung infections, such as bacterial pneumonia and viral illnesses like influenza, have been associated with these coupled mechanical and inflammatory disruptions. Due to decreased lung capacity and compromised antiviral responses, obese people had increased rates of hospitalization, respiratory failure, and mortality during COVID-19 (Hornung et al., 2021; Popkin et al., 2020).

1.5 Obesity and Susceptibility to Bacterial & Viral Infections

Obesity is associated with a markedly increased susceptibility to a wide range of bacterial and viral infections, reflected in both higher infection rates and more severe clinical outcomes. This vulnerability stems partly from obesity related alterations in the immune system, including impaired

innate and adaptive immune responses and frequent vitamin D deficiency, all of which diminish host defense capacity (Pugliese et al., 2022). There is strong evidence indicating that excess adiposity negatively impacts immune function and host defence in obese individuals, thereby increasing susceptibility to infections (Milner & Beck, 2012).

Obesity has been associated with a significantly higher risk of invasive bacterial disease and complications because of compromised immune function, chronic inflammation, and altered antimicrobial pharmacokinetics. Obesity has been linked to higher incidence, greater disease severity, and poorer prognosis in bacterial infections. Several epidemiological studies have shown that obesity is associated with an increased risk of community-acquired infections, skin and soft tissue infections, and surgical site infections (Dobner & Kaser, 2018).

Furthermore, because obesity alters leukocyte biology, delays pathogen clearance, and increases susceptibility to systemic bacterial dissemination, obese individuals are more likely to develop severe forms of community-acquired bacterial diseases, surgical site infections, and skin and soft tissue infections (Hegde et al., 2013). Obesity per se is associated with altered cytokine synthesis, reduced antigen responses, and diminished function of macrophages and other innate immune cells, which may predispose obese individuals to invasive bacterial infections and sepsis (Dobner & Kaser, 2018; Huttunen & Syrjänen, 2013).

Viral infections follow a similar pattern of exacerbation. Respiratory viral diseases tend to be more severe in individuals with obesity because obesity is associated with impaired antiviral responses, increased viral spread, and enhanced disease severity during respiratory virus infections (Guglielmi et al., 2021). Both human and animal studies demonstrate that obesity is associated with impaired antiviral immune responses, including defective T-cell function and reduced interferon responses, leading to increased viral replication and disease severity (Milner & Beck, 2012; Honce & Schultz-Cherry, 2019). Outcomes of acute viral pneumonia in obese patients are worsened by certain critical care issues, such as intubation, extubation, mask breathing, prone positioning, and the need for higher ventilation pressures. During the 2009 H1N1 influenza pandemic and the COVID-19 pandemic, obesity emerged as a major risk factor for intensive care unit admission and mechanical ventilation (Honce & Schultz-Cherry, 2019; Gleeson et al., 2020). Furthermore, as seen in SARS-CoV-2 infection, visceral fat accumulation appears to be a poor prognostic factor in severe pulmonary insufficiency (Pugliese et al., 2022).

All things considered, obesity appears to significantly increase susceptibility to infectious diseases through a combination of bacterial and viral immune disorders, resulting in more severe clinical

courses across a variety of pathogens. Taken together, excess adiposity represents an independent and clinically relevant risk factor for adverse infectious disease outcomes across a wide spectrum of bacterial and viral pathogens (Milner & Beck, 2012; Dobner & Kaser, 2018).

2. Definition and Classification of Diabetes Mellitus

Diabetes mellitus is not a single disease, but a group of different metabolic diseases characterized by persistently high blood sugar levels (chronic hyperglycaemia). Continuously high blood sugar can lead to microvascular complications that damage the eyes, kidneys, and nerves, and macrovascular complications that affect the heart and major blood vessels, increasing the risk of cardiovascular disease and death. (Genuth et al., 2018).

Diabetes mellitus is classified into four main groups: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types of diabetes. Most diabetic patients fall into the type 1 or type 2 diabetes group. In the past, different terms were used, such as childhood-onset or adult-onset diabetes, insulin-dependent or non-insulin-dependent diabetes. Classifications were also made based on whether or not ketosis occurred. However, these terms are not preferred today because the clinical characteristics of patients are similar and they are not sufficiently differentiating for diagnosis. Therefore, the current classification primarily distinguishes between type 1 and type 2 diabetes (Genuth et al., 2018).

Type 1 diabetes results from damage to the β-cells in the pancreas that produce insulin, caused by the immune system, leading to an almost complete lack of insulin in the body. Latent autoimmune diabetes (LADA) in adults and diabetes caused by certain immunotherapy treatments also fall into this group; because in all of them, the underlying cause is the immune system attacking its own cells (Petersmann et al., 2019).

Type 2 diabetes occurs when the body cannot use insulin effectively enough (insulin resistance) and also when insulin production decreases. Which of these two conditions is more dominant can vary from person to person (Petersmann et al., 2019).

Gestational diabetes, on the other hand, is a condition that appears for the first time during pregnancy and is characterized by elevated blood sugar; that is, it is a sugar metabolism disorder that develops during pregnancy (Genuth et al., 2018).

Other specific types of diabetes are a group of diseases with known causes and arising through different mechanisms. These include genetic disorders of insulin-producing β-cells, hereditary problems that prevent insulin from acting in the body, diseases of the exocrine part of the pancreas, certain hormonal diseases, and diabetes caused by certain drugs or chemicals (Petersmann et al., 2019).

2.1 Epidemiology

Type 2 diabetes is considered a significant public health problem with serious consequences for human health and healthcare systems. Rapid economic development and urbanization have led to an increase in the prevalence and burden of diabetes in many parts of the world. Diabetes reduces individuals' ability to perform daily activities and their quality of life, leading to serious health problems and increased premature deaths (Khan et al., 2019). Furthermore, the International Diabetes Federation identifies diabetes as one of the fastest-growing global health problems of the 21st century (IDF Diabetes Atlas, 2025).

Globally, the number of individuals diagnosed with diabetes has quadrupled in the last thirty years, and today one in every 11 adults has diabetes. Approximately 90% of individuals with diabetes have type 2 diabetes. Furthermore, in 2017, it was reported that approximately 462 million people, representing 6.28% of the world's population, were affected by type 2 diabetes, corresponding to a prevalence of 6059 cases per 100,000 people (Zheng et al., 2018; Khan et al., 2019). In addition, the International Diabetes Federation estimates that approximately 589 million adults aged 20–79 worldwide live with diabetes, and this number is projected to increase significantly in the coming years (IDF Diabetes Atlas, 2025).

When regional distribution is considered, Asia is one of the regions where type 2 diabetes is increasing most rapidly globally, with China and India standing out as countries with the highest disease burden. In contrast, in developed regions such as Western Europe, diabetes prevalence remains high and continues to increase despite current public health practices. However, the rapid rise in diabetes prevalence in low-income countries is considered a worrying trend indicating a significant change in the global distribution of the disease. This increase is particularly pronounced in low- and middle-income countries undergoing rapid socioeconomic transformation (Zheng et al., 2018; Khan et al., 2019).

Assessments based on age groups reveal that the frequency of type 2 diabetes increases significantly with age, reaching its highest prevalence between the ages of 55 and 59. These findings indicate that type 2 diabetes is a disease closely related to age. Furthermore, previous community-based studies have reported that the prevalence of type 2 diabetes increases significantly with age and is higher in individuals living in cities compared to those living in rural areas (Khan et al., 2019; Chen et al., 2012). However, it is noted that the increasing aging of the global population plays a significant role in the rise of diabetes prevalence (IDF Diabetes Atlas, 2025).

Finally, long-term projections indicate that the global prevalence of type 2 diabetes will reach 7079 cases per 100,000 people by 2030, and the disease will continue to increase worldwide. Consistent with these predictions, current studies show that the incidence of type 2 diabetes mellitus continues to rise globally, particularly in developing countries experiencing rapid socioeconomic change (Khan et al., 2019; Ma & Tong, 2024). Accordingly, a significant increase in the number of individuals living with diabetes worldwide is projected in the coming years (IDF Diabetes Atlas, 2025).

2.2 Pathophysiology and Insulin Resistance

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders. This disease is caused by a combination of dysfunction in insulin secretion by pancreatic β cells and the inability of insulin-sensitive tissues to respond appropriately to insulin. Since insulin synthesis, secretion, and action are essential for maintaining glucose homeostasis, defects in the molecular mechanisms regulating these processes lead to metabolic imbalance and contribute to the development of the disease (Galicia-Garcia et al., 2020). From a pathophysiological perspective, malfunctioning feedback loops between insulin action and insulin secretion lead to elevated blood glucose levels. β-cell dysfunction reduces insulin release and limits the capacity to maintain physiological glucose concentrations, whereas insulin resistance contributes to increased hepatic glucose production and decreased glucose uptake in skeletal muscle, liver, and adipose tissue. Although both processes occur early in disease pathogenesis, their coexistence amplifies hyperglycaemia and drives disease progression (Galicia-Garcia et al., 2020; Zheng et al., 2018).

β-cells play a central role in glucose regulation through insulin production and secretion. Insulin is synthesized as pre-proinsulin, processed within the endoplasmic reticulum to proinsulin, and subsequently cleaved into insulin and C-peptide in the Golgi apparatus before storage in secretory granules. Insulin release is primarily triggered by elevated glucose concentrations and involves glucose uptake via glucose transporter 2 (GLUT2), activation of glucose metabolism, increased intracellular ATP/ADP ratio, membrane depolarization, calcium influx, and insulin exocytosis (Galicia-Garcia et al., 2020).

β-cell dysfunction in T2DM has traditionally been associated with β-cell loss; however, accumulating evidence indicates that dysfunction also arises from complex interactions between environmental factors and intracellular stress pathways. In states of chronic nutrient excess, such as obesity, hyperglycaemia and hyperlipidaemia promote insulin resistance and low-grade inflammation, exposing β-cells to endoplasmic reticulum stress, oxidative stress, and metabolic

stress. Lipotoxicity, glucotoxicity, and glucolipotoxicity impair β-cell integrity, disrupt islet architecture, and compromise coordinated insulin secretion, thereby exacerbating hyperglycaemia and disease progression (Galicia-Garcia et al., 2020).

Insulin resistance represents a core pathophysiological feature of T2DM and is defined as the reduced responsiveness of target tissues to insulin signaling, resulting in impaired glucose utilization and compensatory hyperinsulinaemia. It commonly precedes the onset of overt diabetes and is closely associated with obesity and metabolic syndrome. Inflammation, ectopic lipid accumulation, oxidative stress, and endoplasmic reticulum stress contribute to impaired insulin sensitivity and β-cell dysfunction, reinforcing the reciprocal relationship between metabolic disturbances and disease progression. The combined presence of insulin resistance and β-cell dysfunction is therefore central to the development and progression of T2DM (Zheng et al., 2018; Lu et al., 2024).

2.3 Type 2 Diabetes and Immune Dysfunction

Type 2 diabetes mellitus is a metabolic disease characterized by long-term low-grade inflammation and impaired host defense systems, which significantly impact the immune system. Individuals with type 2 diabetes have both innate and adaptive immune responses affected, contributing to immune dysregulation and increased susceptibility to infections (Berbudi et al., 2020). Increased adipose tissue, insulin resistance, and hyperglycemia all contribute to the development of a persistent inflammatory milieu that modifies immune cell activity and disturbs normal immunological balance (Berbudi et al., 2025).

Innate changes in the immune system are a key component of the immune disorders associated with type 2 diabetes mellitus. Monocytes and macrophages obtained from individuals with type 2 diabetes have been shown to secrete high levels of inflammation-promoting cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), thus exhibiting a significant pro-inflammatory response. However, impairments are observed in the essential functions of neutrophils, such as chemotactic response, phagocytosis capacity, and microbicidal activity; this leads to a weakening of the early defense response against pathogens (Berbudi et al., 2020). These innate immune abnormalities are closely linked to metabolic disturbances and contribute to sustained inflammatory activation (Berbudi et al., 2025).

Type 2 diabetes mellitus is associated with structural and functional changes in the acquired immune response, beyond the innate disorders of the immune system. In particular, an imbalance between pro-inflammatory T cells and regulatory T cells has been reported in T lymphocyte subpopulations;

this imbalance is said to contribute to the persistence of the inflammatory process and the weakening of immune functions (Berbudi et al., 2020). Chronic metabolic stress and inflammatory signals negatively affect adaptive immune responses, leading to impaired immune regulation and altered immune surveillance (Berbudi et al., 2025).

The combined effects of metabolic inflammation, impaired innate immune responses, and adaptive immune dysregulation have important clinical consequences. Individuals with type 2 diabetes have a significantly increased risk of developing infections; these infections tend to present with more severe clinical manifestations, and the incidence of sepsis is higher. Immune dysfunction associated with obesity and type 2 diabetes makes it difficult to effectively control infections and, in the presence of infection, leads to an excessive inflammatory response, negatively impacting disease severity and clinical prognosis (Frydrych et al., 2018).

2.4 Type 2 Diabetes and Susceptibility to Infections

Type 2 diabetes mellitus is associated with a marked susceptibility to bacterial and viral infections due to chronic hyperglycemia, insulin resistance, and accompanying immune system disorders. Hyperglycemia has been shown to suppress various components of the host defense response, preventing the formation of an effective immune response against pathogens. In this context, individuals with type 2 diabetes have an increased likelihood of developing infections, and these infections present with more severe clinical manifestations compared to individuals without diabetes (Berbudi et al., 2020).

Clinical and epidemiological studies have consistently reported an increased incidence of lower respiratory tract infections, urinary tract infections, and skin and soft tissue infections in patients with type 2 diabetes. Especially it has been reported that infections caused by bacterial pathogens, particularly *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis*, are more common in individuals with diabetes; the rates of complications associated with these infections are increased, and the recovery time is prolonged. Hyperglycemia negatively affects essential immune processes such as cytokine response, phagocytosis capacity, elimination of microorganisms, and the redirection of leukocytes to the infection site, thus weakening the early defense response against bacterial agents (Gan et al., 2013; Berbudi et al., 2020).

Several pathophysiological mechanisms have been identified that lead to increased susceptibility to bacterial infections in type 2 diabetes mellitus. Under conditions of persistent hyperglycemia, the chemotactic response, phagocytic capacity, and reactive oxygen species production of neutrophils

decrease; furthermore, structural and functional changes occur in the formation of neutrophil extracellular traps. In addition, macrophage dysfunction, characterized by decreased phagocytosis, impaired antigen presentation, and polarization towards antimicrobially deficient phenotypes, further weakens bacterial clearance. Moreover, hyperglycemia has been shown to negatively affect antibody-mediated opsonization processes through complement system activation, thereby reducing the effectiveness of both innate and humoral immune responses (Gan et al., 2013; Berbudi et al., 2020).

Beyond bacterial infections, type 2 diabetes is also associated with increased susceptibility to viral infections and more severe clinical outcomes. In individuals with diabetes, exposure to viral agents often leads to uncontrolled inflammatory responses, while the effectiveness of protective immune mechanisms targeting viruses may be reduced. Imbalances in cytokine responses, disruptions in interferon signaling pathways, and changes in the functional capacity of natural killer cells and T lymphocytes contribute to the inadequate elimination of viruses. These immune changes are thought to play a role in the more severe course of viral infections, including influenza and other viral respiratory tract infections, in patients with type 2 diabetes (Wensveen et al., 2021).

Individuals with type 2 diabetes mellitus are among the high-risk patient groups for coronavirus disease 2019 (COVID-19). Clinical data reveal that the presence of diabetes is strongly associated with increased hospitalization rates, intensive care unit requirements, and mortality in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Hyperglycemia and chronic inflammation accompanying diabetes suppress antiviral immune responses and trigger excessive inflammatory processes, leading to a more severe course of the disease (Wensveen et al., 2021; van Niekerk et al., 2021). Furthermore, dysregulations in cytokine release, suppression of T lymphocyte function, and inadequate innate immune responses have been associated with ineffective control of viral replication, and are considered to contribute to the development of severe COVID-19 in individuals with diabetes (Wensveen et al., 2021).

The co-occurrence of obesity and type 2 diabetes mellitus significantly increases susceptibility to infections and leads to more severe clinical presentations of the disease. The chronic low-grade inflammatory environment, metabolic endotoxemia, and functional impairments in the immune system associated with obesity deepen the immunodeficiency accompanying diabetes, making effective control of infectious agents more difficult and increasing the likelihood of developing systemic infections. In this context, infections in patients with obesity and type 2 diabetes are more

likely to progress to severe disease, including sepsis, and are associated with poorer clinical outcomes and increased mortality (Frydrych et al., 2018).

Overall, the increased susceptibility to infections observed in type 2 diabetes mellitus reflects the combined effects of hyperglycaemia-induced immune dysfunction, impaired innate and adaptive immune responses, and chronic metabolic inflammation. These changes suppress the host defense response, increasing the frequency of infections in individuals with type 2 diabetes and leading to more severe clinical outcomes (Berbudi et al., 2020; Frydrych et al., 2018).

2.5 Type 2 Diabetes and the Gut Microbiota

The gut microbiota is a highly diverse and complex community of microorganisms that influences human physiology and plays a role in numerous biological functions, including the regulation of immune and inflammatory responses, maintenance of the intestinal barrier, execution of metabolic processes, and synthesis of various metabolites (Galicia-Garcia et al., 2020). The gut microbes in individuals in good health generate a wide range of compounds that support physiological balance. However, alterations in metabolite production and microbiota structure triggered due to environmental and genetic factors, such as aging, diet, lifestyle, genetics, or comorbidities, may disrupt metabolic balance and lead to the development of disease (Galicia-Garcia et al., 2020; Gurung et al., 2020).

Gut microbiota have the ability to alter host glucose homeostasis through multiple mechanisms, including the production of metabolites during fermentation and their secondary effects, activation of inflammatory cascades leading to cytokine release, disruption of intestinal mucosal barrier permeability allowing the influx of toxins, and direct signaling actions through incretin secretion (Cunningham et al., 2021). Increasing evidence suggests that gut dysbiosis plays a key role in the emergence of insulin resistance and the development of type 2 diabetes mellitus; changes in microbiota composition increase metabolic inflammation, predisposing to impaired glucose metabolism (Gurung et al., 2020; Galicia-Garcia et al., 2020).

Significant changes in the function of the gut microbiota have been reported in individuals with type 2 diabetes mellitus (T2DM). In T2DM patients, functions related to the transport of sugars across the cell membrane, the transport of branched-chain amino acids, methane metabolism, the breakdown and metabolism of xenobiotics, and sulfate reduction are observed to be increased. Conversely, decreased processes related to bacterial motility (chemotaxis), flagellum formation, butyrate synthesis, and cofactor and vitamin metabolism have been detected (Cunningham et al., 2021). These

functional shifts are accompanied by reduced production of short-chain fatty acids, which are known to promote gut barrier integrity, pancreatic β-cell proliferation, insulin biosynthesis, and regulation of inflammatory responses (Galicia-Garcia et al., 2020; Iatcu et al., 2021).

Disruptions in the structure of the gut microbiota, particularly changes in the *Bacteroidetes/Firmicutes* ratio, have been associated with weakening of the integrity of the intestinal barrier. This can facilitate the passage of bacterial byproducts into the circulation, leading to increased intestinal permeability and triggering inflammatory responses specific to type 2 diabetes mellitus (Iatcu et al., 2021). On the other hand, some bacterial taxa have been reported to have clearly protective effects, supporting intestinal barrier integrity, reducing pro-inflammatory markers, and improving glucose metabolism and insulin sensitivity. Bacteria such as *Bifidobacterium*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Roseburia intestinalis*, *Akkermansia muciniphila*, and *Bacteroides fragilis* have been shown to be associated with positive metabolic effects and suppression of pro-inflammatory cytokines (Gurung et al., 2020; Iatcu et al., 2021).

In addition to bacterial alterations, recent studies have demonstrated that the diversity of gut viruses is reduced in obese patients with type 2 diabetes mellitus compared with lean controls, while fungal communities also differ between diabetic patients and healthy individuals. It has been reported that in individuals with type 2 diabetes, the abundance of *Malassezia furfur* and unclassified *Davidiella* species is increased, while the abundance of unclassified *Basidiomycota* species is decreased. These findings demonstrate that the gut microbiota is a complex and multi-component ecosystem playing a significant role in the pathogenesis of type 2 diabetes mellitus (Zhou et al., 2022).

The gut microbiota produces numerous metabolites of great importance to host physiology, primarily short-chain fatty acids and bile acids. Short-chain fatty acids provide an energy source for colonocytes and enhance the antimicrobial effects of macrophages and regulatory T cells, while bile acids, metabolized by gut microbes, regulate intestinal barrier integrity, and these processes are disrupted in diabetes. The reciprocal and cyclical relationship between the production of microbial metabolites, shaped by dietary intake, and the gut environment directly affects the structure and function of microbial communities. This interaction demonstrates that maintaining gut homeostasis is critical for preventing metabolic diseases and improving gut microbiota composition even in the presence of insulin resistance and type 2 diabetes mellitus (Baars et al., 2024).

3. Overview of the Human Microbiota

Microorganisms are an essential part of life on Earth and can exist in association with virtually any living organism. The human microbiota is defined as the collection of organisms inhabiting and interacting with the human body, and these interactions may be commensal, mutualistic, or pathogenic. The microbes present in the human body play a crucial role in maintaining overall health, while the environmental microbiota also influences the composition of the human microbiota. Depending on their anatomical location, microbial communities are classified as gut, oral, respiratory, or skin microbiota. These communities live in symbiosis with the host, contributing to homeostasis and regulating immune function. The human microbiota constantly evolves in response to host-related factors. Age, nutrition, lifestyle, hormonal changes, inherited genetics, and underlying diseases are major determinants shaping microbiota composition at any given time. However, alterations in the structure or function of the human microbiota, known as dysbiosis, can lead to serious or even life-threatening conditions. A balanced microbiota plays a key role in the maintenance of human health. The gut contains the largest concentration of microorganisms in the human body, and these communities are major contributors to sustaining metabolic, immune, and physiological stability (Ogunrinola et al., 2020)

4. Gut Microbiota

The human gastrointestinal tract hosts an extremely dense and diverse microbial community comprised of bacteria, archaea, fungi, and viruses. Although the adult gut microbiota generally exhibits a stable structure, it continues to be shaped throughout life by numerous endogenous and exogenous factors such as mode of birth, genetic characteristics of the individual, immune responses, diet, breastfeeding or formula feeding, antibiotic use, infections, circadian rhythm and microbial exposures in the environment (Lynch and Pedersen, 2016). The gut microbiota consists of approximately 100 trillion symbiotic microorganisms, significantly more than the total number of human cells. These microorganisms maintain high population densities in the intestinal lumen by utilizing indigestible food residues, intestinal mucus, and shed epithelial cells as their primary source of nutrients (Liu et al., 2021).

In healthy adults, the gut microbiota consists primarily of the phyla *Firmicutes* and *Bacteroidetes*, with *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia* present in lower amounts but contributing important functional contributions (Liu et al., 2021). Extensive microbiota studies have consistently shown that these two phyla form the fundamental structural backbone of the intestinal ecosystem. Species and strain diversity varies greatly from individual to individual, and each individual's microbiota is taxonomically unique; however, the metabolic and immunological functions carried out by microbial communities largely overlap in most people (Lynch & Pedersen, 2016).

A key characteristic of a healthy gut microbiota is high microbial diversity. This diversity increases the ecosystem's resilience to environmental and biological stresses and allows the system to return to its normal balance after temporary disruptions, such as antibiotic use or dietary changes. However, low microbial diversity has been associated with various chronic diseases, including obesity and metabolic disorders. In general, the adult gut microbiota is a complex and dynamic ecosystem dominated by *Firmicutes* and *Bacteroidetes*, functioning in an anaerobic environment and playing a decisive role in host metabolism, immune regulation and physiological homeostasis (Liu et al., 2021).

4.1 Core Functions of the Gut Microbiota

The gut microbiota carries out a wide range of essential physiological functions that support host metabolism, immune response, and overall homeostasis. These microorganisms ferment indigestible dietary components to produce short-chain fatty acids (SCFAs), such as acetate, propionate, and

butyrate. These microorganisms provide important energy sources for colonocytes, strengthen intestinal barrier integrity, and exert anti-inflammatory effects on the host immune system (McBurney et al., 2024).

The gut microbiota ferments indigestible carbohydrates to produce short-chain fatty acids (SCFAs). These molecules provide the body with an additional energy source and influence metabolic function and satiety signals. SCFAs have been shown to have protective effects against diet-induced obesity. Diet is one of the most important factors determining the diversity and structure of the microbiome. Therefore, it has been suggested that changes in dietary habits may affect the gut microbiota and be used as a strategy in the management of obesity. Among diet types, one of the most prominent approaches in obesity management is ketogenic diets, which have very low carbohydrate availability (Green et al., 2020). Because carbohydrates are the main substrates used by microbes for energy production, this diet type leads to a decrease in overall microbial diversity (Zhu et al., 2022).

In addition to SCFAs, the gut microbiota contributes to the synthesis of numerous vitamins, such as vitamin K and several B vitamins, and produces numerous bioactive metabolites that influence host gene expression, immune signaling pathways, and metabolic processes (McBurney et al., 2024).

The gut microbiota plays a central role in the development and regulation of the immune system by shaping both innate and adaptive immune responses, supporting the maturation of mucosal immunity, and conferring colonization resistance against pathogenic organisms. Alterations in gut microbial communities can lead to abnormal immune activity in the gut-associated lymphoid tissue, weakening systemic immune responses. Because the immune system in the intestinal mucosa is constantly stimulated by dietary antigens and resident microorganisms, maintaining a tight balance between immune tolerance and effective defense is essential (Micic et al., 2023).

Microbial communities, in constant interaction with epithelial and immune cells, regulate the host's inflammatory balance and contribute to the maintenance of immune tolerance. The gut microbiota also contributes significantly to energy production and metabolic regulation; certain microbial compositions have been shown to have the capacity to extract more calories from the diet, and this has been associated with an increased risk of obesity in both experimental models and human studies. Overall, these functions demonstrate that the gut microbiota is a central regulator of metabolic homeostasis, immune competence, and intestinal barrier integrity. Disruption of these essential functions can lead to metabolic disorders, inflammation, and increased susceptibility to disease (Sanmiguel et al., 2015).

4.2 Gut Microbiota and Metabolic Regulation in Obesity

The gut microbiota plays a critical role in metabolic regulation and, together with changes in its structure, is significantly associated with the development of obesity. One of the most common microbial profiles associated with obesity is associated with a change in the distribution of bacterial phyla, characterized by a decrease in Bacteroidetes and an increase in Firmicutes. This compositional shift is linked to increased energy extraction from nutrient substrates and a higher caloric availability for the host (Liu et al., 2021). Experimental studies support this relationship, showing that obese mice have a significantly increased Firmicutes/Bacteroidetes ratio and an increased capacity of the microbiota to extract energy from indigestible polysaccharides. A similar phenomenon has been observed in obese human populations (Sanmiguel et al., 2015).

Changes in specific microbial taxa are known to affect host metabolic pathways. Decreases in beneficial species such as Bacteroidetes and Lactobacillus paracasei are associated with lower metabolic efficiency, while increased levels of Lactobacillus reuteri and Lactobacillus gasseri are linked to higher adiposity. Furthermore, several studies have highlighted the metabolically protective effects of Bifidobacterium species, demonstrating that reduced abundance of these taxa is common in obese individuals and may contribute to metabolic dysregulation. These microbial changes can affect host energy balance and lipid storage by altering carbohydrate fermentation, short-chain fatty acid profiles, and bile acid metabolism. (Liu et al., 2021).

Visceral adiposity exacerbates metabolic disorders associated with alterations in the gut microbiota. Excess visceral adipose tissue is closely associated with chronic low-grade inflammation, altered microbial metabolite production, and reduced metabolic flexibility, reinforcing the complex interactions between the gut microbiota, adipose tissue biology, and systemic metabolic dysfunction. Overall, these findings suggest that obesity is not solely a calorie imbalance but is also shaped by gut microbial composition, metabolic signaling pathways, and dynamic host-microbe interactions (Zhang et al., 2023).

4.3 Dysbiosis in Obesity

Dysbiosis in the gut microbiota contributes to the development of obesity by exerting broad effects on energy metabolism, appetite regulation, inflammation, and the circadian rhythm. Microbial perturbations caused by genetic and environmental factors can increase energy absorption through alterations in gene expression and excessive accumulation of short-chain fatty acids (SCFAs). Dysbiosis can also increase central appetite via the gut-brain axis, gut hormones, and

neuromodulators. Fat storage can be increased by affecting transcription factors and lipoprotein lipase, and chronic inflammation can be exacerbated by upregulating inflammatory gene expression and lipopolysaccharide (LPS). Alterations in the gut microbiota can also affect the circadian rhythm, disrupting rhythm-related transcription factors, epigenetic modifications, and bile acid and SCFA synthesis. All of these processes are considered critical mechanisms that increase susceptibility to obesity (Liu et al., 2021).

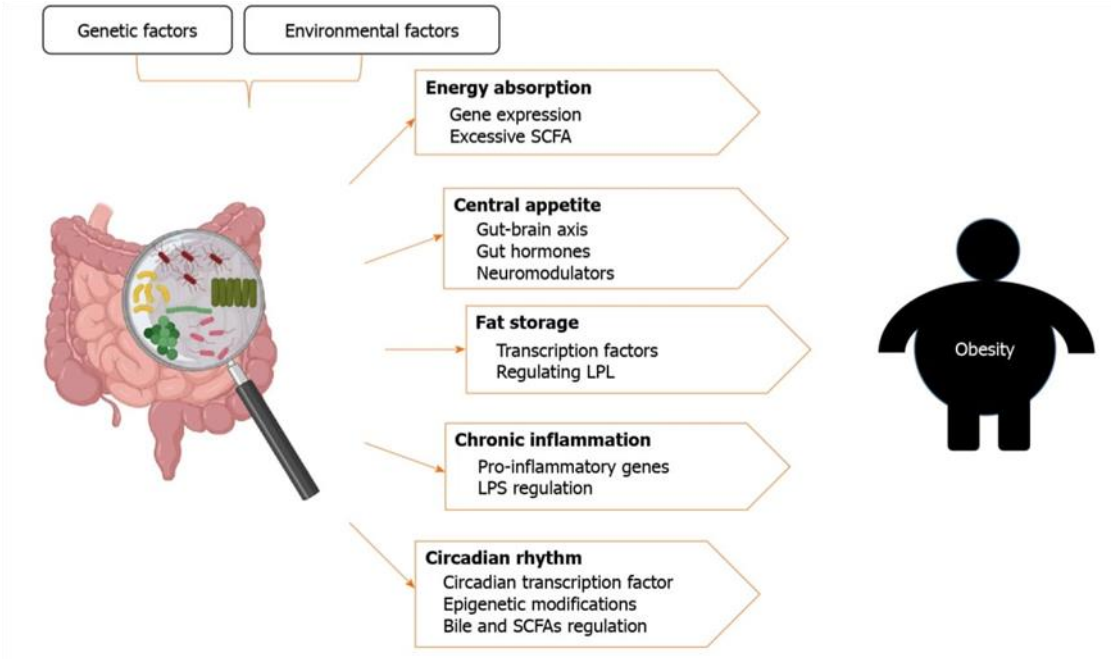

Figure 1. Dysbiosis of gut microbiota (Liu et al., 2021).

4.4 Microbiota-Derived LPS and Metabolic Endotoxemia

Intestinal barrier integrity is critical for maintaining immune homeostasis, nutrient absorption, and preventing the passage of microbial products into the circulation. Obesity-associated changes in the gut microbiota weaken this barrier structure, impairing epithelial function and increasing intestinal permeability. As a result, microbial components such as lipopolysaccharide (LPS) can enter the circulation and contribute to low-grade systemic inflammation that exacerbates the pathophysiology of obesity. Gram-negative bacteria release LPS, which is a type of endotoxin, and higher levels of LPS in the intestine can result from the expansion of Gram-negative bacteria in obese individuals, such as *Veillonella*. An increased intestinal LPS load can damage the gut barrier by activating the TLR4/MyD88/IRAK4 signaling pathway in intestinal epithelial cells, leading to the translocation of LPS from the gut into the systemic circulation. Moreover, the decreased abundance of *Akkermansia*

muciniphila contributes to the passage of microbial byproducts due to its role in maintaining gut barrier integrity. A high-fat diet further favors the incorporation of LPS into chylomicrons, promoting its intestinal absorption and transport into systemic circulation through lymphatic fluid. Once in systemic circulation, LPS is able to initiate immune responses in adipose tissue and the liver. It activates TLR4 on macrophages and adipose tissue, contributing to the secretion of proinflammatory cytokines and chemokines, including TNF-α, IL-6, and MCP-1. Short-chain fatty acids (SCFAs), particularly butyrate, represent another important link between inflammatory responses and the gut microbiota due to their anti-inflammatory properties (Cheng et al., 2022).

4.5 Gut Microbiota and Susceptibility to Infection

Disruption of the gut microbiota in obesity leads to a decrease in protective metabolites and an imbalance in the immune system. This creates an environment where antimicrobial defenses are weakened at the intestinal surface, negatively impacting host defenses. Dysbiosis weakens mucosal immunity and impairs systemic immune function by disrupting gut-immune system interactions and causing abnormal activity in gut-associated lymphoid tissue (Liu et al., 2021; Micic et al., 2023; McBurney et al., 2024).

These disruptions in the microbiota lead to intrinsic weaknesses in antibacterial defenses. The alteration of the microbial balance in obesity hinders pathogen clearance, weakens the intestinal barrier, and facilitates the passage of bacterial products into the bloodstream. Therefore, obesity confers a significantly higher risk for invasive bacterial infections and their associated complications. Obese individuals are at higher risk for bloodstream infections, postoperative infectious complications, and more severe forms of community-acquired bacterial infections due to impaired leukocyte recruitment and altered cytokine responses (Hegde et al., 2013).

Viral infections are similarly affected by obesity-associated microbiota disruption. Alterations in microbial composition and inflammation-induced immune dysfunction impede antiviral responses, attenuate interferon signaling, and promote prolonged viral shedding; all of these combine to worsen the clinical course of respiratory viral diseases (Guglielmi et al., 2021). These mechanisms have become more clearly evident during the COVID-19 pandemic. Higher rates of hospitalization, greater respiratory involvement, and more severe immune dysregulation in obese individuals have all suggested that disrupted microbiota–immune interactions worsen the course of viral disease (Pugliese et al., 2022).

Collectively, these findings suggest that disruptions in the gut microbiota directly contribute to increased susceptibility to infection in obesity. Through weakened mucosal immunity, impaired antimicrobial responses, dysfunctional antiviral signaling, and increased systemic inflammatory activation, dysbiosis constitutes a central mechanistic link between obesity and the increased risk and severity of both bacterial and viral infections. Throughout life, the composition of the gut microbiota is further affected by diet, associated geography, exercise levels, antibiotic usage, and the usage of other medications (Lynch and Pedersen, 2016).

Beyond these general mechanisms, certain infections interact directly with the gut microbiota. Viral infections, viral hepatitis, and urogenital bacterial infections are associated with specific and long-term alterations in gut microbiota composition and immune function (Berbudi et al., 2020; Gan et al., 2013; Wensveen et al., 2021; Baars et al., 2024).

4.5.1 Herpetic Infections and the Gut Microbiota

Herpesviruses are common human pathogens that can cause lifelong infections. Recent findings indicate that viral infections can affect the composition and ecological balance of the gut microbiota. The gastrointestinal microbiota represents a complex microbial ecosystem that can be affected by systemic infections even if the gut is not the primary site of viral replication. Viral infections have been reported to cause significant shifts in gut microbial communities by contributing to the development of dysbiosis and altering the relative abundances of bacterial species (Campbell et al., 2023; Mizutani et al., 2022).

Experimental and observational studies have shown that herpesvirus infections are associated with significant changes in fecal microbiota composition. In particular, herpes simplex virus infection has been shown to disrupt gut bacterial communities by leading to changes in dominant bacterial phyla and a decrease in microbial taxa associated with gut barrier integrity. These findings suggest that herpesvirus infections may play a role as a regulatory factor in the gut microbial ecosystem, independent of the host metabolic state. In addition to the direct effects of infection, it has been reported that antiviral approaches commonly used in the treatment of herpesviruses can also affect the structure of the gut microbiota. Acyclovir and intravenous immunoglobulin administration have been shown to cause significant shifts in bacterial taxa involved in maintaining intestinal balance and can trigger gut dysbiosis on their own. These results highlight that both herpesvirus infection itself and clinical treatment processes can contribute to changes in the fecal microbiota, and are of considerable importance for microbiome research based on fecal samples (Ramakrishna et al., 2020).

Recent human-based genetic studies further strengthen the association between herpesvirus exposure and specific gut microbiota taxa. Mendelian randomization studies have identified associations between gut microbiota composition and herpesvirus-specific serological markers, suggesting that some microbial taxa may be linked to herpesvirus exposure. These findings provide strong evidence that herpesvirus–microbiota interactions can be assessed at the population level using genetic data on gut microbiota (Tan et al., 2024).

Although information regarding the mechanism has largely been obtained from experimental models, current evidence consistently demonstrates that herpesvirus infections can affect gut microbial balance. Studies using animal models have shown that herpesvirus infections are associated with gut microbiota dysbiosis and that targeted modulation of the gut microbiota can alter disease outcomes. These findings further reinforce the view that herpesvirus infections are associated with changes in gut microbial profiles (Li, F., et al., 2023; Shan et al., 2023).

4.5.2 Viral Hepatitis and Gut Microbiota Alterations

Viral hepatitis, caused by hepatitis B and C viruses, is considered a serious public health problem worldwide due to its potential to cause chronic liver damage and progressive loss of liver function; this disease process can progress to advanced and life-threatening conditions such as cirrhosis and hepatocellular carcinoma. Recent research has revealed the existence of a critical interaction between the liver and gut microbiota, known as the gut-liver axis, highlighting the close anatomical and functional connection between these two systems (Neag et al., 2021; Milosevic et al., 2021).

It has been reported that hepatitis B and C virus infections lead to significant changes in the structure of the gut microbiota, including a decrease in beneficial and protective gut microorganisms and alterations in microbial diversity. This imbalance in the gut microbiota has been associated with weakened intestinal homeostasis and changes observed during the progression of chronic liver disease (Neag et al., 2021; Yang et al., 2023). Furthermore, many studies have shown that gut dysbiosis, particularly in hepatitis B and C infections, is associated with viral hepatitis, and that metabolic products and cellular components derived from the gut microbiota can influence pathological processes developing in the liver (Milosevic et al., 2021).

Changes in the diversity and composition of the gut microbiota are reported throughout the different stages and clinical presentations of viral hepatitis. Although hepatitis B and C virus infections have been shown to affect gut microbial diversity, the direction and extent of these effects may vary depending on the patient population and the level of disease progression. Gut microbiota dysbiosis

has been identified in both chronic HBV and chronic HCV infections; differences in microbial structure have been shown to be associated with disease progression and liver pathology (Neag et al., 2021; Milosevic et al., 2021; Yang et al., 2023).

Therapeutic approaches used in the treatment of viral hepatitis are also reported to affect the structure of the gut microbiota. Treatments such as entecavir and directly acting antiviral agents have been shown to cause changes in the relative abundance of certain bacterial groups, particularly members of the Lachnospiraceae family, which play a role in short-chain fatty acid production and maintaining host metabolic balance. Some studies have reported that improvement in gut microbiota structure after antiviral treatment occurs more frequently in patients with mild hepatic fibrosis, suggesting that the microbiota's response to treatment may vary depending on the disease stage (Neag et al., 2021).

In summary, current findings indicate that hepatitis B and C infections are associated with significant changes in gut microbiota composition, microbial diversity, and functional characteristics. Although the mechanisms underlying gut-liver interactions in viral hepatitis are not yet fully understood, current studies strongly support the existence of a bidirectional communication between the gut microbiota and the liver. This reciprocal interaction is thought to have significant implications for disease progression and the shaping of treatment approaches (Neag et al., 2021; Milosevic et al., 2021; Yang et al., 2023).

4.5.3 Bacterial Urinary Tract Infections and the Gut Microbiota

Many clinical studies have shown that the gut plays a key role in the development of urinary tract infections (UTIs) because the gut can act as a reservoir for uropathogens responsible for infection, particularly *Escherichia coli* (Meštrović et al., 2020; Worby et al., 2022). In most cases, the mechanism of UTI development begins with the transport of these pathogens, which are resident in the gut, to the periurethral region; followed by colonization of the urethra and upward progression of microorganisms towards the bladder (Meštrović et al., 2020).

Gut microbiota dysbiosis is associated with changes in intestinal microbial diversity and bacterial abundance, which can lead to an increase in potentially pathogenic microorganisms in the gut. Dysbiosis has been reported to create an inflammatory environment in the gut, weaken mucosal barrier integrity, and increase intestinal permeability; this allows bacteria, toxins, and other microbial components to translocate across the intestinal boundaries (Iqbal et al., 2024). Furthermore, dysbiosis is defined by a disruption of the ecological balance between gut microbial species, which can lead to the overgrowth of uropathogenic bacteria in the gut (Iqbal et al., 2024).

Numerous studies have shown that patients with urinary tract infections (UTIs) have lower gut microbiota diversity and increased gut *E. coli* abundance compared to healthy individuals. It has been shown that uropathogenic *E. coli* strains are resident in the gut and are detected at high rates during periods of UTI development; these findings reinforce the view that the gut ecosystem can serve as a source for UTI-causing pathogens (Meštrović et al., 2020; Worby et al., 2022).

Antibiotics are the primary agents commonly used in the treatment of urinary tract infections (UTIs). However, frequent or repeated antibiotic exposure is reported to negatively impact the composition of the gut microbiota and promote gut dysbiosis. Changes in microbial diversity and abundance caused by antibiotics have been associated with decreased colonization resistance and overgrowth of pathogenic bacteria. This can contribute to a mutually reinforcing cycle between dysbiosis and infection, facilitating the development of recurrent UTIs (Iqbal et al., 2024).

In conclusion, the current findings support a significant link between gut microbiota structure and susceptibility to urinary tract infections. Depending on the diversity and balance of the microbial community, the gut can serve as a source for uropathogenic bacteria or function as a protective ecosystem that suppresses the proliferation of pathogens. These findings highlight the importance of changes in the gut microbiota in the context of bacterial UTIs (Meštrović et al., 2020; Worby et al., 2022; Iqbal et al., 2024).

4.6 Microbiota-Gut–Brain Axis

The microbiota–gut–brain axis is a multicomponent network that facilitates bidirectional communication between the gut microbiota and the central nervous system via neural, endocrine, immune, and metabolic pathways. Signaling in this flora occurs through various mechanisms, including immune system activation, host neurochemical signaling, enteric nervous system pathways, vagus nerve transmission, and bacterial metabolite production (Long-Smith et al., 2020). While a "healthy microbiota profile" has not yet been established, each individual's gut microbiota is unique, and diversity and stability are considered essential characteristics. Commensal gut microorganisms perform a variety of vital functions, including immune system development, the breakdown of non-fermentable carbohydrates, and the synthesis of vitamins and secondary bile acids (Cryan et al., 2019).

The bidirectional communication between the gut and the brain is particularly evident in the regulation of hunger and satiety, as gastrointestinal-derived signals rapidly influence short-term food intake, modulating central appetite-regulating circuits. Furthermore, the observation of behavioral

changes in both organic gastrointestinal diseases and functional gastrointestinal disorders, which, despite the absence of structural abnormalities, have a strong psychological component, supports the relationship between gut physiology and the central nervous system. One of the main elements influencing the diversity and composition of the gut microbiota is nutrition; even brief dietary modifications can quickly change microbial profiles, which can have an impact on gut–brain axis transmission. One well-defined pathway of this axis is enteroendocrine communication, where gut hormones carry information from the gastrointestinal tract to appetite-regulating centers in the central nervous system via vagal or non-vagal afferent signals or via the circulation (Buhmann et al., 2014).

Neural centers in the hypothalamus, including the arcuate nucleus, paraventricular nucleus, ventromedial nucleus, dorsomedial nucleus and lateral hypothalamic area, play crucial roles in controlling energy balance. Complex neural networks distributed throughout the forebrain and brainstem regulate feeding behavior and energy homeostasis (Lee et al., 2025; Cryan et al., 2019).

Another crucial element of gut–brain connection is microbial metabolites. After entering the bloodstream, the fermentation of indigestible dietary fibers produces short-chain fatty acids (SCFAs), which include butyrate, propionate, and acetate. These metabolites affect the maturation of microglia, control neuroimmune functions, and aid in the synthesis of neurotransmitters that regulate hunger. Additionally, enterochromaffin cells are stimulated by SCFAs to generate serotonin and regulate intestinal motility. In addition to SCFAs, other microbial products such as secondary bile acids, tryptophan metabolites, glutamate, GABA, dopamine, norepinephrine, serotonin, and histamine also participate in communication with the brain (Longo et al., 2023; McBurney et al., 2024).

The neural pathways in the axis are primarily mediated by the afferent and efferent fibers of the vagus nerve. The vagus nerve consists of 80% sensory and 20% motor fibers. Afferent fibers transmit signals from the gut and gut microbiota to the brain, while efferent fibers carry signals from the central nervous system to enteroendocrine and enterochromaffin cells and the mucosal immune system. The vagus nerve has been shown to detect microbial activity and transmit this information to the central nervous system; these signals are processed in the central nervous system to produce appropriate physiological or behavioral responses (McBurney et al., 2024).

Microbiota–brain contact is also facilitated by non-neuronal pathways. While immunological pathways affect central nervous system activity through cytokine signaling and inflammatory mediators, humoral pathways function by releasing gut hormones and microbial metabolites into the bloodstream. One of the main endocrine pathways by which gut microbiota interacts with the brain

and influences stress reactions and systemic homeostasis is the hypothalamic–pituitary–adrenal (HPA) axis (Longo et al., 2023).

All these mechanisms demonstrate that the microbiota–gut–brain axis is an integrated network that links gut microorganisms to the central nervous system through multifaceted pathways. This axis plays a fundamental role in appetite, mood, cognitive function, and systemic physiological balance (Cryan et al., 2019; McBurney et al., 2024).

4.7 Microbiota-Gut–Brain Axis in Obesity

Obesity is characterized by significant disruptions in the gut-brain axis, which regulates communication between the gastrointestinal tract and the central nervous system. Gut microbiota and microbial metabolites regulate metabolism, adiposity, homeostasis, energy balance, and central appetite and reward signaling, all of which play critical roles in obesity (Asadi et al., 2022). Disruptions in this axis affect homeostatic and hedonic feeding pathways, leading to impaired appetite control and increased energy intake (Cryan et al., 2019; Lee et al., 2025).

One of the main mechanisms impaired in obesity is vagal signaling. Under normal circumstances, nutrient and hormone signals are transmitted via the vagus nerve to the brainstem, where appetite-regulating circuits are activated. Vagotomy has been shown in animal models to reduce anorexigenic hormone signaling, leading to increased food intake and weight gain. This suggests that reduced vagal sensitivity in obesity contributes to impaired satiety (Buhmann et al., 2014).

Gut hormones also play a key role in this axis. The secretion of GLP-1, ghrelin, PYY, and leptin can be modified by specific gut bacteria. The central nervous system receives signals from these hormones about hunger and fullness. The neuroendocrine mechanisms that decrease appetite cannot work normally in obesity due to reduced release of these hormones (Asadi et al., 2022).

Microbial metabolites also play a significant role in obesity. Microbiota-derived short-chain fatty acids (SCFAs) can alter hormone release by binding to receptors on enteroendocrine cells (Longo et al., 2023). Due to alterations in microbial composition, these usually beneficial metabolites may have distinct physiological consequences in obesity. For example, the altered microbiota leads to increased acetate levels, leading to increased insulin release, increased ghrelin, increased parasympathetic activity, and obesity (Asadi et al., 2022).

Gut bacteria also influence central appetite control by producing neuroactive compounds. Neuroactive metabolites that influence central appetite control, such as serotonin and GABA, are produced by gut bacteria (Asadi et al., 2022). While GABA is required for normal energy regulation, serotonin plays a central role in the control of hunger and satiety. Microbial dysregulation of these neuroactive chemicals is linked to metabolic imbalance and abnormal eating patterns in obesity (Cryan et al., 2019; Lee et al., 2025).

Reward-based feeding pathways are also significantly altered in obesity. Abnormal hedonic drive and increased motivation are two key components of the reward system and play a significant role in obesity. Obese individuals have been shown to exhibit increased reward responses to high-calorie foods and decreased striatal dopamine D2 receptor binding potential. These neural changes trigger overeating behavior independent of metabolic demand (van Son et al., 2021).

Other significant factors in obesity include increased intestinal permeability and the entry of microbial compounds into the bloodstream. Increased intestinal permeability is caused by alterations in the gut flora in obesity. As a result, inflammatory cascades are started when lipopolysaccharide (LPS) enters the systemic circulation. Neuroinflammation can also be brought on by LPS. Leptin resistance develops as a result of this neuroinflammation's disruption of hypothalamic appetite circuits. One of the main pathophysiological characteristics of obesity is leptin resistance (van Son et al., 2021).

Finally, the obesity-specific microbiota contributes to increased energy production by the host. The microbiota linked to obesity increases the synthesis of several fermentation enzymes and nutrient transporters, which gives the host extra energy. This sustains obesity by preserving a favorable energy balance (Asadi et al., 2022).

Taken together, these findings suggest that obesity leads to multifaceted disruptions in the gut-brain axis. Alterations in the microbiota structure impair the regulation of appetite, energy balance, and feeding behaviors by affecting the release of gut hormones, SCFA signaling, vagal transmission, neuroactive metabolite production, dopaminergic reward pathways, and inflammatory processes. Therefore, the gut-brain axis plays a critical role in the pathophysiology of obesity (Cryan et al., 2019; Lee et al., 2025; Asadi et al., 2022).

5. Dietary Interventions and Very Low Calorie Ketogenic Diet (VLCKD)

Diet directly affects energy balance, metabolism, and the gut microbiota, and plays a crucial role in the prevention and treatment of obesity. Different nutritional approaches can influence body weight, insulin sensitivity, inflammation, and appetite control, both through direct metabolic effects and via the gut microbiota. Among these approaches, low-carbohydrate and ketogenic diets, especially very low-calorie ketogenic diets (VLCKD), have gained more attention in recent years due to their ability to provide rapid weight loss, improve metabolic indicators, and affect gut microbiota and gut-brain communication. This section will first address the relationship between diet and obesity, and then examine the effects of VLCKD as a therapeutic nutritional approach on the body and gut microbiota (Green et al., 2020; Asadi et al., 2022; Portune et al., 2017).

5.1 Diet and Obesity: Relationships

Obesity arises from a complex interplay of genetic, behavioral, environmental, and socioeconomic factors. Rapid urbanization and globalization have accelerated this process by increasing the consumption of high-calorie diets and sedentary lifestyles. As diets worldwide have shifted toward high-calorie, processed foods and beverages, and as physical activity levels have declined due to increasingly sedentary occupations and lifestyles, many populations have experienced a sustained caloric surplus conducive to weight gain. The modern environment creates an "obesity-prone" environment due to the easy availability of cheap, energy-dense foods and the reduction of opportunities for physical activity. These environmental pressures interact with individual biological predispositions. Genetic, endocrine, and metabolic factors modulate one’s propensity to gain weight, and genetic predisposition significantly influences appetite regulation and fat storage. However, the emergence of these predispositions generally requires favorable environmental conditions, such as access to high-fat/sugar foods. Therefore, the global increase in obesity reflects the interaction between biological predispositions and modern lifestyles (Ahmed & Mohammed, 2025).

Unhealthy dietary habits constitute a major driver of obesity. Excessive consumption of calorie-dense but nutrient-poor foods, particularly high-fat, high-sugar products that are widely marketed and readily accessible stimulates overeating and leads individuals to consume more calories than required. Large portion sizes, frequent snacking, and an increase in processed foods further fuel this trend. Individuals who frequently consume fast food, sugary drinks, and highly processed snacks have a significantly increased risk of obesity (Ahmed & Mohammed, 2025).

Diet is also a key determinant of gut microbiota composition. Diet provides specific nutritional substrates and triggers environmental changes in the gut ecosystem, such as pH and bile acid shifts, shaping microbial diversity and function. Both long-term dietary patterns and short-term dietary interventions can rapidly alter microbiota composition; these changes can be detected within 24 hours of a dietary change. Populations consuming plant-rich, fiber-dense diets exhibit distinct microbial signatures compared to individuals consuming Western diets rich in fat and protein. Sustained dietary habits can even drive the loss of key bacterial strains across generations, particularly fiber-fermenting species essential for metabolic health (Portune et al., 2017).

Dietary fats have additive effects on microbiota composition and obesity risk. Habitual consumption of animal-fat-rich diets has been associated with increased abundance of *Bacteroides*, while short-term consumption of animal-based diets elevates *Alistipes* and *Bilophila* and reduces key *Firmicutes* involved in fiber fermentation. High fat intake leads to increased adiposity, chronic low-grade inflammation, insulin resistance, and increased bile acid production, contributing to outcomes such as obesity, metabolic syndrome, type 2 diabetes, and fatty liver disease. Moreover, microbial metabolism of dietary lipids can generate bioactive metabolites that stimulate inflammatory T-cell responses, demonstrating a mechanistic link between dietary fat, gut microbiota, and obesity-associated inflammation (Portune et al., 2017).

Experimental studies reinforce the role of diet in determining obesity. Switching obese mice consuming a high-fat diet (HFD) to a normal-fat diet (NFD) resulted in significant reductions in weight gain, adipose tissue weight, and fat accumulation. In this mouse model, this improvement is attributed to enhanced lipolysis in adipose tissue and the liver and to the inhibition of adipocyte hypertrophy. Sustained high-fat feeding also leads to adipose tissue dysfunction and ectopic fat deposition in the liver and muscle, contributing to metabolic disorders. Dietary reduction of energy intake improves glucose metabolism, reduces visceral fat mass, and reduces fatty liver (Ji et al., 2023).

Dietary fiber has significant effects on metabolic health, both directly and via the microbiota. Fiber regulates nutrient absorption and increases the production of short-chain fatty acids (SCFAs) through microbial fermentation. SCFAs meet the energy needs of the intestinal epithelium and immune cells, regulate inflammation, and influence the expression of microbial virulence genes. These metabolites also serve as energy sources and signaling molecules in host tissues (Portune et al., 2017).

Finally, dietary modulation of the microbiota is increasingly recommended as a treatment strategy for obesity. Functional foods containing prebiotics and probiotics stand out as a promising approach

because the microbiota influences energy harvesting, fat storage, and inflammation. Prebiotics increase the fiber content of foods, improving their nutritional quality and reducing their fat, sugar and energy content. Regular consumption of such functional foods may significantly increase daily fiber intake and help remodel the gut microbiota toward a metabolically healthier profile (Green et al., 2020).

5.2 Principles of Ketogenic Diets

The Ketogenic Diet The ketogenic diet (KD) was first used in the treatment of epilepsy in 1921. It was suggested to mimic the effects of fasting. The KD is a normocaloric, low-carbohydrate, and high-fat diet. The fat/protein+carbohydrate ratio is 4:1, and lipids constitute almost 90% of the calories. There are variations of the ketogenic diet: the low-calorie ketogenic diet (LCKD), which consumes 800–1200 kcal per day, and the very-low-calorie ketogenic diet (VLCKD), which consumes less than 800 kcal per day (Guarnotta et al., 2022).

It promotes a metabolic shift from glucose utilization to fat oxidation. Its primary goal is to reduce carbohydrate availability sufficiently to support the production of ketone bodies as alternative energy substrates, as the body normally uses carbohydrates (glucose) for energy. It is possible to achieve this goal with the ketogenic diet (Baylie et al., 2024).

Classic ketogenic diet protocols are structured to create a balanced state of dietary ketosis. They typically consume approximately 1 g of protein per kilogram of body weight, 10–15 g of carbohydrate per day, and provide the remaining calories from fat. This macronutrient distribution is designed to mimic the metabolic state of fasting while maintaining adequate nutrient intake, thereby shifting the primary energy pathway from glycolysis toward ketogenesis (Roehl and Sewak, 2017).

Under normal circumstances, the body relies primarily on carbohydrates for energy production. Insulin functions to extract and store the energy derived from glucose. When carbohydrates are depleted, insulin secretion decreases. Initially, stored glucose in the form of glycogen can be used as fuel, but this is depleted after three to four days. Ketosis develops when carbohydrate intake is restricted enough to lower circulating glucose and insulin levels. As hepatic glycogen stores are depleted, free fatty acids released from adipose tissue undergo β-oxidation in the liver, producing acetyl-CoA, which is then converted to ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) (McGaugh and Barthel, 2022).

Ketone body production is governed by the balance between ketogenesis and ketolysis, These ketone bodies enter the systemic circulation and serve as efficient mitochondrial fuel for extrahepatic tissues such as the brain, heart, and skeletal muscle. Under physiological conditions, ketosis remains a controlled metabolic state distinct from pathological ketoacidosis, with ketone concentrations rising within a safe and adaptive range (Drabińska et al., 2021).

5.3 Very Low-Calorie Ketogenic Diet (VLCKD)

A Very Low-Calorie Ketogenic Diet (VLCKD) is a clinically structured ketogenic protocol defined by a significant reduction of total energy intake (usually ≤800 kcal/day), severe carbohydrate restriction, and controlled protein consumption (Barrea et al., 2021).

Ketogenic diets were initially developed to mimic the metabolic processes that occur during fasting. Classic ketogenic diets are normal-energy, high-fat, and very-low-carbohydrate eating patterns, with approximately 90% of total energy coming from fat. This approach is often implemented with a formulation based on a 4:1 fat to protein+carbohydrate ratio (Guarnotta et al., 2022). In contrast, VLCKD applies both carbohydrate restriction and marked caloric restriction, resulting in a more rapid and intense ketogenic response (Moreno et al., 2014).

In VLCKD programs, daily carbohydrate intake is generally kept below 30–50 grams. To maintain lean body mass, protein intake is adjusted to approximately 1–1.5 grams per kilogram. The remaining energy needs are met primarily by high-biological-value protein products, not by consuming free fat. In this respect, VLCKD significantly differs from classic ketogenic diets (Moreno et al., 2014). In terms of nutritional content, the macronutrient distribution of VLCKD consists of approximately 44% fat, 43% protein, and 13% carbohydrate. Total daily energy intake is limited to a maximum of 800 kcal (Guarnotta et al., 2022).

In VLCKD, glycogen stores are rapidly depleted, leading to a metabolic shift. When carbohydrate intake decreases, liver glycogen is depleted within 3–4 days, insulin secretion decreases, and glucagon levels increase (McGaugh and Barthel, 2022). This promotes β-oxidation of free fatty acids in the liver and their mobilization from adipose tissue. Consequently, the accumulated acetyl-CoA is converted into acetoacetate, β-hydroxybutyrate, and acetone, which are alternative energy substrates for extrahepatic tissues such as the heart, brain, and skeletal muscle (Drabińska et al., 2021).

The production of ketone bodies during VLCKD is significantly greater than during classical ketogenic diets. This is because, as glycolysis slows down, oxaloacetate is directed towards

gluconeogenesis and more acetyl-CoA becomes available for ketogenesis (Guarnotta et al., 2022). Ketone bodies are then transported through the bloodstream and metabolized to acetyl-CoA via ketolysis, supporting mitochondrial ATP production (Drabińska et al., 2021).

VLCKD is primarily indicated for individuals with obesity (BMI ≥ 30 kg/m²) or overweight with metabolic complications, but additional applications include metabolic syndrome, dyslipidemia, type 2 diabetes, polycystic ovary syndrome, and several neurological or inflammatory conditions (Guarnotta et al., 2022; Zambrano et al., 2023).

Finally, VLCKD promotes rapid weight loss through the appetite-suppressing effect of ketone bodies. This effect reduces hunger and improves diet adherence by reducing the impact of external stimuli that trigger overeating (Guarnotta et al., 2022).

5.4 VLCKD and the Gut Microbiota

The very-low-calorie ketogenic diet (VLCKD) differs from other diets in that it incorporates very low carbohydrates, high fats, and moderate protein. This diet provides approximately 70% of energy from fat, 20% from protein, and 10% from carbohydrates. Daily carbohydrate intake is generally below 50 grams. This macronutrient distribution leads to nutritional ketosis, where the liver produces more ketone bodies such as acetoacetate and β-hydroxybutyrate (βOHB). Fat and protein sources in VLCKD are generally animal and plant-based products such as nuts, seeds, butter, cheese, cream, red meat, chicken, olive oil, and fish oil. Current findings suggest that VLCKD maintains ketosis more consistently and improves metabolic indicators more effectively than low-carbohydrate or low-fat diets. Therefore, VLCKD is considered an effective nutritional approach for weight loss (Alsharairi, 2021).

Because the ketogenic diet is so low in carbohydrates, the liver breaks down fat and produces acetoacetate and β-hydroxybutyrate (βOHB). The body uses these two ketones for energy. Therefore, VLCKD promotes ketosis. Intestinal bacteria ferment fiber to produce SCFAs (acetate, propionate, and butyrate). Butyrate is produced primarily by *Firmicutes* (*Ruminococcaceae* and *Lachnospiraceae*). It is suggested that butyrate may interact with VLCKD to promote ketosis. SCFAs play a critical role in metabolism because acetate and butyrate are used in lipid synthesis, while propionate is a source for gluconeogenesis (Alsharairi, 2021).

Diet is one of the most important factors affecting the composition of the gut microbiota. The balance of macronutrients in the diet (e.g., plant-based or animal-based, high or low fat, and carbohydrate

levels) significantly alters the microbiota. The very low amount of nondigestible carbohydrates (NDCs) in VLCKD leads to a significant reduction in the microbiota. Because NDCs are the primary fermentation source for colonic bacteria, bacterial counts naturally decline when these nutrients are reduced. It has been reported that the ketogenic diet (KD) may have an antimicrobial effect in the short term by reducing total bacterial counts. The most prominent example of this is the significant reduction in carbohydrate-dependent *Bifidobacterium* species. Furthermore, decreases in the levels of butyrate-producing *Eubacterium rectale*, *Roseburia*, and, in some cases, *Faecalibacterium prausnitzii* are also observed. The primary reasons for this decrease are low fiber intake and a decrease in *Bifidobacterium*. Additionally, it has been experimentally demonstrated that βOHB can directly suppress *Bifidobacterium* growth (Rew et al., 2022).

The typical microbiota structure seen in obesity is characterized by a high *Firmicutes*/*Bacteroidetes* ratio, increased *Actinobacteria* levels, and decreased microbial diversity. Species such as *Actinomyces odontolyticus*, *Streptococcus thermophilus*, and *Collinsella aerofaciens* are more prevalent in obese individuals, while *Alistipes shahii*, *Alistipes senegalensis*, and some *Lachnospiraceae* species are more prevalent in healthy individuals (Lim et al., 2022).

In this regard, the ketogenic diet (KD) has been investigated as an effective option for the treatment of obesity. Clinical studies show that KD is as successful as a low-fat diet + orlistat diet in weight loss and also provides improvements in blood pressure, lipid profile and glycemic indicators. VLCKD applications have been reported to decrease *Firmicutes*, increase *Bacteroidetes*, decrease Actinobacteria levels, and increase microbial diversity. These changes are interpreted as a shift in the microbiota toward the pattern more commonly seen in lean individuals (Lim et al., 2022; Attaye et al., 2021; Rondanelli et al., 2021).

In VLCKD, when carbohydrate intake is severely reduced, glycolysis decreases while lipolysis, glycogenolysis, and gluconeogenesis increase. These processes result in the production of ketone bodies with high energy efficiency. Clinical studies demonstrate that VLCKD results in significant weight loss, reduced fat mass, and improved cardiometabolic indicators in obese individuals (Alsharairi, 2021; Rondanelli et al., 2021; Attaye et al., 2021)

In terms of microbiota, VLCKD is associated with an increase in beneficial bacterial groups such as *Oscillospira*, *Butyricimonas*, *Alistipes*, *Parabacteroides*, *Akkermansia*, and *Christensenellaceae*. Conversely, decreases in *Firmicutes*, *Actinobacteria*, and some *Lactobacillus* species have been reported. These changes are considered positive for the correction of obesity-related microbiota disruption (Zambrano et al., 2023; Rondanelli et al., 2021; Casillo et al., 2024).

The gut microbiota is vital in influencing health and disease. Diet or diet-based therapies like VLCKD can influence microbial composition and diversity, and targeting the gut microbiota may help treat diseases and improve overall health. Diet influences the composition and activity of the microbiota (Zambrano et al., 2023; Casillo et al., 2024)

Studies have shown that obese individuals have higher abundance of *Firmicutes* and lower abundance of *Bacteroidetes*, and KD may alter gut microbiota composition by decreasing the abundance of Firmicutes and increasing the abundance of *Bacteroidetes* to restore balance to the gut ecosystem. Additionally, research has shown how VLCKD can improve microbiota homeostasis and increase the abundance of bacteria associated with good health (Lim et al., 2022; Rondanelli et al., 2021).

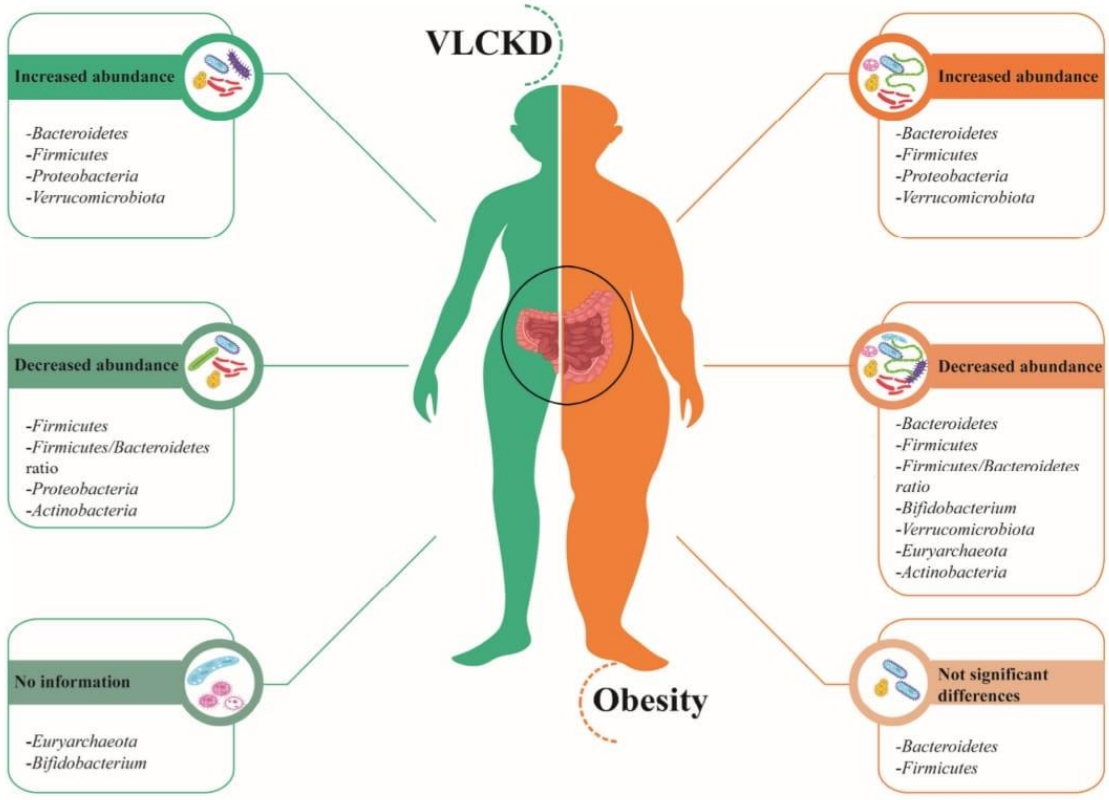

Figure 2. Gut microbiota alterations in VLCKD and obesity (Zambrano et al., 2023)

5.5 VLCKD and Susceptibility to Infection

Obesity increases susceptibility to infections by negatively affecting the intestinal barrier, immune cell balance, and inflammation. VLCKD's ability to improve these disrupted processes may

contribute to a reduced risk of infection. Obesity alters the composition of the gut microbiota and increases the permeability of the intestinal barrier, allowing microbial products such as lipopolysaccharide (LPS) to pass into the bloodstream. This leads to metabolic endotoxemia. Increased permeability leads to low-grade chronic inflammation, activation of proinflammatory signaling pathways such as NF-κB, and elevation of cytokines such as IL-1β and TNF-α. Furthermore, obesity is associated with a Th1/Th17 imbalance, which exacerbates inflammation in the gut. Decreased Treg, Th17, and IgA levels further weaken the intestinal barrier, increasing dysbiosis and facilitating the passage of microbial molecules into the bloodstream (Shemtov et al., 2022).

In addition to microbiota changes, obesity significantly impairs immune cell function. Obese individuals exhibit altered circulating immune cell ratios, including granulocytes, monocytes, and lymphocytes; CRP and proinflammatory cytokines are also elevated. A decreased immune response to vaccines is also a marker of the immune dysfunction observed in obesity. When monocyte subtypes are examined, it is seen that intermediate (IM) and non-classical (NCM) monocytes increase in obesity, while classical monocytes (CM) decrease. VLCKD administration positively affected this distribution, increasing CM levels and decreasing IM and NCM levels. These results suggest that VLCKD may have immune-modifying and anti-inflammatory potential in obesity (Curreli et al., 2025).

Obesity is also a major risk factor for severe infectious disease outcomes, including COVID-19, due to chronic inflammation, impaired respiratory mechanics, and co-morbidities such as diabetes, NAFLD and metabolic syndrome. Ketogenic diets, including VLCKD, are known to promote weight loss and metabolic improvement. It has also been suggested that these diets may reduce NLRP3 inflammasome activity, decrease chronic activation of immune cells, and enhance protective T-cell responses against infections (Gangitano et al., 2021).

Finally, VLCKD significantly modulates the gut microbiota. While obesity is often associated with dysbiosis, VLCKD has been shown to direct the microbiota toward a healthier balance through changes such as an increase in Bacteroides and a decrease in Firmicutes. Restoring microbial balance may contribute to improved immune regulation and reduced inflammation. It is also reported that VLCKD leads to changes in short-chain fatty acid (SCFA) profiles and strengthening of intestinal barrier integrity. These effects may further support host defense mechanisms (Casillo et al., 2024).

5.6 Clinical limitations of VLCKD

Ketone bodies, which are normally produced during the active phase of VLCKD, are excreted via frequent and increased urination, which can lead to dehydration and electrolyte loss. Dehydration-related disorders typically include dry mouth, headache, dizziness or orthostatic hypotension, lethargy, and visual disturbances. For this reason, adequate water intake, particularly during ketosis, is recommended. Headache is common during the first week and may require mild analgesics, preferably in pill form to avoid sugar-containing liquid formulations. Hypotension has also been observed, making blood pressure monitoring and salt adjustment advisable. Halitosis is very frequent due to increased acetone levels during ketosis. Moreover, Gastrointestinal complaints such as nausea, vomiting, diarrhea, and constipation are common in VLCKD and may lead to diet discontinuation in some individuals, which is detrimental to the diet's sustainability. Diarrhea is generally caused by inadequate fat absorption or fat intolerance, while the high fat content of the diet can slow gastric emptying, triggering gastroesophageal reflux, nausea, and vomiting. Constipation may occur due to decreased water or fiber intake, and may require increased fluid intake, dietary fiber supplementation, or laxative use when necessary (Barrea et al., 2021).

Transient hypoglycemia may occur at the onset of ketosis or during the initial phase of refeeding. This may be due to the sudden calorie restriction and the insulin-increasing effects of ketone bodies. It is usually mild, but if symptoms occur, a carbohydrate-containing drink may be necessary. Additionally, elevated uric acid levels (hyperuricemia) may develop, particularly in individuals with a history of gout, and may require specialized treatment (Muscogiuri et al., 2021).

Safety concerns remain central when prescribing VLCKD. Conditions in which the diet is contraindicated include type 1 diabetes, recent cardiovascular or cerebrovascular disease, severe liver or kidney failure, gout, kidney stones, electrolyte imbalances, severe psychiatric illnesses, substance abuse, and pregnancy and breastfeeding. Furthermore, because caloric intake is so low, adequate micronutrient supplementation must be provided (Castellana et al., 2020; Muscogiuri et al., 2021).

Despite its clinical benefits, VLCKD should not be used as a routine first-line therapy but only in carefully selected patients and under strict medical supervision, due to risks such as electrolyte imbalance, lipid profile changes, and potential increases in total cholesterol and triglycerides. Close clinical and laboratory monitoring is crucial to prevent complications (Castellana et al., 2020; Muscogiuri et al., 2021).

Additionally, some studies have shown that ketogenic diets may increase apoB-containing lipoproteins and lead to cardiovascular problems in individuals without diabetes or metabolic syndrome. In such cases, alternative dietary patterns with better long-term safety data may be more appropriate (O'Neill and Raggi, 2020).

Finally, the existing evidence has several limitations. For example, most studies originate from a single geographic location, the samples are largely comprised of middle-aged adults, and both study designs and definitions of ketogenic diets vary widely. Due to these inconsistencies, it becomes difficult to generalize findings and compare results across different studies (Li, S., et al., 2023).

6. Aim of the Study

The primary objective of the SIDERALE study is to analyze differences in the composition of the gut microbiota in relation to the presence of chronic bacterial and/or viral infections in a cohort of patients affected by obesity and type 2 diabetes mellitus (T2DM), compared with subjects with obesity and T2DM without chronic infections.

The secondary objective is to assess whether there is an association between the microbiological "signature" and the clinical characteristics of the enrolled patients.

7. Materials and Methods

7.1 Study Design

The SIDERALE project, promoted by the SS. Antonio and Biagio and Cesare Arrigo Hospital of Alessandria, is an observational study (cases: patients with obesity, T2DM, and chronic infections at baseline; controls: patients with obesity and T2DM without a history of chronic infections), open-label, non-interventional, and prospective longitudinal.

The investigator was limited to observing differences between the two cohorts in relation to the presence of the event of interest, namely changes in microbiota composition over time in subjects with or without a history of chronic infection. For the study of gut microbiota, fecal samples were collected from patients.

Patients were advised to maintain their usual diet. Pharmacological therapy for T2DM and its comorbidities was administered according to clinical judgment based on current disease guidelines.

The study, based on 41 patients (14 women and 27 men) , involved baseline monitoring through stool analysis. The total amount of samples was 41.

Patients meeting the inclusion and exclusion criteria defined by the project were recruited from the Endocrinology Unit of the University of Eastern Piedmont and divided into two groups based on the characteristics of interest.

Inclusion Criteria

1. Ability to understand the study procedures and objectives.
2. Male or female subjects aged between 18 and 65 years.
3. Diagnosis of class I–II obesity (BMI 30–40 kg/m²).

4. Diagnosis of type 2 diabetes mellitus.

The two groups were differentiated based on the presence or absence of concomitant chronic infections (e.g., asymptomatic bacteriuria, urinary tract infections, pyelonephritis, periodontitis, herpetic infection, varicella-zoster virus infection). The case group consisted of subjects affected by the aforementioned chronic infections, whereas the control group was defined by the absence of such infections. The control sample was selected to be comparable to the case group in terms of age, sex, and hypoglycemic therapy at the time of recruitment.

Exclusion Criteria

1. Any psychological, psychiatric, or other medical condition that could compromise understanding of the nature, purpose, and potential consequences of the study.
2. Subjects following dietary regimens different from Mediterranean or Western diets (e.g., ketogenic diet, FODMAP diet, vegan/vegetarian diet), in order to avoid bias in the interpretation of microbial signatures (diet-related microbial signatures for Western and Mediterranean diets are well documented in the literature).
3. Subjects at high risk of developing diabetic foot within the following 12 months (macrovascular damage of the lower limbs, Charcot foot, sensorimotor polyneuropathy of the lower limbs, absence of pedal pulses, therapy with drugs for neuropathic pain).
4. Wound or ulcer infections, as these are acute, often recurrent infections requiring repeated antibiotic or antiviral therapies.
5. Pregnancy or breastfeeding.
6. Recent history of alcohol or drug abuse.
7. Previous bariatric surgery.
8. Vaccination with live attenuated viruses within 12 weeks prior to trial initiation.
9. Hematological or oncological diseases.
10. Patients undergoing dialysis or awaiting kidney transplantation.
11. Hypogonadism or hormone replacement therapy with estrogen-progestin compounds.
12. Severe immunosuppression.

7.2 DNA Extraction

For the analysis of the intestinal microbiota, microbial DNA was extracted from fecal samples using the QIAamp® PowerFecal Pro DNA Kit (Qiagen, Milan, Italy), a patented method specifically designed for the isolation of high-quality microbial genomic DNA from fecal material.

This kit incorporates an inhibitor removal technology and is optimized for samples containing substances commonly present in feces that may interfere with downstream applications, including polysaccharides, bile salts, and heme compounds.

DNA extraction was performed following the manufacturer's instructions. Briefly, fecal material was transferred into PowerBead Pro Tubes containing ceramic beads and subjected to combined mechanical and chemical lysis to ensure efficient disruption of microbial cells. Subsequently, a precipitation reagent was used to remove non-DNA organic and inorganic material. Total DNA was then bound to a silica membrane spin column, washed using dedicated buffers and an ethanol-based solution, and finally eluted using the provided elution buffer.

The resulting DNA was of sufficient purity and quality for downstream molecular applications, including PCR amplification and next-generation sequencing.

The Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Monza, Italy) was used to quantify the extracted DNA in accordance with the manufacturer's instructions.

7.3 Library Production

Amplification of bacterial DNA was performed using the Microbiota Solution B kit (Arrow Diagnostics Srl, Genoa, Italy). This kit enables PCR amplification of the hypervariable regions V3–V4–V6 of the bacterial 16S rDNA gene for the characterization of intestinal bacterial communities.

The amplification protocol consisted of two distinct PCR reactions:
(i) a target PCR for amplification of the selected 16S rDNA gene regions;
(ii) an index PCR for sample barcoding through the incorporation of index sequences.

Following each PCR reaction, amplification products were evaluated by agarose gel electrophoresis using a fluorescent double-stranded DNA intercalating dye (SYBR Safe®), allowing visualization of DNA bands under UV illumination.

Subsequently, amplified DNA was purified using Agencourt® AMPure® beads to remove residual primers and amplification by-products.

7.4 Next-Generation Sequencing

Next-generation sequencing was performed using the MiSeq platform (Illumina Inc., San Diego, CA, USA) with the MiSeq Reagent Nano Kit v2 (500 cycles) and PhiX as an internal sequencing control.

For sequencing, purified and quantified libraries from each sample were pooled at equimolar concentrations, denatured, diluted to the appropriate loading concentration, and combined with

denatured PhiX control. The final library pool was loaded into the MiSeq reagent cartridge, and sequencing was carried out using Illumina's standard automated workflow.

7.5 Bioinformatic and Statistical Analysis

For each sample, sequencing generated paired-end reads (forward and reverse), which were provided in FASTQ format, including both nucleotide sequences and corresponding base quality scores.

MicrobAT software (SmartSeq Srl, Novara, Italy), a bioinformatic pipeline intended for sequencing read alignment against a reference database, was used to handle raw sequencing data. MicrobAT bases its taxonomy assignment on the Ribosomal Database Project (RDP). Sequence similarity was used to determine taxonomic categorization after each sequence was aligned to the RDP database.

A web-based platform for thorough statistical, visual, and meta-analysis of microbiome data, MicrobiomeAnalyst, was used to import the output files produced by MicrobAT for statistical analysis. Constant characteristics and traits seen in a single sample were eliminated before analysis. In order to reduce biases associated with sequencing depth, low-abundance and low-variance characteristics were eliminated, and data were standardized. P-values were used to determine statistical significance, with a cutoff of $p < 0.05$.

Statistical results are thus obtained based on the uploaded raw data (FASTQ files).

- Alpha-diversity. This analysis shows the biodiversity within a single sample. Alpha diversity allows to evaluate the richness and the diversity of the microbial community in a selected sample. In particular, alpha diversity was characterized by the total number of distinct species actually detected in a sample (Observed index) and by other diversity indices: Shannon index that account for both richness and evenness (higher values indicate greater community diversity) and Simpson index that measures the probability that two individuals, randomly selected from a sample, belong to the same species (values range between 0 and 1; the closer the value is to 1, the greater the biodiversity).
- Beta-diversity. This analysis measures the biodiversity comparing species composition in different samples.
- LDA-LEfSe analysis. This method is a linear discriminant that allows the identification of the signatures associated to different parameters, such as species, and it is used to analyse the microbiome. In our case, the analysis includes the application of FRD (False Discovery Ratio) adjusted, which increases the precision of the results by controlling for false positives that may arise from multiple comparison, and the LDA score that quantifies the effect size

and a higher LDA score corresponds to a stronger biological relevance of that microbial signature, helping to distinguish the most meaningful taxa contributing to the observed differences between groups.

8. Results

The findings refer to 41 patients enrolled in the SIDERALE study at the Endocrinology Unit of the University of Eastern Piedmont and SS. Antonio and Biagio and Cesare Arrigo Hospital in Alessandria. All participants provided baseline (T0) fecal samples for gut microbiota analysis.

Patients were stratified according to the presence or absence of chronic bacterial and/or viral infections, in accordance with the predefined study criteria. Subjects affected by chronic infections constituted the case group, whereas patients without a history of chronic infections were included in the control group. For analytical purposes, participants were further stratified by sex into four subgroups:

- T0_F_NO (females without chronic infections);
- T0_F_SI (females with chronic infections);
- T0_M_NO (males without chronic infections);
- T0_M_SI (males with chronic infections).

The analysis aimed to evaluate potential differences in gut microbiota composition at baseline associated with chronic infectious exposure in patients with obesity and type 2 diabetes mellitus (T2DM).

The results of microbiota analysis are presented below, including alpha diversity analysis and differential abundance analysis using Linear Discriminant Analysis Effect Size (LEfSe).

8.1 Biodiversity of Microbial Communities

Alpha diversity analysis was performed at the species taxonomic level to evaluate within-sample microbial richness and diversity at baseline (T0) in patients with obesity and type 2 diabetes mellitus (T2DM), stratified according to sex and chronic infectious exposure (T0_F_NO, T0_F_SI, T0_M_NO, T0_M_SI).

This approach provides a characterization of the heterogeneity of the gut microbial community within each fecal sample by assessing both the total number of detected species and the relative distribution of their abundances.

Species richness was assessed using the Observed index, representing the total number of species identified in each sample, while microbial diversity and evenness were evaluated using the Shannon and Simpson indices.

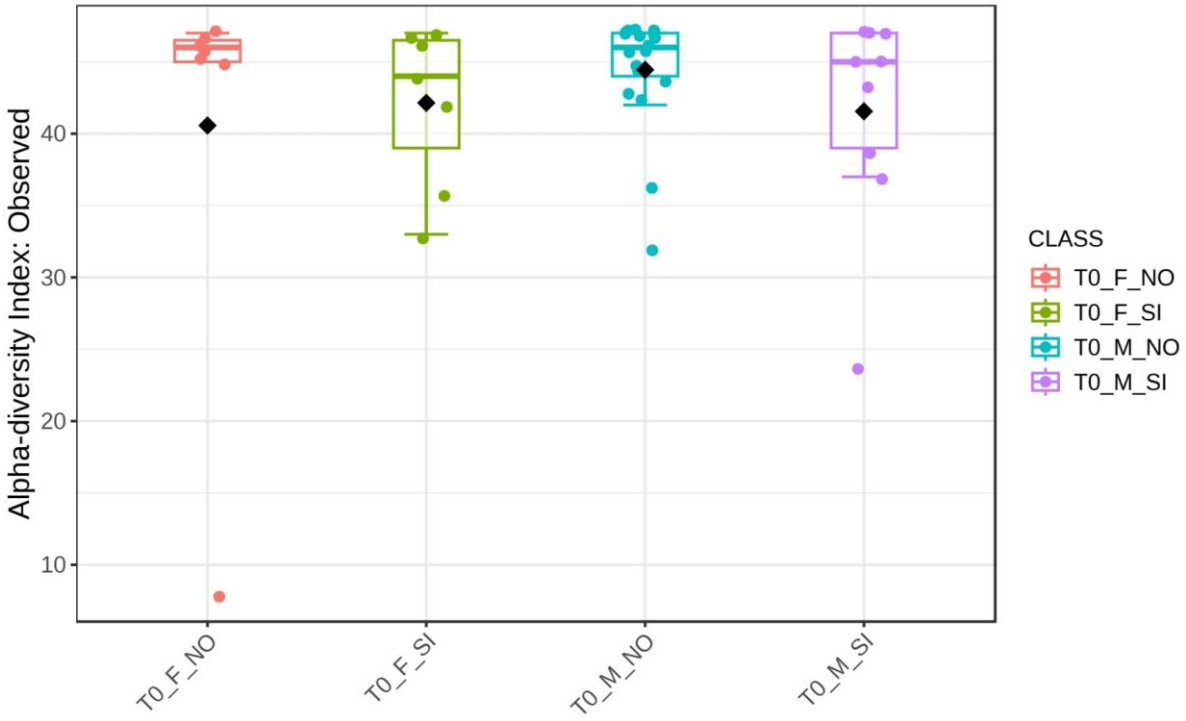

Figure 3. Baseline (T0) alpha diversity of the gut microbiota measured by the Observed index in patients with obesity and T2DM, stratified by sex and chronic infectious exposure (T0_F_NO, T0_F_SI, T0_M_NO, T0_M_SI). Dots represent individual fecal samples; boxplots indicate median and interquartile ranges; black diamonds represent mean values. p = 0.65294.

Analysis of species richness using the Observed index demonstrated comparable distributions across all baseline subgroups. Statistical analysis did not reveal significant differences in species richness between patients with and without chronic infectious exposure (p = 0.65294). Isolated low-value outliers were observed in selected subgroups; however, these did not influence the overall statistical outcome.

Despite this, a possible trend was observed indicating a decrease in the Observed index among patients, both males and females, who were exposed to infections.

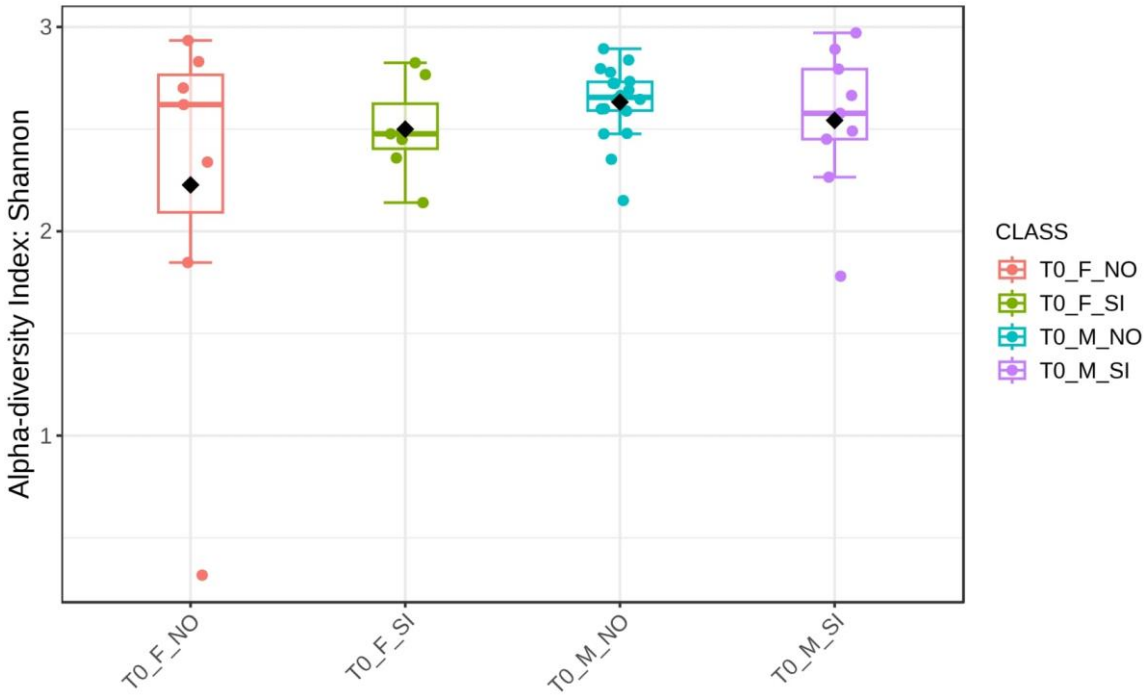

Figure 4. Baseline (T0) alpha diversity expressed as the Shannon index in patients with obesity and T2DM, stratified by sex and chronic infectious exposure (T0_F_NO, T0_F_SI, T0_M_NO, T0_M_SI). Dots represent individual fecal samples; boxplots indicate median and interquartile ranges; black diamonds represent mean values. $p = 0.632$.

Alpha diversity assessed using the Shannon index, which accounts for both species richness and evenness, demonstrated similar diversity patterns across all baseline subgroups. Median values were comparable among groups, and variability ranges largely overlapped. Statistical analysis did not reveal significant differences in Shannon index values between patients with and without chronic infectious exposure ($p = 0.632$).

However, a potential trend toward a reduction in the Shannon index was observed among both male and female patients who were exposed to infections.

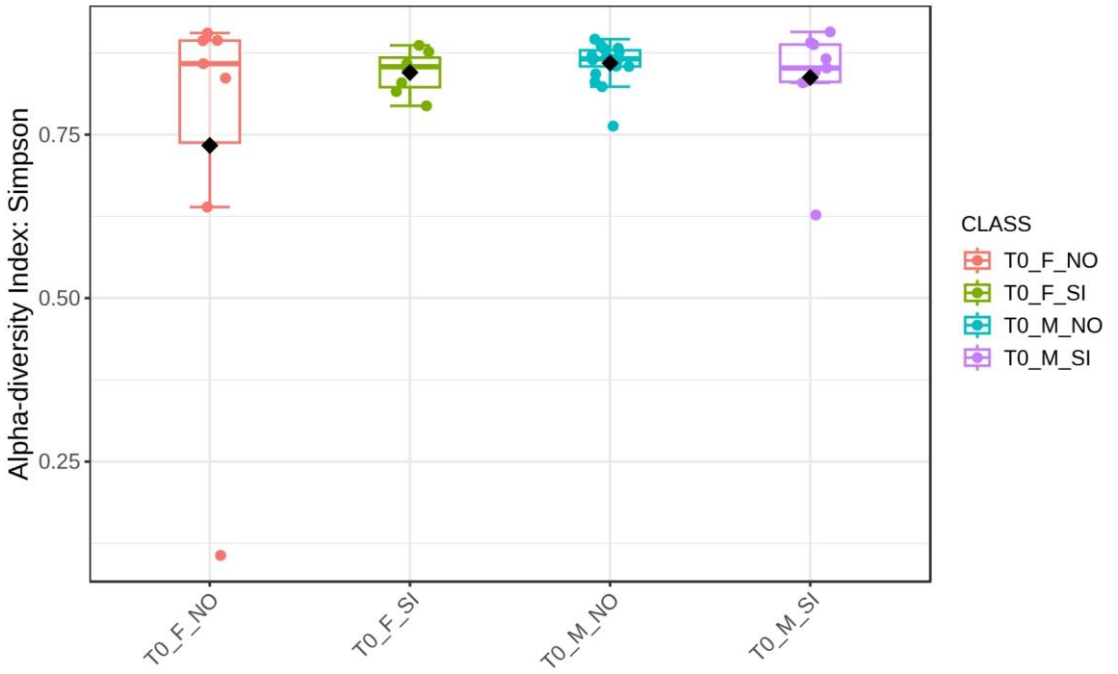

Figure 5. Baseline (T0) alpha diversity expressed as the Simpson index in patients with obesity and T2DM, stratified by sex and chronic infectious exposure (T0_F_NO, T0_F_SI, T0_M_NO, T0_M_SI). Dots represent individual fecal samples; boxplots indicate median and interquartile ranges; black diamonds represent mean values. p = 0.77709.

Alpha diversity assessed by the Simpson index, which reflects the probability that two randomly selected individuals belong to the same species, showed consistently high values across all baseline subgroups. Distribution patterns were comparable among groups, and no evident dominance shifts were observed. Statistical analysis did not demonstrate significant differences in Simpson index values between patients with and without chronic infectious exposure (p = 0.77709).

Similarly, although less pronounced, a possible trend toward a decrease in the Simpson index was observed in the T0_M_SI and T0_F_SI groups.

8.2 Signature Description

Differential abundance analysis at the species taxonomic level was performed using Linear Discriminant Analysis Effect Size (LDA-LEfSe) to identify bacterial taxa potentially associated with chronic infectious exposure at baseline (T0).

At the species level, two taxa (*Blautia intestinalis* and *Dorea* sp.) exhibited Linear Discriminant Analysis (LDA) scores greater than 3.0, indicating potential differences in relative abundance among the study subgroups. Examination of mean relative abundance values showed that the identified species presented higher levels in the non-infectious subgroups (T0_F_NO and T0_M_NO), compared with the corresponding infectious subgroups (T0_F_SI and T0_M_SI).

However, after adjustment for multiple comparisons using the False Discovery Rate (FDR), none of the identified taxa remained statistically significant (FDR > 0.05).

The table below provides an overview of the species that were shown to be differentially abundant by LDA-LEfSe analysis.

IDENTIFIED SPECIES	Pvalues	FDR	T0_F_NO	T0_F_SI	T0_M_NO	T0_M_SI	LDAscore
Blautia intestinalis	0.027526	0.80582	2394.8	1943.8	5360.6	3089	3.23
Dorea sp.	0.059399	0.80582	3627.2	2488.2	6417.5	4500.6	3.29

Table 1. Differentially abundant bacterial species identified by LDA-LEfSe analysis.

9. Discussion

The gut microbiota represents one of the most complex and physiologically relevant microbial ecosystems in the human body, playing a central role in metabolic regulation, immune signaling, and inflammatory homeostasis. Evidences indicate that obesity and type 2 diabetes mellitus (T2DM) are associated with alterations in gut microbial diversity and composition, often characterized by shifts in key microbial taxa and ecological instability (Sasidharan Pillai et al., 2024). Within this metabolically dysregulated environment, chronic infectious exposure may represent an additional biological stressor capable of influencing microbial community structure. Persistent viral or bacterial infections are known to promote immune activation and inflammatory signaling, which may further modulate host–microbiota interactions and contribute to dysbiotic microbial configurations (Velikova et al., 2026).

In the present study, we evaluated baseline gut microbiota composition in individuals with obesity and T2DM, stratified by sex and chronic infection status, in order to investigate whether infection history could act as a modifier of microbial community structure. Although no statistically significant differences were observed, several consistent directional patterns emerged across biodiversity and taxonomic analyses, suggesting subtle but biologically plausible microbial modulation associated with chronic infectious exposure.

Alpha diversity analysis (Observed, Shannon, Simpson indices) demonstrated broadly comparable distributions across the four study subgroups. However, directional shifts were evident when infection status was examined within sex strata. For all biodiversity indices, a tendency toward lower richness and diversity was observed in infectious groups compared with non-infectious groups. Importantly, these trends were not isolated to a single diversity metric but appeared consistently across independent measures of richness and evenness. In a cohort of 41 participants further divided into four subgroups, such directional coherence may indicate subtle ecological variation that does not reach statistical thresholds but remains biologically plausible. The trends observed in the analysis of biodiversity indices appear to suggest a form of dysbiosis associated with exposure to infections, consistent with previous reports describing reduced microbial richness and evenness in chronic viral infections (Yang et al., 2023).

Reductions in microbial richness and evenness have previously been reported in chronic infection settings, where persistent immune activation may contribute to microbiota instability (Yang et al., 2023). Similarly, in metabolic and inflammatory conditions such as obesity and T2DM, microbiota

alterations are frequently characterized by modest but coherent ecological shifts rather than uniformly significant differences across cohorts (Yu et al., 2025). In this context, the absence of statistical significance in the present cohort may largely reflect the limited sample size and the additional stratification into multiple subgroups. Nevertheless, the consistent directional tendencies observed across independent diversity metrics support the hypothesis that chronic infectious exposure may exert additional ecological pressure on an already metabolically altered gut microbiota. These consistent directional trends across independent analytical approaches may represent preliminary but promising signals that require confirmation in larger and more statistically robust populations.

Species-level differential abundance analysis provided further insight. LEfSe identified two taxa, *Blautia intestinalis* and *Dorea* sp., with LDA scores greater than 3.0, suggesting non-random separation between groups. Examination of mean relative abundance values revealed consistently higher levels of both taxa in the non-infectious subgroups (T0_F_NO and T0_M_NO) compared with the corresponding infectious subgroups (T0_F_SI and T0_M_SI). Although these differences did not remain statistically significant after FDR correction, the magnitude of the LDA scores and the consistency of direction across sex-stratified comparisons indicate potentially meaningful microbial signals.

The biological relevance of these taxa strengthens the interpretation of the observed trends. *Blautia intestinalis* is a commensal anaerobic bacterium isolated from human fecal samples and considered a member of the human gut microbiota (Wang et al., 2021). Experimental studies have also reported that *B. intestinalis* can participate in microbial consortia associated with short-chain fatty acid production and reduced chronic inflammation in metabolic models (Koh et al., 2025). Similarly, reduced abundance of *Dorea* sp. has been reported in metabolic and dysbiotic conditions. In a microbiome study comparing individuals with type 2 diabetes mellitus and healthy controls, *Dorea* sp. was found to be significantly less abundant in diabetic subjects, while higher levels were observed in controls. As a short-chain fatty acid–producing bacterium, its depletion may reflect microbiota alterations associated with chronic inflammation and metabolic dysfunction (Ting et al., 2024).

Taken together, the lower relative abundance of these metabolically relevant taxa in individuals exposed to chronic infections may indicate a shift away from bacterial groups typically associated with microbial homeostasis. Since short-chain fatty acid–producing bacteria play an important role in maintaining intestinal barrier integrity and regulating host metabolic pathways, their depletion

may contribute to the inflammatory and metabolic disturbances commonly observed in obesity and T2DM (Anachad et al., 2023).

The interaction between chronic infection and gut microbiota composition may also be mediated through sustained immune activation. Chronic viral exposure has been associated with long-term inflammatory responses and immune signaling pathways that can influence microbial ecology within the gastrointestinal tract. Rather than inducing dramatic changes in overall biodiversity, such immune-mediated effects may manifest as subtle quantitative alterations in specific microbial taxa (Yang et al., 2023). This interpretation is consistent with the patterns observed in the present study, where modest changes in microbial abundance occurred without large-scale shifts in global diversity indices.

Sex-specific microbial variation may represent an additional factor influencing these interactions. Previous studies in both human and animal models have demonstrated that biological sex can shape the structure and diversity of the gut microbiota. These differences are thought to arise from complex interactions between sex hormones, immune regulation, and microbial ecology. Consequently, the response of the gut microbiota to metabolic and inflammatory stressors may vary between males and females (Kim et al., 2020). Although the present study was not powered to formally test sex-dependent effects, the stratified analysis suggests that infection-related microbial modulation may occur within a sex-dependent biological context.

Several limitations should be considered when interpreting the present findings. Most importantly, the relatively small cohort size (n = 41), further subdivided into four analytical groups, may have limited the statistical power to detect subtle microbiota differences. Although statistically significant differences were not observed, consistent directional patterns emerged across both alpha diversity indices and species-level analyses. These coherent trends may represent biologically meaningful microbial signals that did not reach statistical significance due to the limited sample size. Increasing the cohort size and including a larger number of participants may therefore help determine whether these patterns represent reproducible microbial signatures associated with chronic infectious exposure.

High inter-individual variability in gut microbial composition is a well-recognized feature of microbiome studies and may further complicate the identification of statistically significant patterns in relatively small cohorts (Yu et al., 2025). In addition, the heterogeneity of chronic infections within the study population may have influenced microbial responses. Different viral or bacterial pathogens may impose distinct immunological and ecological pressures on the gut environment, and grouping

these conditions under a single category of chronic infection may obscure pathogen-specific microbial signatures (Velikova et al., 2026).

Finally, the present analysis was limited to taxonomic profiling at baseline and did not include functional metagenomic or metabolomic assessments. Integrating pathway-level microbiome analyses and measurements of microbial metabolites such as short-chain fatty acids could provide deeper insight into microbiota–host metabolic interactions, as functional alterations may occur independently of compositional changes (Sasidharan Pillai et al., 2024).

Future studies involving larger cohorts and longitudinal follow-up will be necessary to determine whether the promising directional trends observed in this study translate into statistically significant microbial signatures associated with chronic infectious exposure. Integrating clinical parameters, inflammatory biomarkers, and functional microbiome profiling may further clarify whether chronic viral exposure represents a clinically relevant modifier of gut microbiota structure in individuals with obesity and type 2 diabetes.

Overall, while statistically significant differences were not demonstrated at baseline, the convergence of consistent alpha diversity tendencies and the identification of metabolically relevant taxa with elevated LDA scores suggest that chronic infectious exposure may exert subtle yet biologically coherent modulatory effects on gut microbiota composition in individuals with obesity and T2DM. Expanding the study population may clarify whether these observed trends represent reproducible infection-associated microbial patterns.

10. Conclusion

The present study investigated the baseline gut microbiota composition in patients with obesity and type 2 diabetes mellitus (T2DM), exploring whether chronic infectious exposure could influence microbial community structure. Although no statistically significant differences were identified in alpha diversity indices or differential abundance analyses, consistent directional patterns emerged across several analytical approaches. In particular, patients with chronic infections showed a tendency toward reduced microbial richness and diversity compared with non-infected individuals. While these trends did not reach statistical significance, their consistency across multiple diversity metrics suggests the presence of subtle ecological variations in the gut microbiota associated with infectious exposure.

Species-level analysis further highlighted potentially relevant microbial signals. The taxa *Blautia intestinalis* and *Dorea* sp. exhibited elevated LDA scores and showed consistently higher relative abundance in non-infectious groups compared with infectious groups. Both taxa have been associated with beneficial metabolic functions, including short-chain fatty acid production and anti-inflammatory activity. Their reduced abundance in patients exposed to chronic infections may therefore reflect a shift away from microbial groups linked to intestinal homeostasis and metabolic health. These findings suggest that chronic infectious exposure may contribute to modest compositional alterations in the gut microbiota within an already metabolically compromised host environment.

Several limitations, including the relatively small cohort size and the heterogeneity of chronic infections within the study population, may have limited the statistical power to detect significant microbiota differences. Nevertheless, the directional coherence observed across biodiversity metrics and species-level analyses indicates that infection-related microbial modulation may represent a biologically plausible phenomenon. Future studies involving larger cohorts, longitudinal analyses, and integrated functional microbiome approaches will be necessary to clarify whether chronic infectious exposure represents a reproducible modifier of gut microbiota composition in individuals with obesity and T2DM.

Overall, the results of this study highlight the presence of coherent directional trends in gut microbiota composition associated with chronic infectious exposure in individuals with obesity and T2DM. Although statistical significance was not achieved, likely due to the limited sample size, the

observed patterns suggest potentially meaningful microbial signals that warrant further investigation in larger and more statistically robust populations.

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