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*Baseline Oral Microbiota Profiling in Obese Patients with Type 2 Diabetes Mellitus in
Relation to Infectious History*

(SIDERALE Project)

Candidate: Muhammed Melih Güneştekin

Supervisor: Prof. Elisa Bona



Firmato digitalmente da Elisa Bona
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Co-supervisor: Chiara Bazzano

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Abstract

Obesity and type 2 diabetes mellitus (T2DM) are long-term metabolic diseases characterized by immunological dysregulation and low-grade inflammation. These diseases can affect host-microbiota interaction and vulnerability to persistent infections. The aim of this study was to determine whether individuals with obesity and T2DM have different baseline oral microbiota compositions depending on their history of bacterial and/or viral infections. This observational, non-interventional study included a total of 39 patients (14 women and 25 men) with T2DM and obesity, classified according to the presence or absence of chronic infections. Baseline saliva samples were collected twice from each patient, in the morning and at night, while fasting, and 16S rDNA gene sequencing was used for analysis. Microbial diversity was assessed using alpha diversity indices (Observed, Shannon and Simpson), beta diversity was assessed using principal coordinate analysis, and differential abundance was examined using linear discriminant analysis effect size (LDA-LEfSe). Compared to individuals without a history of chronic infection, patients with a history of chronic infection showed significant changes in oral microbiota diversity and community composition; these changes were particularly pronounced in male patients. Different clustering patterns were shown between study groups using beta diversity analysis, indicating changes in community structure. In addition, LEfSe analysis revealed 4 types of bacterial taxa differing between infected and uninfected participants, indicating differences in baseline oral microbiota signatures associated with infection history. Overall, our results suggest that baseline oral microbiota profiles in individuals with obesity and T2DM are altered depending on the presence of chronic infections and may be indicative of underlying immunometabolic imbalance. Oral microbiota profiling may be a useful, non-invasive method for identifying infection-related susceptibility in metabolically impaired groups, even if causality cannot be determined. Further long-term research integrating functional microbiome analysis with clinical outcomes is needed.

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

1. Obesity

The Lancet Commission on Diabetes and Endocrinology states that obesity should be re-evaluated not only as an issue related to body mass index (BMI), but as a chronic, systemic disease characterized by excess fat that has the potential to negatively affect the functioning of tissues, organs, and even the entire organism (Rubino et al., 2025). According to the Commission, it is defined in two main categories as follows; preclinical obesity and clinical obesity (Rubino et al., 2025).

- Preclinical obesity is a condition in which an individual accumulates excessive amounts of fat while still being able to maintain organ and tissue functions (Rubino et al., 2025).
- Clinical obesity in contrast is defined as when excess fat in the body causes detectable organ and tissue dysfunction and can cause organ damage such as kidney disease, fatty liver, cardiovascular disease or metabolic disorders (Rubino et al., 2025).

It is now suggested that to more accurately determine obesity, individualized and now traditional BMI alone should no longer be sufficient; this well-known method should be used in conjunction with at least one other method. This means that at least one additional anthropometric criterion (such as waist circumference, waist-to-hip ratio, or waist-to-height ratio) or direct measurements of body fat (such as dual-energy X-ray absorptiometry) should be used in addition to BMI, and obesity cutoffs should be adjusted according to age, gender, and ethnicity (Rubino et al., 2025). Individuals with very high BMIs (e.g., $>40 \text{ kg/m}^2$) can be assumed to be overweight without further confirmation (Rubino et al., 2025).

The goal of these redefinitions is to more accurately distinguish and classify individuals based on their risk and provide more personalized treatment options. Patients with preclinical obesity who are not yet considered patients should receive the best possible treatment and preventative measures, i.e., potential patients should be monitored; however, patients with clinical obesity and associated organ dysfunction often require prompt, evidence-based treatment (Rubino et al., 2025).

To better coordinate the diagnosis, prognosis, and treatment of obesity, this new perspective transforms obesity from a simple BMI-based disease label to a disease model that distinguishes between individuals at latent risk (preclinical) and those experiencing true functional impairment (clinical) (Carruba et al., 2021).

1.2 Epidemiology

Unfortunately, an increasing portion of the world's population still faces the risk factor of increasing obesity. According to a modeling study using data from the Noncommunicable Disease Risk Factor

Collaboration (NCD RiskC), the prevalence of obesity ($\text{BMI} \geq 30$) has increased from approximately 6.44% in 1990 to 16.06% in 2022. If current negative trends continue, the prevalence is projected to reach 38.96% by 2040 (Liu et al., 2025). This model predicts a shift towards lower-income countries over time, despite high-income countries historically having the highest obesity rates. This is evidence that health inequalities between high-income and low-income countries have also increased over time (Liu et al., 2025).

It's not hard to say that the scope of the issue becomes even more evident as we continue to examine in detail the causes and effects that can or may cause obesity. It's known that by 2022, more than 1 billion people worldwide, or approximately 13% of the adult population, were struggling with obesity (Ahmed & Mohammed, 2025). The most common factors causing the increase in obesity are various variables such as genetic predisposition, poor eating habits, sedentary lifestyle, urbanization, stress and socioeconomic status (Ahmed & Mohammed, 2025). Most importantly, it has been calculated that if these negative trends are not brought under control, approximately 25% of the world's adult population could be affected by this by 2035 (Ahmed & Mohammed, 2025).

When we look at the situation only in Europe, we see the difference between Northern and Eastern Europe. Looking at the latest epidemiological data from a large-scale study, the lowest rates are seen in Northern European countries, while the highest rates are seen in Eastern Europe (Diamantis et al., 2022). The study also found that important factors such as education level and socioeconomic differences, as well as differences such as urban or rural residence, are important factors affecting the risk of obesity (Diamantis et al., 2022). These findings can actually be considered positive, we must understand that region-specific public health interventions, such as those adapted to local, social and economic conditions, should be implemented (Diamantis et al., 2022).

When all this epidemiological data is brought together, a striking picture emerges: Not only is obesity an increasingly prevalent and increasing disease worldwide, but its burden also varies regionally, disproportionately affecting regions with previously low prevalence rates, as in the case of Northern and Eastern European countries. Complex social, economic, and behavioral factors contribute to the disease's negative consequences, and in scenarios where appropriate public health interventions are not implemented, both clinical and economic outcomes are projected to be significantly worse. Future prevalence projections highlight the urgent need for preventive measures, particularly in regions where obesity is predicted to increase (Liu et al., 2025).

1.3 Pathophysiology

Obesity is a chronic, relapsing disease characterized by abnormalities in various regulatory systems controlling metabolic balance, neuroendocrine signaling, and energy homeostasis, and its prevalence is

increasing. Recent data and current research indicate that obesity is not solely caused by excess caloric intake but rather results from a complex interplay of biological, behavioral, and environmental factors. Neurohormonal mechanisms maintain higher weight targets and promote weight regain after weight loss (Lin & Li, 2021).

Hypothalamic circuits, which integrate peripheral metabolic signals from outside the brain and spinal cord such as leptin, insulin, ghrelin, and peptide YY to ensure their functioning as a harmonious whole, are largely responsible for the central regulation of hunger and energy expenditure. Dysregulated appetite regulation and reduced energy expenditure result from hypothalamic inflammation, microglial activation the process by which microglia, the brain's resident immune cells, become activated in response to injury, disease, or threat, impaired intracellular signaling pathways of leptin and insulin, and chronic overfeeding and increased adipose tissue in obesity. Experimental and clinical findings suggest that this neuroinflammatory state disrupts neuronal connections in the arcuate nucleus of the hypothalamus, often disrupting central feedback mechanisms that limit food intake (Thaler et al., 2012).

The fact that adipose tissue also plays a significant pathogenic role cannot be ignored. When fat cells grow excessively, tissue oxygenation decreases, and this decrease increases mechanical stress, leading to endoplasmic reticulum stress, mitochondrial dysfunction, and the intervention of immune cells such as proinflammatory M1 macrophages. This structural change in adipose tissue leads to a systemic, low-grade inflammatory state that releases cytokines such as TNF- α , IL-6, and MCP-1, inhibiting insulin signaling pathways and leading to metabolic problems. Insulin resistance leads to endothelial dysfunction, a condition in which the cells lining the inner surface of blood vessels malfunction, and cardiometabolic risks. This may be exacerbated by altered adipokine profiles, particularly elevated leptin and decreased adiponectin produced by adipose tissue (Kirichenko et al., 2022).

Obesity-related immunometabolic dysfunction has consequences for metabolic and chronic diseases, as well as susceptibility to infection. Immune system cell deterioration, such as decreased macrophage activity and T-cell dysfunction, are consequences of chronic inflammation impairing innate and adaptive immune responses. Clinical observations and experiments indicate that obese individuals are more susceptible to respiratory viral infections, have delayed viral clearance (the time it takes for our immune cells to eliminate a viral infection), and have suboptimal vaccination responses. These findings also suggest a broader immunological dysregulation associated with excess adiposity (Lin & Li, 2021).

Individual obesity risk is also influenced by genetic and epigenetic factors. Alterations in genes such as FTO and MC4R, which influence appetite, satiety, and energy control, have been identified through whole-genome analyses and genome-wide association studies. Gene expression in metabolic pathways

is frequently altered by epigenetic changes influenced by maternal nutrition during pregnancy, early-life exposures, and gut microbiota composition. These mechanisms may partially explain the differential response to dietary interventions and the interindividual heterogeneity in obesity susceptibility (Thaler et al., 2012).

To summarize, neuroendocrine changes, immunological dysregulation, adipose tissue dysfunction, and genetic predisposition all combine to cause obesity. These interconnected biological processes create a self-reinforcing cycle of increased inflammation, weight gain, and metabolic dysfunction, highlighting the need for multimodal and long-term treatments that may vary from person to person.

1.3.1 Energy Homeostasis & Hypothalamic Regulation

The hypothalamus, which integrates hormonal, nutritional, and neural cues to control hunger, thermogenesis, and metabolism, serves as the primary controller of energy balance. To maintain energy balance, arcuate nucleus neurons, which express genes called pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP), respond to environmental cues such as insulin and leptin. However, chronic low-grade inflammation in the hypothalamus disrupts these circuits in obesity, making it difficult for them to sense and respond effectively (Sonnefeld et al., 2023).

Leptin and insulin resistance in the brain occurs as a result of the activation of glial cells, particularly microglia and astrocytes. These cells secrete proinflammatory cytokines and activate molecular pathways such as nuclear factor kappa B (NF- κ B) and junctional kinase (JNK) (Sonnefeld et al., 2023). As leptin resistance increases, the hypothalamus loses its ability to control energy expenditure and suppress hunger, causing chronic hyperphagia and weight gain, meaning the person feels excessive and abnormally hungry (Sonnefeld et al., 2023).

This pathophysiological pathway is also supported by human neuroimaging studies. Obese patients show signs of hypothalamic gliosis, a marker of glial activation and inflammation determined by in vivo MRI, and such gliosis is associated with metabolic disorders such as insulin resistance, independent of adiposity (Sewaybricker et al., 2023). Gliosis, a structural change in the hypothalamus itself, may disrupt neuroendocrine feedback loops over time and lead to the development of a more maintained body weight (Sewaybricker et al., 2023). Therefore, hypothalamic inflammation creates a cycle in which neuroendocrine dysregulation continues and obesity is reinforced, while also impairing the perception of peripheral energy signals and paving the way for exponentially increasing problems (Sonnefeld et al., 2023).

1.3.2 Adipose Tissue Dysfunction

Obesity is characterized by adipose tissue dysfunction that significantly disrupts metabolic homeostasis through a network of cellular stress, inflammation, and maladaptive remodeling. White adipose tissue (WAT) serves as a resilient energy buffer in health by secreting advantageous adipokines, expanding through hypertrophy and hyperplasia, and safely storing lipids. However, persistent nutrient overload in obesity disrupts these adaptive mechanisms, leading to endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and adipocyte stress. Stress disrupts metabolic signaling pathways by causing the unregulated release of proinflammatory cytokines and adipokines (Bou Matar et al., 2025).

Stressed and hypertrophic fat cells release more proinflammatory cytokines (including TNF- α and IL-6) and chemokines, which attract immune cells such as macrophages to fat depots. The interaction between immune cells and dysfunctional fat cells creates a self-reinforcing inflammatory environment (Bou Matar et al., 2025). This low-grade chronic inflammation also affects local insulin signaling, further exacerbating insulin resistance and metabolic diseases (Bou Matar et al., 2025).

Another important issue is mitochondrial dysfunction in fat cells. The lipid buffering ability of dysfunctional adipose tissue is compromised by decreased mitochondrial oxidative capacity, increased reactive oxygen species (ROS) production, and metabolic stiffness (Bou Matar et al., 2025). This inefficiency can cause ectopic lipid accumulation (lipotoxicity) in other organs, increasing metabolic stress (Bou Matar et al., 2025).

Adipose dysfunction, cellular aging, and impaired fat formation worsen. As fat stem/progenitor cells lose their ability to regenerate and differentiate, the tissue becomes reliant on hypertrophy of existing fat cells, exacerbating stress and inflammation (Bou Matar et al., 2025). In addition, chemical mediators that initiate and maintain a proinflammatory response that enhances local immunological activation are secreted by senescent fat cells that develop a senescence-associated secretory phenotype (SASP) (Bou Matar et al., 2025).

Additionally, this dysfunction affects brown and beige adipose tissue, which normally facilitates energy dissipation through thermogenesis. In chronic obesity, key thermogenic regulatory proteins such as PGC-1 α (peroxisome proliferator-activated receptor-gamma coactivator 1-alpha) and PRDM16 (PR domain-containing protein 16) are disrupted, reducing the thermogenic capacity of these depots and, consequently, whole-body energy expenditure (Bou Matar et al., 2025).

Considering all this, adipose tissue dysfunction in obesity is an active and maladaptive process involving factors such as cellular stress, inflammatory signaling, reduced regenerative capacity, and

impaired mitochondrial function. Because adipose tissue disrupts systemic metabolic balance in addition to disrupting adipose function itself, it is a significant contributor to obesity-related comorbidities, such as heart disease, type 2 diabetes, and hypertension (Bou Matar et al., 2025).

1.3.3 Genetic and Epigenetic Mechanisms

Obesity susceptibility is largely influenced by genetic predisposition, but epigenetic factors also play an undeniably significant role, as it is increasingly understood that epigenetic processes link environmental exposures to long-term body weight management. A recent comprehensive study has shown that single nucleotide polymorphisms (SNPs), including variations in genes such as FTO, MC4R, and LEP, found in genome-wide association studies (GWAS), constitute polygenic risk for obesity, the cumulative effect of many different genetic variants rather than a single gene, and explain a significant portion of interindividual variability (Smith et al., 2023). Rather, it highlights the importance of epigenetic control in understanding this disease, as genetic risk alone does not fully reflect heredity.

In metabolically critical tissues such as adipose tissue, the hypothalamus, and the liver, epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNAs (such as microRNAs) actively control gene expression (Smith et al., 2023). For example, obesity is associated with differential methylation of CpG sites in the promoters of genes involved in inflammation (e.g., TNF- α), adipogenesis (the formation of new fat cells) (e.g., PPAR γ), and appetite regulation (e.g., POMC, NPY), which can permanently affect energy intake and storage (Smith et al., 2023).

Epigenetic expression associated with obesity is also significantly influenced by early-life exposures and maternal nutrition. Recent research has shown, for example, how prenatal overnutrition and a high-fat maternal diet can alter epigenetic traits in offspring, leading to altered methylation of obesity-related genes and long-term susceptibility to obesity (Smith et al., 2023). Similarly, later-life outcomes and environmental influences, such as physical activity and diet quality in adulthood, influence chromatin states and gene expression in fat cells through epigenetic drift (Smith et al., 2023).

Furthermore, the difficulty of losing weight that is, losing it through diet or exercise may be due to epigenetic "memory." They even report that long-lasting changes in DNA and histone methylation in adipose tissue occur even after weight loss, and this may predispose fat cells to accumulate fat more readily in response to a high-calorie stimulus (Smith et al., 2023). This metabolic "memory" may explain why weight gain is so much easier than weight loss.

These epigenetic marks are reversible, and therapeutic interventions such as dietary modification, physical activity, or medication can re-establish maladaptive epigenetic marks (Smith et al., 2023). In

other words, in addition to contributing to the pathophysiology of obesity, epigenetic mechanisms can be used as a guiding biomarker for more accurate and permanent treatment approaches.

1.4 Immunometabolism and Immune Dysregulation

Obesity is considered a chronic immunometabolic disease in which prolonged overnutrition alters immune cell function, leading to systemic inflammation and increased susceptibility to infection. Macrophages in the adipose tissue of obese patients adopt an M1 phenotype due to increased TNF- α and IL-6 production, increased glycolysis, and decreased mitochondrial respiration (Shaikh et al., 2024). This metabolic restructuring, one of the key mechanisms linking obesity to infection susceptibility, perpetuates inflammation while simultaneously reducing the elimination and effective destruction of pathogens (Shaikh et al., 2024). As these macrophages accumulate in fat depots, they trigger inflammatory pathways mediated by JNK (c-Jun N-terminal kinase) and NF- κ B (nuclear factor kappa B). These pathways spread throughout the body, disrupting the immune system's internal balance, or immune homeostasis (Shaikh et al., 2024).

Numerous effects of this immunometabolic imbalance occur with viral infections. In obese individuals, despite having higher levels of the hormone leptin, type I interferon (IFN- α/β) responses in airway macrophages are reduced during influenza infection (Almond et al., 2023). Elevated leptin levels due to obesity inhibit interferon signaling through metabolic mechanisms that restrict oxidative phosphorylation in pulmonary immune cells (Almond et al., 2023). The result is longer viral shedding, delayed antiviral response, and more severe clinical disease (Almond et al., 2023). Similar pathways have been reported for SARS-CoV-2 infection; obesity puts patients at risk for decreased viral clearance, endothelial dysfunction, and increased inflammatory responses. Immunometabolic stress weakens early antiviral defenses and primes innate immune cells for hyperinflammation, increasing the likelihood of adverse outcomes (Khwatenge et al., 2021).

Obesity-induced immunometabolic abnormalities also have a significant impact on bacterial infections. Obese individuals exhibit decreased neutrophil chemotaxis the movement of neutrophils, a type of infection-fighting white blood cell, toward the site of inflammation under the influence of chemical signals; impaired phagocytosis and altered glucose metabolism; and delayed bacterial killing associated with increased accumulation of neutrophil reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂) or hydroxyl ion (OH⁻) (Shaikh et al., 2022). Research suggests that metabolic overload impairs neutrophil mitochondrial activity, leading to poor bacterial clearance and increased susceptibility to infections such as *Helicobacter pylori*, *Staphylococcus aureus*, and periodontal pathogens (Shaikh et al., 2022). Decreased autophagy, where macrophages in obese patients self-degrade and remove dysfunctional parts, and weakened lysosomal activity inherently further limit bacterial destruction (Shaikh et al., 2022).

Additionally, adaptive immunity, or the acquired immune mechanism, is also weakened. Decreased antibody levels and impaired T-cell activation in obese patients are indicators of weakened immunity after influenza and COVID-19 vaccination (Shaikh et al., 2022). Obesity alters T-cell metabolism by shifting cells toward glycolytic dependence, limiting memory T-cell formation, and reducing vaccine responsiveness (Shaikh et al., 2022). Furthermore, obesity-associated leptin resistance provides an additional mechanism for immune impairment by impairing TH1-type antiviral responses and T cell maturation (Almond et al., 2023).

Obesity-associated immunometabolic changes affect mucosal barriers as well as the systemic immune system. For example, the ability of the respiratory and gastrointestinal systems to produce antimicrobial peptides is inhibited by persistent inflammation or dysregulated immunological signaling (Shaikh et al., 2022). This weakens epithelial defenses and promotes bacterial adhesion and colonization, contributing to recurrent infections (Shaikh et al., 2022). Impaired mucosal immunity increases the likelihood of serious lower respiratory tract disease by increasing virus entry and replication in viral infections (Almond et al., 2023).

A paradox results from immunometabolic abnormalities in obesity: immune cells that are malfunctioning and persistently inflammatory. Immune cells are driven into proinflammatory phenotypes by metabolic stress, which prevents them from mounting effective defenses against infections (Shaikh et al., 2024).

Bacterial infections are made worse by impaired neutrophil and macrophage activity, but viral infections like influenza and SARS-CoV-2 show decreased interferon signaling and increased inflammation. Because of this combination, obese people are more prone to infection and have a far higher chance of developing a major infectious disease (Khwatenge et al., 2021).

1.4.1 Immün Dysregulation in Obesity

Obesity significantly alters the immune system, causing chronic inflammation in the body while simultaneously weakening host cell defenses (Fei et al., 2025). The fat tissue of obese individuals secretes higher amounts of chemokines such as MCP-1 and proinflammatory cytokines such as TNF- α and IL-6. This attracts and polarizes immune cells toward a proinflammatory phenotype. This chronic, low-grade inflammation, in addition to excess adiposity, results from a reorganization of innate immune function and a disruption of the proper balance between inflammatory overactivation and basal defenses (Khan et al., 2021).

Furthermore, metabolic disorders caused by obesity impair the function of innate immune cells. The altered immune metabolism of macrophages in obese individuals' adipose tissue weakens their first

line of defense against infection and results in a reduced ability to switch between pro-inflammatory and anti-inflammatory states, meaning they lose their ability to differentiate over time (Nance et al., 2022). The metabolic rigidity of innate cells reduces their response to new infections and hinders the resolution of inflammation, increasing the risk of infection and prolonging inflammatory damage (Nance et al., 2022).

Beyond innate immunity, obesity significantly alters adaptive immune responses. T-cell populations from obese individuals often show signs of aging or exhaustion, reduced proliferative potential, and altered cytokine profiles (Colamatteo et al., 2025). B-cell functions, such as reduced antibody production and inadequate vaccination responses, are also compromised. The body's ability to mount and maintain effective defenses against infectious threats is further constrained by these adaptive deficiencies (Colamatteo et al., 2025).

Lastly, even after weight loss, the immunological dysregulation associated with obesity may persist. Recent research suggests that changes in immune cell structure and function, such as persistent proinflammatory macrophage morphologies, may persist and represent an immunological “memory” of obesity (Fei et al., 2025). The significant consequences of this persistent immune dysregulation on infection risk and long-term immune health in individuals who have lost weight highlight the need for therapeutic approaches that restore immune homeostasis rather than simply reducing adiposity (Fei et al., 2025).

1.5 Infection Susceptibility

The persistent, low-intensity inflammatory environment caused by obesity significantly alters host defenses and makes the individual more vulnerable to bacterial and viral diseases. In obesity, high levels of proinflammatory cytokines (TNF- α , IL-6) and chemokines (MCP-1) are produced by adipose tissue, which attracts immune cells with proinflammatory phenotypes. This ongoing systemic inflammation impedes coordinated antimicrobial responses at tissue barriers and in the circulation (Miron et al., 2024).

One of the primary mechanisms linking obesity to more frequent and severe infections is innate immune deficiency. Obese hosts often exhibit blunted neutrophil chemotaxis, phagocytosis, and oxidative burst, and macrophages in their expanding fat depots are polarized toward a proinflammatory M1 profile with metabolic reprogramming (increased glycolysis, mitochondrial stress) that reduces their capacity to effectively eliminate pathogens. These intrinsic deficiencies prolong inflammatory tissue damage during infections and increase the likelihood of initial infection (reduced pathogen killing at portals of entry) (Vrieling & Stienstra, 2023).

Obesity also impairs adaptive immunity, which has significant implications for vaccine efficacy and infection control. B-cell antibody responses and post-vaccine persistence may also be diminished in obese T-cell populations, which often show signs of fatigue or aging, decreased memory development, and altered metabolic flexibility, leading to weaker antigen-specific responses. Taken together, these adaptive defects contribute to the clinical findings of higher rates of breakthrough or severe disease and lower vaccine responses in obese populations (Miron et al., 2024; Vrieling & Stienstra, 2023).

These mechanistic insights are supported by epidemiological and meta-analytic data: obesity is associated with higher rates of surgical site and skin/soft tissue infections, more serious respiratory viral infections (such as COVID-19 and influenza), more hospitalizations and intensive care unit admissions, and worse outcomes for many bacterial infections (Jiang et al., 2022). A comprehensive systematic review and meta-analysis indicates that obesity significantly increases the risk of wound complications and infectious complications across all settings, highlighting the robustness of the link between pathogens and clinical conditions. Based on these converging lines of evidence, obesity should be considered an independent risk factor for infection, and therapeutic intervention should include tailored management techniques and obesity-sensitive prevention (vaccination strategies, preoperative planning) (Miron et al., 2024).

1.5.1 Viral Infections Associated with Obesity

Obesity is a consistent and independent risk factor for worse outcomes from respiratory viral infections such as SARS-CoV-2 and influenza. According to large cohort studies and meta-analyses, higher BMI is associated with a higher likelihood of hospitalization, the need for invasive ventilation, and mortality from COVID-19, with the risk increasing across obesity classes (Singh et al., 2022). Mechanistically, obesity-associated metabolic comorbidities and persistent low-grade systemic inflammation intensify the host response to viral infection, resulting in a maladaptive hyperinflammatory state in extreme cases (Jaisinghani & Kumar, 2023).

Obesity weakens early innate antiviral defenses at the cellular level. In influenza and other respiratory viruses, obese hosts exhibit prolonged viral replication and shedding due to reduced type I interferon (IFN- α/β) responses in relevant tissues (e.g., lung), delayed activation of antiviral genes, and reduced antiviral activity of pulmonary macrophages and dendritic cells (Shaikh et al., 2022). These abnormalities are associated with both leptin-mediated signaling that dysregulates interferon pathways and altered immunometabolism (e.g., increased glycolysis, mitochondrial stress) in innate immune cells (Miron et al., 2024; Shaikh et al., 2022).

Similar effects are seen in adaptive immunity. Metabolic fatigue and reduced memory development are characteristics of T-cell responses in obesity; cytotoxic CD8⁺ T-cell performance may be reduced,

and vaccine-induced cellular immunity is often weaker and less durable in obese individuals (Honce & Schultz-Cherry, 2019). Clinically, this manifests as a reduced functional T-cell response after some vaccines and a more rapid decline in serologic protection after influenza vaccination. These findings support the findings of increased breakthrough infection rates and reduced vaccine efficacy in populations with high body mass index (Honce & Schultz-Cherry, 2019).

SARS-CoV-2 (COVID-19) has been a notable example of this: mechanistic studies have linked endothelial dysfunction, prothrombotic tendencies, dysregulated interferon responses, and potential adipose tissue permeability to viral persistence leading to severe disease and predicting worse clinical outcomes during the pandemic, even after adjusting for obesity, age, and comorbidities (Nagar et al., 2022; Singh et al., 2022). There is ongoing research on the possibility that expanded fat depots may serve as viral reservoirs (or at least sites of persistent immune activation), with important implications for long-term COVID and viral clearance (Nagar et al., 2022).

Obesity may impact systemic viral infections, as well as respiratory viruses, through metabolic changes that promote viral replication. Many viruses exploit host lipid metabolism to form envelopes and assemble replication complexes. This process may be facilitated by obesity-associated increases in intracellular lipid availability and modified lipid droplet biology, which may enhance viral production and pathogenicity (Miron et al., 2024). Taken together, epidemiological and mechanistic evidence suggests that obesity alters the risk and severity of viral infections, highlighting the need for clinical vigilance and personalized prophylaxis (e.g., vaccination) in obese populations (Nagar et al., 2022; Singh et al., 2022).

1.5.2 Bacterial Infections Associated with Obesity

Obesity impairs wound healing through various local and systemic mechanisms and increases the risk of skin infections. Clinically, obese patients have higher rates of maceration in skin folds, delayed wound healing, and skin colonization by opportunistic bacteria; these changes collectively increase the frequency and severity of skin and soft tissue infections (STIs). The review highlights that inadequate host defense and wound repair processes in obesity are due to immunological dysfunction (including altered neutrophil and macrophage activity), local tissue hypoperfusion, and mechanical factors in fat-rich tissues (Frasca & Strbo, 2022).

According to evidence from Mendelian randomization, obesity and SSTIs are causally associated. Using genome-wide tools for BMI and related obesity traits, researchers discovered that higher BMI and a range of obesity measures were genetically linked to higher odds of impetigo, skin abscesses, cellulitis, and other localized SSTIs. Many of these associations persisted after controlling for type 2 diabetes and peripheral vascular disease, suggesting that obesity itself increases the risk of SSTIs.

These genetic findings support observational correlations and suggest that biological pathways mediated by obesity contribute to susceptibility to bacterial skin diseases (Hu et al., 2022).

Additionally, obesity is consistently associated with an increased risk of surgical site infections (SSI) across various surgical specialties. According to recent systematic reviews and meta-analyses combining recent research, obesity remains a significant and potentially modifiable perioperative risk factor. The increased combined risk of SSI in obese patients is likely due to a combination of technical/surgical factors (greater subcutaneous fat thickness, longer operative times), impaired tissue oxygenation and perfusion, and obesity-related immune dysfunction that impairs bacterial clearance. Our results support obesity-sensitive perioperative planning (wound care, antibiotic dosage, and preventative measures) to reduce postoperative infectious complications (Abi-Jaoude et al., 2023).

Obese individuals are more likely to develop oral bacterial diseases, including periodontitis, which is often more severe. According to large cross-sectional analyses and population data sets, higher body mass index and waist circumference are associated with higher risks of periodontitis; mechanistic studies link worsening periodontal outcomes in obesity to adipokine-mediated dysregulated inflammation, altered oral microbial communities (oral dysbiosis), and weakened host immune responses. The oral-metabolic axis is a clinically important pathway through which localized bacterial disease may exacerbate systemic inflammation in obese patients due to the systemic dissemination of periodontal pathogens and associated inflammatory mediators (Liu et al., 2024).

Finally, obesity-associated gut dysbiosis and increased intestinal permeability create a microbiome-mediated pathway that increases the risk of bacterial translocation, metabolic endotoxemia, and specific gastrointestinal bacterial complications (including increased susceptibility to *Clostridium difficile* infection in dysbiosis states). Reviews summarizing mechanistic and translational research link obesity to a gut environment that promotes pathogenic bacterial overgrowth and systemic proinflammatory signaling through diet-driven changes in community structure, loss of colonization resistance, and altered microbial metabolites (e.g., decreased short-chain fatty acids (SCFAs), increased LPS-bearing taxa). Microbiome-targeted prevention or restoration therapies (dietary fiber, probiotics, and FMT in select settings) are being considered as prospective ways to reduce infection-related effects in obese populations (Acevedo-Román et al., 2024).

1.6 Interactions between Microbiota and Obesity

1.6.1 Gut Microbiota & Obesity

The gut microbiota plays another important role in the pathophysiology of obesity. The role of the gut microbiota in the pathophysiology of obesity is well understood, as gut bacteria extract energy from

fibrous foods, processes affecting intestinal barrier integrity, and systemic inflammation. Gut microbial dysbiosis, characterized by decreased intestinal microbial diversity, an increased *Firmicutes/Bacteroidetes* ratio, and an increase in molecules and cells that trigger and amplify inflammation, is frequently observed in obese individuals. These factors combine to improve dietary energy utilization and promote obesity (Romaní-Pérez et al., 2025).

This increased inflammation results in increased intestinal permeability, or “leaky gut,” or perforations in the gut, a key pathogenic mechanism. Dysbiosis weakens the tight junctions between the epithelial cells lining the gut, allowing bacterial endotoxins such as lipopolysaccharide (LPS) to leak from the gut and into the bloodstream (Romaní-Pérez et al., 2025). Toll-like receptor 4 (TLR4) signaling in metabolic tissues is consistently activated by elevated serum LPS levels, leading to low-grade systemic inflammation that exacerbates insulin resistance and adipose tissue dysfunction (Romaní-Pérez et al., 2025).

In addition, the gut microbiota produces bioactive compounds that alter host metabolism through various pathways, such as tryptophan derivatives, secondary bile acids, and short-chain fatty acids (SCFAs). These products can have positive or negative consequences, but in obese patients, they generally lead to negative outcomes, such as increased energy intake or inflammation (Romaní-Pérez et al., 2025). For example, SCFAs (such as acetate, propionate, and butyrate) can both promote and prevent fat accumulation. Although they support intestinal barrier integrity and control hunger via enteroendocrine hormones, excessive SCFA production in obesity can lead to the delivery of excess energy substrate to adipose tissue (Romaní-Pérez et al., 2025).

Another important link is neuroendocrine signaling, the communication between the nervous and endocrine systems that regulate body functions. In this signaling, gut microorganisms interact with cells in the enteroendocrine pathways, which are produced by endocrine cells, to influence the release of hormones such as GLP-1 (Glucagon-like peptide-1), peptide YY (PYY), cholecystokinin (CCK), and serotonin, which regulate functions such as digestion, appetite, blood sugar, and metabolism (Romaní-Pérez et al., 2025). These enteroendocrine pathways are compromised by dysbiosis in obesity, which reduces satiety signaling and alters gut-brain communication, increasing food intake and disrupting metabolic control (Romaní-Pérez et al., 2025).

Finally, metabolic inflammation is amplified by the immune-modulating function of the microbiota. Proinflammatory immune responses, such as increased activation of Th1 and M1 intestinal macrophages, decreased regulatory T (Treg) cells, and decreased mucosal immunoglobulin A (IgA) production, are triggered by dysbiotic microbial populations (Romaní-Pérez et al., 2025). In addition to compromising intestinal barrier protection, these immunological changes spread inflammation to distant organs such as the liver, skeletal muscle, and adipose tissue (Romaní-Pérez et al., 2025).

In summary, altered microbial balance is linked to increased energy intake, a weakened intestinal barrier, dysregulated neuroendocrine signaling, and systemic inflammation when gut microbiota dysfunction occurs in obesity. The microbiome plays a key role in the pathophysiology of obesity, and these interconnected processes make the development of methods targeting the microbiome to treat obesity compelling. If the microbiome plays such a central role in the obesity process, perhaps the solution lies within (Romaní-Pérez et al., 2025).

1.6.2 Oral Microbiota & Obesity

Beyond its local impact on periodontal disease, which generally results from bacterial accumulation in the mouth and on the teeth, the oral microbiota is increasingly being considered to play a systemic role in the pathogenesis of obesity. Recent research has shown that obese individuals exhibit different salivary microbiome composition and diversity compared to healthy individuals. These changes, similar to those in the gut microbiota, include a higher relative abundance of *Firmicutes* bacteria, a higher *Firmicutes/Bacteroidetes* ratio, and lower microbial diversity, strongly supporting this notion (Schamarek et al., 2023). This similarity suggests a systemic link between the gut and oral microbial communities.

Additionally, the oral microbiome may influence metabolic control by influencing taste perception. Food preferences and consumption habits can be influenced by certain oral bacteria that alter the activity of taste receptor cells (Schamarek et al., 2023). Therefore, dysbiosis within these microbial communities may alter taste sensitivity, which may increase cravings for high-calorie foods and promote excessive calorie consumption.

The oral microbiome and obesity are also linked to inflammatory processes. Dysbiotic oral bacteria, including genera such as *Prevotella* and *Aggregatibacter*, trigger both local and systemic immune responses, while obesity-associated increases in proinflammatory adipokines such as resistin and leptin may worsen periodontal inflammation (Schamarek et al., 2023). Insulin resistance and systemic inflammation may arise as a result of this inflammation feedback into metabolic dysfunction.

Finally, the oral microbiota may indirectly influence distant organs: oral bacteria and their metabolic products can colonize or influence the gut microbiota via swallowed saliva, leading to gut dysbiosis (Schamarek et al., 2023). Therefore, oral microbial dysbiosis may exacerbate obesity-associated metabolic disease through this important but understudied oral-gut microbial axis.

In conclusion, new research suggests that the oral microbiome, contrary to popular belief, plays a more active role than a passive observer and may actively contribute to the development and maintenance of

obesity through taste modification, immunological activation, and cross-colonization. Therefore, treating oral dysbiosis may be a unique therapeutic target within the broader treatment of obesity.

2. Very Low Calorie Ketogenic Diet (VLCKD)

The very-low-calorie ketogenic diet (VLCKD), a nutritional intervention that should be implemented under the supervision of a specialist, targets a very low energy intake, generally not exceeding 400-800 kcal per day, and should include carbohydrate restriction meaning a daily carbohydrate intake of less than 30-50 grams. It also aims to consume sufficient amounts of high-value, bioavailable protein sources to maintain lean muscle mass. With this nutritional intervention, experts predict that obese individuals will lose weight by creating an energy deficit in a short time (Barrea et al., 2024). VLCKD, a temporary nutritional intervention, aims to force obese individuals to metabolize fat instead of glucose for energy. This phenomenon is called ketosis. This means that by consuming very little daily energy, a large energy deficit is created, and the body can now meet its energy needs solely by burning fat. The sustainability of this nutritional intervention is due to the high adequacy of other nutrients and proteins consumed. Another key point is VLCKD's effect on appetite suppression (Barrea et al., 2024). When the body enters a metabolic state called ketosis, the aforementioned primary goal of VLCKD, the liver begins producing ketone bodies. When blood ketone levels rise, certain hormonal and psychological effects are observed. Ghrelin and leptin, which trigger feelings of hunger and fullness, can be directly or indirectly affected in obese individuals undergoing VLCKD. This explains VLCKD's appetite suppression at the hormonal level. Another important aspect of this is its psychological effects. The feeling of fullness felt by obese individuals as a result of VLCKD's appetite suppression, and the fact that they don't get hungry as easily, can help them feel good and increase their motivation throughout the diet process, supporting their weight loss success during the VLCKD intervention (Barrea et al., 2024).

VLCKD is very different from the traditional ketogenic diet (KD), which has long been used to treat epilepsy. For seizure management, the body must enter a state of deep ketosis—a state in which the body produces very high levels of ketone bodies—to maintain this state. Classic KD is generally eucaloric, meaning a person's daily energy intake is approximately equal to their daily energy expenditure, and has a high fat-to-lean ratio (e.g., 4:1). A standard ketogenic diet is high in fat, meaning it contains high fat, very low carbohydrate, and moderate protein intake. VLCKD, on the other hand, is hypocaloric, meaning the person follows a diet containing fewer calories than their daily energy needs and uses a significant caloric loss in addition to carbohydrate restriction to achieve ketosis (Barrea et al., 2024).

The ability of VLCKD to rapidly and clinically significantly reduce weight has led to the increased use of integrative approaches to obesity management. Clinical studies have shown significant improvement in insulin sensitivity, fatty liver, and systemic inflammatory markers during the active ketogenic phase. In obese individuals undergoing bariatric surgery, VLCKD is also used

preoperatively to reduce liver volume and surgical risk. European organizations are increasingly recognizing VLCKD as an acceptable option when used under medical supervision with clear contraindications and refeeding guidelines (Muscogiuri et al., 2021).

The emergence of very-low-calorie diets (VLCDs) in the 1970s, which rapidly gained popularity due to their rapid weight loss but also presented problems with their safety, side effects, and nutritional deficiencies, marked the beginning of the very-low-calorie ketogenic diet (VLCKD). Improved formulations, expert approaches, clinical monitoring techniques, and the adoption of ketogenic principles in the light of research and studies conducted in the following decades have transformed the VLCD into the modern VLCKD. Clinical research and randomized trials have demonstrated the very positive effects of VLCKD since the 2000s, including its effects on hepatic fat mobilization (the breakdown, transport, and processing of fat stored in adipose tissue to the liver to meet the body's energy needs), metabolic signaling pathways, and the reduction of visceral fat. Furthermore, several guidelines supporting VLCKD as a structured treatment were published between 2020 and 2024, confirming its reliability (Muscogiuri et al., 2021).

To understand why VLCKD is preferred in the treatment of obesity and metabolic diseases, its benefits can be summarized as follows: first, it provides rapid weight loss; second, it produces rapid reductions in liver steatosis and abdominal fat; third, it allows for better glycemic control due to lower insulin levels; and fourth, it provides an anti-inflammatory effect by reducing inflammation through ketone bodies, particularly β -hydroxybutyrate, which act on the NLRP3 inflammasome mechanism. The aforementioned ketosis also promotes appetite suppression, improving short-term compliance with the obese patient's diet. Because of these results, VLCKD is a strategic and highly effective solution for metabolic syndrome, severe obesity, and pre-operative optimization (Verde et al., 2024).

VLCKD causes rapid and significant changes in gut and oral microbial composition, often due to abrupt shifts in macronutrient availability and caloric intake. Studies have revealed alterations in the production of key microbial metabolites (such as SCFAs and bile acids), reductions in microbial diversity, and changes in dominant bacterial taxa. Epithelial barrier integrity, mucosal immunity, and susceptibility to bacterial and viral infections may be affected by these microbial changes. VLCKD is a mechanistic axis through which diet-induced microbial restructuring may influence infection risk and inflammatory pathways (Balestra et al., 2024).

2.1 Composition and Metabolic Logic of VLCKD

The classic ketogenic diet is defined as a daily carbohydrate intake of no more than 30 to 50 grams. VLCKD provides less than 800 kcal and 30-50 grams of carbohydrates per day. For both obese men and obese women, the recommended daily protein intake by specialists or dietitians is known to be

1.2-1.5 grams per kilogram of ideal body weight. While general diet programs recommend four meals per day for obese women, a five-meal-per-day meal plan is recommended for men. Dietary records for obese patients undergoing the VLCKD diet are generally structured as follows: White or red meat, fish, eggs, smoked salmon, ham, or canned fish with a vegetable side dish for lunch and dinner, and meal replacements for breakfast and snacks. Extra virgin olive oil can also be used, provided it doesn't exceed 30 grams per day. Olive oil, bicarbonate, minerals (potassium 2000 mg and magnesium 375 mg), omega-3 fatty acids (1 g), and typical sugar-free multivitamins can be used throughout this dietary intervention. It is also strongly recommended that individuals drink a sufficient amount of water (2-2.5 L) daily (Di Rosa et al., 2022).

To fully understand the content of this dietary intervention, we must also consider the approximate energy ratios of macronutrients. VLCKD limits daily carbohydrate intake to less than 30 g (approximately 13% of total energy intake), increases fat (~44%) and protein (~43%), and aims to maintain daily energy intake below 800 kcal. This dietary intervention resembles a classic fasting state. The VLCKD regimen emphasizes whole foods and a diet rich in high-biodegradable protein preparations, using proteins derived from milk, pea, whey, and soy. Each protein preparation contains approximately 100-150 kcal and contains 18 g of protein, 4 g of carbohydrates, and 3 g of fat (mostly high oleic vegetable oils) (Muscogiuri et al., 2019).

Experts prioritize high bioavailability of protein in nutrition programs designed for obese individuals, and protein-sparing formulations are often prepared. Due to the synergistic effects of the reduction in visceral fat (improvement in insulin resistance, overall improvement in metabolic health, etc.) and the proinflammatory state associated with obesity, another benefit of ketogenic diets is the preservation of muscle mass, which in turn correlates positively with muscle strength. The handgrip strength (HGS) test is the most common strength test and is frequently used as a standalone measure of total muscle strength (Barrea et al., 2022).

The VLCKD diet provides a daily calorie intake of less than 700-800 kcal, 30-50 g of carbohydrates (ideally less than 30 g), and 30-40 g of fat (mostly from extra virgin olive oil). This is a typical protein diet; in fact, to maintain free fat mass, it should provide high levels of biological protein, in the range of 0.8-1.5 g per kg of ideal body weight. Bicarbonate, vitamins, and omega-3 fatty acids should be added to VLCKD and should only be used for short periods of time (8-16 weeks) (Di Rosa et al., 2020).

To maintain lean muscle mass and meet the body's minimum daily needs, obese patients undergoing VLCKD should consume high-biovalue protein between 0.8 and 1.2 g per kilogram of ideal body weight. This nutritional intervention is divided into three phases. In the first phase, patients consume low-glycemic vegetables and high-biovalue protein meals five times a day. The second phase involves

replacing one of the protein servings at lunch or dinner with natural proteins such as meat, eggs, or fish. In the third phase, the second serving of the biological protein preparation is replaced with a second serving of low-fat natural protein. Furthermore, as previously mentioned, international guidelines recommend micronutrient supplementation (complex B vitamins, vitamins such as vitamins C and E, potassium, salt, minerals such as magnesium and calcium, and omega-3 fatty acids) because VLCKD is a very low-calorie diet (Muscogiuri et al., 2019).

Ketosis is a metabolic state where the body switches from using glucose to fat for energy, producing ketone bodies like acetoacetate, beta-hydroxybutyrate and acetone. This happens during low carbohydrate availability such as VLCKD. To discuss ketosis and ketone bodies (KBs), which are direct consequences of VLCKD, it's first necessary to understand the physiological process of ketosis. Briefly, it proceeds as follows: Glucose and insulin levels decrease: When carbohydrate intake is low, blood sugar levels and insulin levels decrease. This initiates the fatty acid breakdown phase: low insulin signals the body to break down stored fat into free fatty acids. Ketogenesis then begins in the liver: the liver converts these fatty acids into ketone bodies such as acetoacetate, beta-hydroxybutyrate, and acetone. β -hydroxybutyrate, acetoacetate, and acetone make up ketone bodies (KBs), a group of fuel molecules that act as a substitute for glucose. The liver primarily produces KBs from fatty acids during fasting and extended or strenuous physical activity or nutritional interventions such as VLCKD (Dąbek et al., 2020).

Physiological ketosis and ketoacidosis are commonly mistaken with each other. Ketoacidosis is a pathological state where ketone synthesis becomes uncontrolled, leading to a significant drop in blood pH. It is most typically seen in persons with uncontrolled type 1 or advanced type 2 diabetes, due to a lack of insulin. Physiological ketosis, on the other hand, is a normal and healthy metabolic state that happens with prolonged exercise, fasting, or VLCKD (Hashim & VanItallie, 2014).

All living organisms, including prokaryotes, archaea, and eukaryotes, possess ketone bodies (KB) as a result of lipid metabolism, particularly β -oxidation. These very low-molecular-weight intermediates, such as acetone (Ac), β -hydroxybutyrate (BHB), and acetoacetate (AcAc), serve as energy fuel substitutes for glucose (Puchalska & Crawford, 2017).

In humans, plasma KB concentrations can range from 0.05 to 0.1 mM (millimolar) under physiological conditions. However, under conditions of prolonged exercise, fasting, carbohydrate restriction/ketogenic diets (VLCKD), or increased KB production caused by insulin deficiency, levels can reach 5-7 mM and even 20 mM in some cases; this concentration is indicative of diabetic ketoacidosis, although ketoacidosis is a pathological condition (Gershuni et al., 2018).

To understand the relationship between carbohydrate restriction and immune function, it's helpful to reinforce the following terms: Hepatocyte mitochondria utilize a metabolic process called ketogenesis to convert fatty acids to ketone bodies via β -oxidation. The catabolic process that utilizes ketone bodies to generate energy in peripheral tissue mitochondria is called ketolysis (Di Rosa et al., 2022). It has been understood that beta-hydroxybutyrate (BHB), one of the ketone bodies produced as a result of ketogenesis, can inhibit the NLRP3 inflammasome through its direct effects (Muscogiuri et al., 2019). The NLRP3 inflammasome is a multiprotein complex essential for controlling inflammatory signals and the innate immune system. The proinflammatory cytokines IL-1 β and IL-18 are processed and released by NLRP3, which oligomerizes and activates caspase-1 in response to Pathogen-Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs) (Barrea et al., 2022).

It is crucial to understand that the physiological ketosis that occurs with fasting and/or ketogenic diet or VLCKD has multiple metabolic effects, of which the increase in circulating BHB is only one, even if BHB is directed at directly suppressing the overactivation of the NLRP3 inflammasome (Muscogiuri et al., 2019). The mechanism by which BHB prevents overactivation of the NLRP3 inflammasome is thought to be by altering an unidentified upstream process related to potassium ion influx and ASC (apoptosis-associated speck-like protein containing a CARD) oligomerization/punctate formation during NLRP3 inflammasome formation (Barrea et al., 2022).

The very-low-calorie ketogenic diet (VLCKD) also has strong relationships with the microbiota. VLCKD has been shown to improve insulin sensitivity, regulate gut flora, and possess anti-inflammatory and antioxidant properties. Oxidative stress and intestinal dysbiosis are positively affected by VLCKD (Di Rosa et al., 2020). The relationship between VLCKDs and the microbiota has been supported by recent clinical studies; both human and animal studies suggest that VLCKDs have beneficial effects on improving intestinal biological function and reorganizing bacterial composition, leading to an increase in the number of anti-inflammatory microorganisms. Through metabolites produced by various bacteria, VLCKDs can affect the gut microbiota, leading to increased hydrogen sulfide, decreased lactate levels, and increased short-chain fatty acid production (Dąbek et al., 2020).

Microbiota bacteria produce a wide variety of metabolites, including nitric oxide, serotonin, and short-chain fatty acids (SCFAs). Due to their similar chemical structures, these can bind to host cell receptors and initiate hormonal signaling. For example, Lactobacilli strains have been shown to produce γ -amino butyric acid (GABA), an inhibitory neurotransmitter whose regulation can influence anxiety and depression. The most common members of the human gut microbiota are *Firmicutes* and *Bacteroidetes*. VLCKD generally reduces the number of these bacteria (Hashim & VanItallie, 2014).

Another study on VLCKD used next-generation sequencing. They also examined the effect of intermittent fasting (IF) on the microbiome. Scientists discovered a higher percentage of the *Akkermansia* and *Bifidobacterium* genera, as well as the *Faecalibacterium prausnitzii* species, but they did not detect any significant changes in the overall bacterial population. Obesity and chronic inflammation have been associated with decreased abundance of *Faecalibacterium prausnitzii*. Similarly, some studies have linked obesity to decreased abundance of the genera *Akkermansia* and *Bifidobacterium* (Hashim & VanItallie, 2014).

To summarize Very Low Calorie Diets (VLCDs) do not increase the number of beneficial bacteria; on the contrary, these diets may significantly reduce the number of some beneficial bacterial species, such as *Lactobacillus* and *Bifidobacterium*, due to reduced fiber and carbohydrate intake. The Very Low Calorie Diet (VLCD) positively impacts the gut microbiota not by directly increasing the number of “good bacteria,” but by altering the negative traits associated with obesity and improving overall metabolic health.

2.2 Ketone Body Metabolism and Epigenetic Effects

In animals, ketone body metabolism involves shuttles between organs and cells, controlled by integrated regulatory mechanisms. Because circulating free fatty acids have a limited capacity to provide energy to neurons, ketone bodies are considered alternative energy sources for the neurological system, particularly during periods of low glucose levels. Kidney, heart, and skeletal muscle contribute to the elimination of ketone bodies. The synthesis of ketone bodies from fatty acids in the liver hepatocytes and their absorption and excretion by extrahepatic organs constitute the metabolism of ketone bodies (Balasse & Féry, 1989).

Fatty acids produced in adipose tissue are cleaved from triacylglycerols (TAGs) via lipolysis. Carnitine palmitoyltransferase 1 (CPT1) then transports these fatty acids to the liver and the mitochondrial matrix of hepatocytes. Most ketone bodies are produced in hepatocytes from acetyl coenzyme A (acetyl-CoA) derived from fatty acid β -oxidation and are then transported to extrahepatic tissues for final oxidation. Hepatic glucose fluxes, mitochondrial redox potential, β -oxidation rates, concentrations of tricarboxylic acid (TCA) cycle intermediates, and glucagon and insulin balance influence ketogenesis rates in addition to fat cell lipolysis. Ketogenesis is also controlled at the transcriptional and posttranslational levels, although the precise and cell-autonomous regulatory mechanisms are not yet fully known (Puchalska & Crawford, 2021).

In healthy humans, the daily circadian concentration of ketones ranges from 50 to 250 μ M and contributes up to approximately 5% of total energy expenditure in the fed state and up to approximately 20% in the fasted state (Cox et al., 2016). Fluctuations in circulating ketone body levels

may increase during fasting, following a low-carbohydrate, high-fat diet, immediately after physical activity, during the neonatal period, or in late pregnancy. In pathological conditions (diabetic ketoacidosis), ketone body concentrations can reach as high as 20 mM, while prolonged activity or 24-hour fasting can elevate circulating ketone body levels above 1 mM (Puchalska & Crawford, 2021). Ketone production and consumption are balanced in physiologic ketotic states, which generally result in steady-state total circulating ketone body concentrations of 4–5 mM. Extrahepatic tissues rapidly deplete ketone bodies, continuing until saturation and oxidation occur. Only a small amount of ketones are excreted in the urine; the majority are oxidized in proportion to circulating concentrations. Ketonuria and the pleasant odor produced by the spontaneous decarboxylation of acetoacetate (AcAc) to acetone can be used as a surrogate for ketoacidosis in humans (>7 mM), and urinary ketone concentrations increase in ketotic states where circulating ketones exceed 1 mM (121, 136). In adults fasting overnight, the rate of ketogenesis is approximately 0.25 mmol/min, increasing to 1–2 mmol/min after five days (Balasse & Féry, 1989). In extrahepatic tissues, AcAc is directed toward terminal oxidation or anabolic pathways. However, the capacity for terminal oxidation is tenfold greater than that of anabolic excretion pathways, and loss of oxidative capacity leads to ketoacidosis, indicating that anabolic excretion alone is insufficient to support effective ketone conversion (Cotter et al., 2011). The fate of AcAc also involves its excretion, so although circulating AcAc and D- β -hydroxybutyrate (D- β OHB) exchange nearly at equilibrium, the two do not fully equilibrate (Balasse & Féry, 1989).

With comparable maximum rates of ketone body appearance and excretion of 1–2 mmol/min/1.73 m² and similar oscillations in circulating concentrations across the feeding-fasting cycle, mice remain an excellent model of the human ketone cycle, despite a more rapid induction in response to fasting compared to humans (see also the sidebar titled Feeding Provocation of Endogenous Ketogenesis) (Sunny et al., 2011). Although ketogenic delays in response to fasting tend to increase with the transition from infancy to maturity, the transition from the perinatal to neonatal phase is associated with a significant increase in ketogenic ability in both mice and humans. The impact of later aging is less clear, as there are conflicting findings regarding differences in ketogenesis or circulating ketones in aging mice and humans. Furthermore, even if there is evidence for sex-related differences in circulating free fatty acids, there are insufficient data to support sex-related differences in the ketogenic rate in humans (Puchalska & Crawford, 2021).

Extrahepatic tissues take up and oxidize ketone bodies at a high rate of delivery at low circulating concentrations; however, at higher concentrations, metabolic clearance rates decrease because uptake and oxidation become saturable. Consequently, there is an exponential relationship between ketone body formation and circulating concentrations; as ketogenesis rates increase, circulating concentrations also increase, reaching a maximum clearance rate at approximately 6 mM (Balasse &

Féry, 1989). Furthermore, there may be different relationships between ketone concentration applied to different tissues and metabolic clearance rate. At low circulating amounts, skeletal muscle consumes ketones rapidly; at high concentrations, this rate decreases. Conversely, the brain can adjust during periods of famine by maintaining a more constant clearance rate across ketone concentrations. It also inhibits adipose tissue lipolysis when ketone body levels rise by activating the cell surface receptor GPR109A, which, in a negative feedback loop, reduces the availability of circulating free fatty acids (Taggart et al., 2005). However, this pathway is not dominant, because in diabetic ketoacidosis, the reduced or absent insulin, dehydration, and sympathetic activity lead to uncontrolled fat cell fatty acid lipolysis, which increases ketogenesis while decreasing ketone excretion rates. This mismatch results in acidosis and massive hyperketonemia (≥ 20 mM). The multiple sources of unbuffered protons generated during fatty acid hydrolysis via ketogenesis may be responsible for the acidosis itself (Green & Bishop, 2019).

Ketone bodies also serve as epigenetic regulators. Histone acetylation patterns are influenced by changes in acetyl-coenzyme A and NAD^+ levels, which are directly linked to cellular metabolic status and gene regulation. Here, the ketone body D- β -hydroxybutyrate (BHB), in addition to being an energy substrate, serves as an endogenous and specific inhibitor of class I histone deacetylases (HDACs). Clinical trials have shown strong increases in global histone acetylation in various mouse tissues in experimental settings that increase circulating BHB, such as fasting and calorie restriction. This suggests that ketosis directly alters chromatin structure, altering epigenetic architecture and linking fuel availability to transcriptional output (Shimazu et al., 2013).

The HDAC inhibitory effect of BHB, which initiates transcriptional changes that occur when genetic information is transferred from DNA to RNA, is followed by extensive changes and regulation, particularly the activation of genes associated with resistance to oxidative stress. Among these, when BHB increases histone acetylation in promoter regions, FOXO3A and MT2 become highly activated. The specificity of the pathway is confirmed by the similar upregulation of the same genes upon selective depletion of HDAC1 or HDAC2. Functionally, this epigenetic reprogramming results in enhanced cellular resilience: A recent study found that BHB-treated mice exhibited significant protection against oxidative stress, which was associated with higher antioxidant defenses mediated by FOXO3A and MT2. All these results indicate that ketone bodies are powerful metabolic cues that can coordinate transcriptional and epigenetic changes that influence tissue stress tolerance (Shimazu et al., 2013).

The relevance of this section to the project is the discovery that the immunomodulatory effects of ketone bodies could potentially interact with both viral and bacterial infections.

2.3 Metabolic Effects of VLCKD

Nutritional ketosis, which is characterized by high circulating ketone bodies and significant changes in energy metabolism, is induced by the very low-calorie ketogenic diet (VLCKD), which is characterized by a drastic reduction in caloric intake paired with very low carbohydrate consumption (<50 g/day). In people with obesity and metabolic dysfunction, this metabolic state improves insulin sensitivity and glycaemic management while promoting rapid weight loss through increased fatty acid oxidation and large reductions in visceral adipose tissue. Research shows that VLCKD interventions significantly lower systemic inflammation markers, such as high-sensitivity C-reactive protein (hs-CRP) and other pro-inflammatory cytokines, in comparison to baseline values or comparator diets. This suggests an anti-inflammatory effect associated with both adiposity loss and ketone body-mediated mechanisms (Rondanelli et al., 2024). Mechanistically, it has been demonstrated that the predominant ketone body β -hydroxybutyrate (BHB) inhibits the activation of the NLRP3 inflammasome, which reduces the secretion of important pro-inflammatory cytokines like interleukin- 1β and contributes to a shift towards lower chronic inflammation independent of calorie restriction alone (Youm et al., 2015). When taken as a whole, these metabolic effects improvements in insulin resistance, decrease of adipose tissue inflammatory burden, and attenuation of systemic inflammation establish VLCKD as a potent therapeutic strategy for managing obesity and modulating metabolic diseases.

2.3.1 Insulin Resistance

A recent clinical study examining the metabolic effects of VLCKD on obese individuals and insulin sensitivity showed rapid improvements in anthropometric and biochemical markers following the VLCKD diet intervention. Table 1 shows the significant improvements in values following the VLCKD diet intervention, which shows the values of obese individuals before and after the dietary intervention. Obese individuals who underwent VLCKD demonstrated statistically significant reductions in weight, body mass index (BMI), fat mass (FM), and visceral adipose tissue (VAT). Furthermore, following the diet, these individuals also demonstrated significant improvements in biochemical markers such as glycemic and lipid profiles. Following the diet, participants' adiponectin levels significantly increased, while their CRP (C-reactive protein) levels significantly decreased, demonstrating the anti-inflammatory properties of the increased adiponectin levels resulting from the VLCKD intervention. The researchers noted that the results of this study revealed a gender dimorphism between men and women, which may be due to distinct physical differences. Specifically, they found that adiponectin levels were higher in obese male VLCKD participants than in obese female VLCKD participants, both before and after the dietary intervention. After VLCKD, a significant increase in adiponectin levels was observed in both men and women. Furthermore,

adiponectin was positively correlated with HDL (good cholesterol) but negatively correlated with visceral adipose tissue (VAT), CRP levels, glycated hemoglobin(HbA1c), and lipid profiles. Furthermore, a significant decrease in serum TNF- α levels was observed in subjects following VLCKD. This suggests a decrease in inflammation, which could have both positive and potentially negative effects on immune system function. Additionally, a significant increase in interleukin-10 (IL-10) levels was observed, a key indicator of the immune system's efforts to suppress and control inflammation. In light of these results, it's safe to say that VLCKD reduces insulin resistance in obese patients in a short time (Monda et al., 2020).

	VLCKD Obese Participants		p-value
	T0	T1	
BMI(kg/m ²)	32.19 $\pm$ 4.78	27.76 $\pm$ 3.6	<0.001
Weight(kg)	91.33 $\pm$ 17.1	78.73 $\pm$ 13.3	<0.001
VAT(g)	1541.55 $\pm$ 141.6	927.79 $\pm$ 104.9	<0.001
HDL(mg/dL)	55.13 $\pm$ 11.1	47.76 $\pm$ 9.1	ns
CRP(mg/dL)	0.89 $\pm$ 0.1	0.48 $\pm$ 0.1	<0.05
Adiponectin (μ g/mL)	10.8 $\pm$ 1.2	25.55 $\pm$ 1.3	<0.001
TNF- α (pg/mL)	345 $\pm$ 6.5	278 $\pm$ 9.2	<0.05
IL-10 (pg/mL)	117 $\pm$ 7	168 $\pm$ 8.8	<0.001
Hba1c (%)	5.65 $\pm$ 0.3	5.38 $\pm$ 0.3	ns

Table 1. Body composition and biochemical features of very low-calorie ketogenic diet (VLCKD) of participants before and after weight-loss (Bosch-Sierra et al., 2024).

2.3.2 Reduction in Inflammatory Markers

In addition to improving metabolism and cardiovascular health, VLCKD also significantly reduces markers of inflammation. The anti-inflammatory effects of VLCKD are further demonstrated by significant reductions in TNF- α and IL-6, two key indicators of systemic inflammation (Bosch-Sierra et al., 2024). Another analysis found that obese patients exposed to VLCKD experienced significant reductions in inflammatory markers such as CRP, IL-6, and TNF-a, suggesting that this nutritional

intervention is an effective nutritional strategy for managing low-grade chronic inflammation associated with obesity (Rondanelli et al., 2024).

2.3.3 Reduction of Adipose Tissue Inflammatory Burden

Very-low-calorie ketogenic diets (VLCKD) have been shown to reduce the inflammatory burden of adipose tissue by altering the structure of immune cells within the tissue and reducing proinflammatory cytokine production. Adipose macrophage polarization shifts from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype as a result of rapid carbohydrate restriction and the resulting increase in ketone bodies, particularly β -hydroxybutyrate, which reduces local cytokine output. Other evidence from clinical and mechanistic studies suggests that VLCKD treatments reduce circulating markers such as CRP, TNF- α , and IL-6, resulting in reduced systemic and fat-induced inflammation. These changes, combined, suggest that VLCKD creates a more physiologically favorable environment in adipose tissue that is less prone to inflammation, which may subsequently confer advantages in terms of metabolic health and susceptibility to infection in obese individuals (Bosch-Sierra et al., 2024).

2.4 VLCKD and the Oral Microbiota

Similar to more general low-carbohydrate, high-fat (LCHF) diets, very-low-carbohydrate ketogenic diets (VLCKD) induce specific changes in the oral microbial environment by altering substrate availability for saccharolytic bacteria, bacteria that produce energy by hydrolyzing carbohydrates. Recent experiments have shown that LCHF intake in a controlled dietary intervention significantly alters the oral microbial community, with increases in bacterial genera such as *Neisseria* and decreases in carbohydrate-fermenting genera such as *Prevotella* and *Veillonella*. Because lower carbohydrate availability favors bacteria that rely on amino acids or alternative metabolic pathways rather than glycolysis, these changes represent a rapid response of the oral microbiome to changes in dietary carbohydrate intake. Such compositional changes are particularly important because VLCKD reflects the same macronutrient composition and, therefore, likely creates similar ecological pressures within the oral cavity. These results highlight how VLCKD may alter the nature of oral microbial communities, leading to a lower prevalence of saccharolytic and acidogenic species (Cato et al., 2023).

Carbohydrate restriction in VLCKD may also affect salivary pH by reducing the growth and metabolic activity of acidogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus* species, which depend on fermentable carbohydrates to produce lactic acid. Individuals on a ketogenic diet experienced measurable increases in salivary pH as their carbohydrate intake decreased, indicating a decrease in acidogenic bacterial activity. Because acid production is a significant pathogenic factor in cariology,

this shift toward a less acidic oral environment could theoretically reduce the likelihood of dental caries and enamel demineralization (Woelber et al., 2021).

VLCKD may reduce acidogenic bacterial species, but its overall impact on periodontal pathogens is not yet clear. Ketogenic diets may indirectly impact periodontal health by altering microbial ecology, food intake, and systemic inflammation. A review has shown that while reducing refined carbohydrates may improve periodontal outcomes by reducing plaque accumulation and gingival inflammation, carbohydrate-restrictive diets, including VLCKD, may also pose nutritional risks if vital micronutrients such as calcium, vitamin D, and antioxidants are not adequately consumed. Both host immunological responses and the integrity of periodontal tissue depend on these nutrients. Furthermore, changes in the oral microbiota under ketogenic conditions may alter the dynamics of microbial competition, which in turn may impact the ecological niches occupied by pathogens such as *Porphyromonas gingivalis*. Current research suggests that VLCKD may alter the risk of periodontal disease through interactions between host immunity, microbial composition, and micronutrient status; however, the data are still evolving, and more research is needed (Taher et al., 2024).

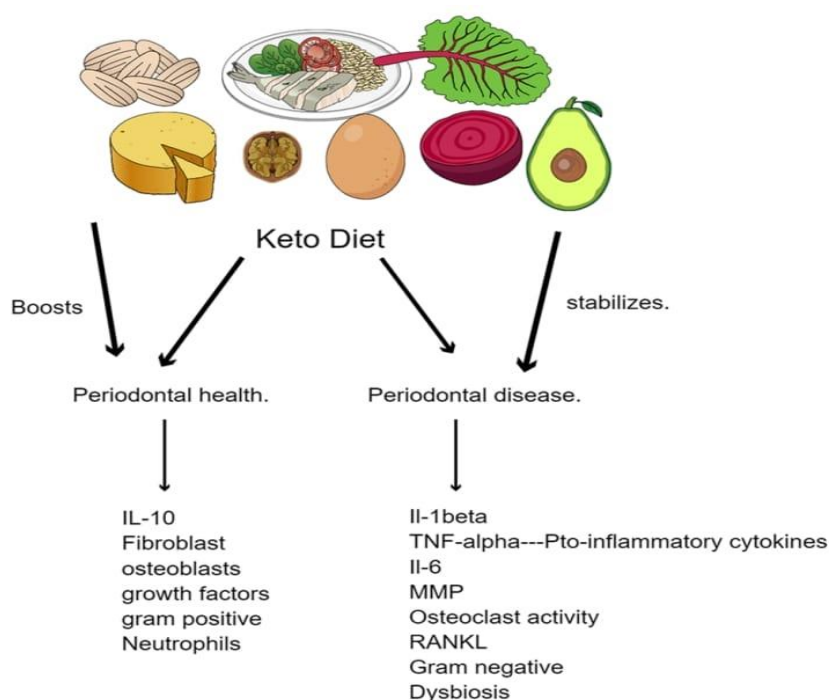


Figure 1. Role of ketogenic diet on periodontal disease progression (Taher et al., 2024).

Nitrate-reducing bacteria, which are critical for maintaining systemic nitric oxide (NO) homeostasis via the nitrate-nitrite-NO pathway, also undergo changes in the oral microbiota during low-carbohydrate or ketogenic diets. Research results indicate that LCHF diets alter many microbial taxa, while the numbers and functional activities of nitrate-reducing bacteria are not significantly affected.

The enzymatic conversion of dietary nitrate to nitrite, the precursor of bioactive NO, which plays a role in vascular control, immunological function, and metabolic health, depends on these bacteria, including *Rothia* and some *Neisseria* species. This metabolic function is preserved under ketogenic conditions, suggesting that beneficial oral microbial pathways are not always adversely affected by VLCKD-induced changes. Given that nitric oxide regulates host defense mechanisms and vascular responses, which can influence both metabolic outcomes and infection susceptibility in obese individuals, maintaining nitrate-reducing ability is crucial. As a result, an increase or decrease in some nitrate-reducing bacteria may occur, but functional capacity can be preserved, thus eliminating any problems in the conversion of dietary nitrate into nitrite, which is the first step in the nitric oxide (NO) production chain, which is important for blood pressure regulation and cardiovascular health (Cato et al., 2023).

2.5 Clinical and Environmental Modulators of VLCKD

2.5.1 Age, Sex and Degree of Obesity as Modulators of VLCKD Response

Clinical responses to VLCKD vary widely among individuals, and characteristics such as age, sex, and baseline obesity class significantly influence outcomes. In controlled interventions analyzed in recent years, men and women exhibited different biochemical processes during VLCKD; women showed relatively slower reductions in visceral adiposity and hepatic steatosis, while men showed more pronounced reductions in liver fat and anthropometric markers. Different baseline inflammatory levels, changes in fat distribution, and hormonal milieu have been identified as contributing factors to these sex-specific differences. Studies have also noted that individuals with higher baseline BMI (Class II-III obesity) exhibited both greater absolute fat mass reductions and more heterogeneous metabolic adaptations. Studies have also observed a slower rate of metabolic recovery in older individuals, likely due to their lower mitochondrial flexibility and altered adipose tissue biology (D'Abbondanza et al., 2020). In general, it would not be wrong to say that the degree of obesity, age and gender are important clinical factors affecting the severity and speed of the physiological reaction to VLCKD in the light of these studies.

2.5.2 Baseline Microbiota Composition as a Predictor of Diet Responsiveness

Another topic that has been investigated in recent years and is gaining increasing evidence is the idea that the metabolic response to diets, particularly VLCKD, may be significantly influenced by the baseline composition of the gut microbiota prior to dietary intervention. A review by (Leshem et al., 2020) suggests that certain microbial profiles are associated with better overall responses to VLCKD, including better weight loss, improved glycemic response, and faster adaptation to carbohydrate restriction (Leshem et al., 2020). Individuals enriched with the *Prevotella* genus or with higher

baseline microbial diversity often show more significant metabolic changes while adhering to carbohydrate-restricted diets. Conversely, individuals with dysbiotic profiles or low-diversity microbiomes respond less effectively than those with other microbiomes, suggesting that the microbiome is both a mediator and predictor of therapeutic success (Leshem et al., 2020). Mechanistically, the core microbiota regulates fermentation capacity, short-chain fatty acid synthesis, intestinal barrier integrity, and systemic inflammatory variables that collectively determine VLCKD outcomes. This study highlights that microbiome-stratified dietary therapies, that is, personalized diets tailored to individuals whose microbiota composition has been studied, may improve therapeutic efficacy.

2.5.3 Metabolic Comorbidities as Determinants of VLCKD Effectiveness

Metabolic diseases such as type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD) have a significant impact on how individuals respond to nutritional interventions in VLCKD. Due to increased hepatic ketogenesis and reduced glucose demand in peripheral nervous system tissues, individuals with type 2 diabetes often experience rapid improvements in insulin sensitivity, reductions in HOMA-IR (Homeostasis Model Assessment of Insulin Resistance), and improvements in glycemic control as a result of VLCKD (Paoli et al., 2023). These results occur within weeks and demonstrate that the metabolic difference between a regular diet and VLCKD is amplified by decreased glucose metabolism. Similarly, VLCKD demonstrates significant and beneficial outcomes in patients with NAFLD; one review found that reductions in liver fat content, systemic inflammation, and anthropometric indicators occurred concurrently with reductions in dietary intervention (D'Abbondanza et al., 2020).

2.5.4 Variability Among VLCKD Protocols as a Source of Clinical Differences

Variations in VLCKD protocols, such as varying caloric intake thresholds, carbohydrate restriction, protein targets during the diet, duration of active ketosis, and refeeding strategies, are another important modifier of clinical outcomes. A study reviewed demonstrates the significant variability of European clinical guidelines or protocols; protein intake is typically maintained at 1.2–1.5 g per kg of ideal body weight, energy intake is set between 600–800 kcal/day, and carbohydrate intake is typically restricted to less than 30–50 g/day (Muscogiuri et al., 2021). However, dietary changes can directly impact ketone body production, nitrogen balance, and the preservation of lean mass (Muscogiuri et al., 2021). Heterogeneity among clinical outcomes across VLCKD studies is further influenced by changes in behavioral monitoring, contraindication management, and supplementation regimens.

3. Oral Microbiota

The oral microbiome, one of the regions of the human body with the second-highest microbial diversity after the gut microbiota, is composed of aggregated microbial communities distributed across various regions within the mouth. A recent study has found that over 600 bacterial taxa inhabit the mouth, and these communities are highly organized and possess unique functional mechanisms (Dewhirst et al., 2010). These bacterial habitats can be found in almost every region of the oral cavity, including the tooth surface, the upper surface of the tongue, the gingival grooves, the buccal mucosa, and salivary secretions. Oxygen levels specific to each region's ecosystem, shear stresses that directly affect microorganisms' ability to form biofilms, nutrient availability, pH, and host immunological factors shape the microbial populations that inhabit them (Dewhirst et al., 2010).

The oral microbiome is a heterogeneous structure composed of diverse, interconnected microbial ecosystems with a high diversity of species. The oral microbiome is not a uniform ecosystem. Due to the diverse conditions offered by different regions, it is possible to observe different microbial populations and proliferations on almost every surface. This is one of the most important characteristics that contribute to the diversity of the oral microbiota (Dewhirst et al., 2010). Due to this diversity, the resilience of oral microbial populations and their capacity to influence systemic health vary (Dewhirst et al., 2010). To fully understand the oral microbiome, it is necessary to understand its diversity, spatial organization, and dynamic interaction with the host environment.

No. (%) of:				
Phylum	Taxa	Named species	Unnamed cultivated taxa	Unnamed uncultivated taxa
Bacteria				
<i>Firmicutes</i>	227(36.7)	120(52.9)	45(19.8)	62(27.3)
<i>Bacteroidetes</i>	107(17.3)	39(36.4)	27(25.2)	41(38.3)
<i>Proteobacteria</i>	106(17.1)	70(66.0)	9(8.5)	27(25.5)
<i>Actinobacteria</i>	72(11.6)	37(51.4)	25(34.7)	10(13.9)
<i>Spirochaetes</i>	49(7.9)	11(22.4)	3(6.1)	35(71.4)
<i>Fusobacteria</i>	32(5.2)	12(37.5)	4(12.5)	16(50.0)
<i>TM7</i>	12(1.9)	0(0.0)	0(0.0)	12(100.0)
<i>Synergistetes</i>	10(1.6)	2(20.0)	0(0.0)	8(80.0)
<i>Chlamydiae</i>	1(0.2)	1(100.0)	0(0.0)	0(0.0)
<i>Chloroflexi</i>	1(0.2)	0(0.0)	0(0.0)	1(100.0)
<i>SR1</i>	1(0.2)	0(0.0)	0(0.0)	1(100.0)
Archaea <i>Euryarchaeota</i>	1(0.2)	1(100.0)	0(0.0)	0(0.0)
Total	619(100)	293(47.3)	113(18.3)	213(34.4)

Table 2. Phylogenetic distribution of 619 taxa in HOMD (Human Oral Microbiome Database) version 10 (Dewhirst et al., 2010).

Because of the diverse microhabitats within the oral cavity, the oral microbiome exhibits a high degree of biogeographic organization. For example, the tongue surface, due to its papillary structure and low-oxygen environment, hosts numerous *Streptococcus*, *Actinomyces*, and *Veillonella* species (Baker et al., 2024).

Dental plaque, on the other hand, is a persistent biofilm attached to the surface, containing nutrient and oxygen gradients. *Fusobacterium*, *Porphyromonas*, and *Prevotella* are among the colonizers that thrive in this ecological niche; many of these organisms exhibit anaerobic metabolism and facilitate the development of biofilms. Inflammation, immunological reactions, and gingival crevicular fluid flow influence the proliferation of periodontal-associated microbes in the deeper, more anaerobic gingival crevicular environment, causing inflammation and damage. Finally, saliva serves as a dynamic microbial reservoir, transporting immunoglobulins, enzymes, and antimicrobial proteins while collecting excreted species from all oral regions (Baker et al., 2024).

There are also communities of microbial taxa or functions consistently present in all individuals, regardless of regional or demographic differences within the oral cavity; this is called the "core microbiome." Genera such as *Streptococcus*, *Veillonella*, *Actinomyces*, *Haemophilus*, and *Neisseria* are commonly found in the core oral microbiome of the oral cavity and are consistent across populations (Sharon et al., 2022).

(Malla et.al) suggested that when defining the core microbiome, the shared functional capacities of classes, in addition to their existence, should be considered. According to the researchers, even though hundreds of different bacterial species in the mouth are constantly changing, essential biological functions essential for oral health, such as nitrate reduction, carbohydrate metabolism, and the synthesis of antimicrobial agents, can be consistently maintained by this core community (Sharon et al., 2022).

3.1 Oral Microbiota Structure in Obese Individuals

Obesity certainly has significant effects on the oral microbiota, and this notion is gaining increasing acceptance. In a study of adolescent girls, Mervish and colleagues discovered significant microbial changes associated with obesity (Mervish et al., 2019). Obese girls showed different patterns of beta diversity and lower alpha diversity compared to their normal-weight counterparts. Alpha diversity refers to the diversity of species within a given region or ecosystem, while beta diversity refers to the difference in species composition between two regions or ecosystems. Higher concentrations of taxa, including *Prevotella* and *Veillonella*, were found in the obese girls' group due to their dietary patterns, suggesting a shift toward more anaerobic or carbohydrate-adapted organisms (Mervish et al., 2019).

Another study supports the impact of obesity on oral microbial structure with data from adults. This analysis, conducted on individuals with type 2 diabetes, found that obesity independently reduces microbial diversity and contributes specifically to the enrichment of *Fusobacterium*, *Prevotella*, and other inflammation-associated taxa. These results suggest that obesity has a significant ecological impact on the oral microbiota, even in the presence of other metabolic disorders such as hyperglycemia (Tam et al., 2018).

3.2 Functional Roles of the Oral Microbiota

3.2.1 Nitrate-Nitrite-NO Conversion in Saliva

Through the nitrate-nitrite-NO pathway found in saliva, the oral microbiota plays a crucial biochemical role in maintaining nitric oxide (NO) homeostasis. Numerous microorganisms capable of metabolizing nitrate are present on the tongue surface, including *Neisseria*, *Rothia*, and *Actinomyces*. These microorganisms convert dietary nitrate, especially abundant in green leafy vegetables, into nitrite using the nitrate reductase enzyme. The ingested compounds are then reduced to NO in the stomach or peripheral tissues. This resulting compound is a crucial signaling molecule in the body (Morou-Bermúdez et al., 2022). NO plays a role in many important processes in the body, such as acting as a vasodilator, lowering blood pressure, and regulating immune responses. The human body normally produces NO through the NOS enzyme, but sometimes the activity of this enzyme may be insufficient, preventing the body from producing sufficient amounts of NO. In such cases, this function of the oral microbiota is vital for maintaining balanced NO levels in the body. For example, the inadequacy of one's own nitric oxide (NO) production, which can be seen during a disease such as obesity, can be eliminated with this alternative pathway provided by beneficial bacteria in the mouth (Morou-Bermúdez et al., 2022).

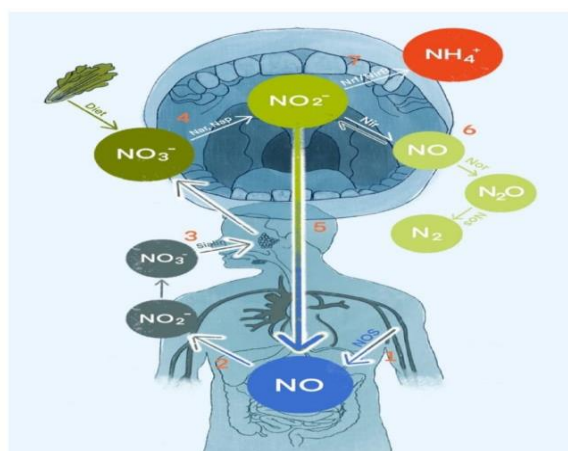


Figure 2. Interconnected microbial and human pathways in nitric oxide (NO) metabolism (Morou-Bermúdez et al., 2022).

3.2.2 Metabolic Products of the Oral Microbiota

The oral microbiota not only produces molecules that provide systemic benefits, such as NO, but also produces a wide variety of substances that can directly affect the internal balance and structure of the mouth. These metabolites affect the mouth's ecological, or habitat, and biochemical balance. For example, dietary carbohydrates are metabolized by streptococci and other saccharolytic, or sugar-degrading, bacteria naturally present in the mouth into lactic acid and other organic acids. This process is essentially a survival process for bacteria, but the byproducts produced during this process are detrimental to oral health. The acids produced in this process lower the pH of the plaque layer on the tooth surface to as low as 4. Normally, the pH of a healthy mouth is close to neutral. When the pH drops, "good bacteria" that dislike acidic environments cannot survive and die, while acidic species proliferate. These acid-loving bacteria cause decay (Cornejo Ulloa et al., 2019). On the other hand, the oral cavity can be balanced by the opposite mechanism, thanks to argininolytic microbes such as *Streptococcus sanguinis*. These species produce ammonia through the breakdown of arginine, thus balancing pH and supporting microbial balance. Additionally, some taxa can produce short-chain fatty acid (SCFA)-like metabolites that can alter epithelial physiology, and oral bacteria produce hydrogen peroxide (H₂O₂), which contributes to interspecies competition. Hydrogen peroxide is essentially a mild antiseptic, meaning bacteria that can produce this substance outcompete other species that cannot, thus protecting their habitat. Microbial stratification is determined by the combination of pH, oxygen tension, and nutrient gradients in this delicately balanced environment (Cornejo Ulloa et al., 2019).

3.2.3 Oral Epithelial Barrier and Immune Homeostasis

The oral epithelium functions as a living and responsive defense system that constantly interacts with local microbial communities, demonstrating that this barrier is not merely a physical barrier but a constantly changing, renewing surface that actively orchestrates immune responses. The mucosal surface, composed of salivary secretory IgA, antimicrobial peptides (AMPs), and stratified squamous cells, controls microbial adhesion and reduces pathogen penetration of the oral epithelium (Liu et al., 2022). The human host influences microbial composition within the mouth through salivary flow, peptide release, and immune surveillance, while commensal bacteria alter epithelial signaling pathways involved in tight junction control, cytokine expression, and cellular renewal. This mutual benefit exists. The host eliminates harmful bacteria through saliva and eliminates the competitive environment. In turn, commensal bacteria strengthen tissue barriers and manage inflammation, helping the human host maintain health. Disruption of this balanced communication, whether through dysbiosis or inflammation, can compromise barrier integrity, making the body more vulnerable to systemic immune activation and local infections (Liu et al., 2022). All of these communication events demonstrates us how oral cavity is an important interface for immunological homeostasis.

3.2.4 Pattern Recognition and TLR-Mediated Immune Responses

Pattern recognition receptors (PRRs), a direct pathway between the immune system and the microbiota, play a crucial role in regulating innate immune responses in the oral cavity. Oral immune cells use TLR sensors to recognize bacteria. In response to this recognition, they produce chemical messengers and killer substances such as cytokines. Through these sensors, oral immune cells maintain microbial homeostasis by determining the overall level of inflammation and which bacterial species will survive (Rajasekaran et al., 2024). Pathogenic species cause more intense reactions associated with tissue damage and periodontal disease, while commensal bacteria generally cause low-level signaling that maintains immunological readiness. This allows the oral immune system to discriminate. The immune system, on the one hand, issues immediate attacks on hostile invaders while, on the other, ensuring that its beneficial inhabitants (the microbiota) remain untouched (Rajasekaran et al., 2024). This dual balance is vital for maintaining oral health, as without beneficial bacteria, overall health would deteriorate.

3.3 Oral Microbiota and Bacterial/Viral Infections

Throughout a person's lifetime, the oral microbiota is a highly dynamic ecosystem that constantly interacts with host immunity, environmental variables, and microbial challenges. Since they may cause both temporary and permanent changes in the makeup and behavior of microorganisms, bacterial and viral infections are among the most powerful modulators of this ecosystem. Through inflammation, immunological activation, or exposure to antibiotics, acute infections may momentarily upset the equilibrium of oral microbiota; however, mounting data indicates that chronic or recurrent infections may cause long-lasting changes in the oral microbiota. A new, stable yet dysbiotic oral microbial state is believed to be the result of these long-term changes, which are believed to be caused by immunological modulation that persists over time, changes in the integrity of the mucosal barrier, and ecological reorganization of microbial communities (Peng et al., 2022).

It has been demonstrated that even after clinical recovery, viral infections in particular continue to affect the oral microbiota. Oral microbial diversity and composition may continue to change for months after the acute illness phase has ended, according to longitudinal studies looking at people recovering from viral infections like SARS-CoV-2. Long-term follow-up periods have shown changes in certain bacterial taxa and decreased microbial resilience, suggesting that viral exposure might have a long-lasting effect on the oral microbial environment (Wang et al., 2025). Crucially, these alterations may continue even after an apparent immunological recovery, indicating that infection-related disruptions have the potential to permanently alter host-microbe interactions in the oral cavity.

By focusing on the cumulative effects of several viral occurrences across the lifespan rather than isolated episodes, the idea of lifetime infection exposure broadens this viewpoint. Chronic inflammatory conditions, recurrent bacterial and viral infections, and repetitive immune activation can all gradually alter the oral microbiota and cause chronic dysbiosis. In people with immunological or metabolic dysregulation, where microbial resistance may already be weakened, such cumulative consequences are especially pertinent. Chronic changes in the oral microbiota brought on by lifetime virus exposure may be linked to immune system dysregulation, systemic inflammation, and heightened vulnerability to inflammatory and metabolic disorders. Together, these results lend credence to the idea that an individual's oral microbiota reflects both their current state of health and their history of infections, highlighting the significance of taking lifetime infection exposure into account when assessing oral microbial profiles in disease-related research (Peng et al., 2022; Wei et al., 2023).

3.3.1 Oral Bacterial Pathogens

Most commensal and pathogenic bacterial communities that make up the oral microbiota are primarily found in the subgingival habitat. *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* are among the best-known examples of pathogenic bacteria and are significant causes of periodontal tissue degradation; these bacterial genera are among those that cause the most clinically significant periodontal infections. Recent studies and research show increasing evidence that the subgingival microbial structure is significantly altered by obesity, and that obesity promotes the enrichment of certain pathogenic species in the subgingival microbial structure. Regardless of periodontal status, systematic studies of the subgingival microbiota have shown that obese individuals have higher levels of *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans* compared to normal-weight individuals; that is, harmful bacterial growth is observed solely due to obesity, regardless of the individual's oral health. This is due to the low-grade chronic inflammation and changes in the immune system that obesity creates in the body. Cytokines secreted by adipose tissue in the body alter the gum tissue's response to bacteria, creating a favorable environment for these pathogenic microorganisms to multiply more easily (Alarcón-Sánchez, 2024).

Periodontal bacteria can proliferate due to obesity, and are also associated with the initiation and exacerbation of systemic infections and other infectious processes that are caused by obesity or that develop at the beginning of obesity and later lead to more severe obesity. In other words, this proves that in addition to the local pathogenic effects of these periodontal bacteria, there is also a negative contribution to obesity, which can be described as a vicious cycle, with a continuous worsening through direct and indirect means. The relationship between the increased cardiometabolic risk and the increased prevalence of periodontal infections in obese individuals highlights a possible oral-systemic disease axis. Endotoxins, which are toxic components found on the outer wall of gram-negative

bacteria such as *P. gingivalis*, and proteolytic enzymes secreted by bacteria to break down proteins are two of the virulence factors that can cause chronic low-grade inflammation when these periodontal bacteria enter the systemic circulation (Leonov et al., 2025). Furthermore, new research suggests that ingested oral pathogens can indirectly affect systemic health by altering the structure and function of the gut microbiota, which in turn increases inflammatory signals in metabolic tissues such as the liver, adipose tissue and vascular endothelium (Xi et al., 2024). The link between obesity, systemic infections and inflammatory disorders and periodontal dysbiosis is explained biologically through these pathways.

The increased risk and severity of periodontal disease in obesity is also an indicator of the enrichment of harmful oral bacteria. Increased dental plaque formation and a high pathogenic organism load in the subgingival region in obese individuals lead to persistent periodontal inflammation and accelerated tissue destruction. According to longitudinal and cross-sectional studies, obesity has consistently been associated with deeper periodontal pockets, more bleeding on probing, and worse attachment loss. These clinical outcomes are closely related to changes in host immunological responses and oral bacterial composition caused by obesity. All these results support the idea that obesity is a distinct risk factor for periodontal disease, partly due to dysregulation of the subgingival microbiota (Alarcón-Sánchez, 2024).

3.3.2 Oral Microbiota and Viral Infections

The oral microbiota is vital in controlling bacterial diseases as well as viral infections in the oral cavity. The oral virus known as herpes simplex virus type 1 (HSV-1), which causes cold sores, settles in nerve cells once it enters the body and remains there for life. It reactivates during periods of weakened immunity or stress, causing cold sores. Recurrent mucosal lesions and local inflammation occur due to the periodic reactivation of HSV-1 and viral replication in the oral epithelial tissues. Studies suggest that the oral microbial environment is a significant factor in HSV-1 reactivation, as microbial dysbiosis can compromise the integrity of the epithelial barrier and alter local immune surveillance. There is strong evidence of a reciprocal relationship between the structure of the oral microbiota and the pathobiology of herpes viruses, as inflammatory conditions in the oral cavity can further promote viral reactivation (Thakkar et al., 2022).

Influenza viruses and other respiratory viruses can enter the body through the oral and oropharyngeal mucosa. Before passing into the lower respiratory tract, viral particles can primarily interact with local bacteria, salivary components, and oral epithelial cells. Besides being a physical entry point, the oral microenvironment is an immunologically active region influencing early host responses, viral binding, and replication. By affecting local inflammatory signaling and epithelial integrity, changes in oral microbial composition can affect the vulnerability of the mucosa to viral invasion. These interactions

highlight the importance of the oral cavity as a critical interface in the early stages of respiratory viral infections such as influenza α (Rajapandi et al., 2025).

Finally, the oral microbiota is crucial in determining antiviral innate immune responses, particularly through its influence on interferon-mediated pathways and immune components in saliva. Defensins, lactoferrin, secretory IgA, and other antimicrobial peptides are among the many antiviral compounds found in saliva that work together to neutralize and eliminate viruses. The structure and metabolic activity of the oral microbiota control the synthesis and function of these immune factors, enabling effective interferon-dependent antiviral defense. These defense mechanisms can be compromised by an imbalance (dysbiosis) of the oral microbiota, increasing vulnerability to viral infections such as influenza and HSV, and weakening antiviral immunity. This underscores the importance of maintaining the balance of the oral microbiota for effective innate immune defense against viral infections (Tada & Senpuku, 2021).

3.3.3 Viral Hepatitis and the Oral–Gut–Liver Axis

One important mechanism that connects liver inflammation and disease development to oral microbial dysbiosis is the oral–gut–liver axis. A significant source of both commensal and pathogenic bacteria that can affect systemic health in addition to local oral diseases is the oral cavity. A growing body of research indicates that changes in the oral microbiota may influence the makeup of gut microbes through ongoing microbial translocation by saliva swallowing, which may then have an effect on hepatic immune responses. Chronic liver inflammation may result from oral pathogenic bacteria's ability to compromise the integrity of the intestinal barrier, induce endotoxemia, and stimulate hepatic immune cells through portal circulation. In chronic liver illnesses, such as viral hepatitis, where inflammatory signals and ongoing immune activation are key components of the disease pathophysiology, this pathway is especially pertinent. Through prolonged microbial and immunological stimulation, oral dysbiosis may consequently function as an upstream modulator of the gut–liver axis, exacerbating hepatic damage (Lei et al., 2023).

Immune dysregulation and the advancement of inflammation have been linked to changes in the gut and oral microbiota in the setting of chronic liver disease. Microbial imbalance is strongly associated with alterations in host immune signaling, such as elevated production of pro-inflammatory cytokines and compromised immunological tolerance, according to research on oral and intestinal microbial populations in liver illnesses. Similar processes are believed to be at work in viral hepatitis, where a persistent viral infection triggers long-term immune activation, despite the fact that a large portion of the material now in publication focuses on autoimmune and metabolic liver disorders. Microbial compounds produced from the oral microbiota may worsen hepatic inflammation by influencing systemic immune responses and strengthening abnormalities in gut permeability. These results

underline the significance of taking oral microbial ecosystems into account when examining the pathophysiology of chronic viral hepatitis and related liver disorders. They also support the idea that oral microbial dysbiosis contributes to liver disease as part of an integrated oral–gut–liver axis rather than as an isolated phenomenon (Lei et al., 2023; Abe et al., 2020).

3.4 Oral Microbiota and Immune System Interactions

Life inside the mouth is based on a biological balance; the mouth is essentially an immunological interface. Food, air, and microbes constantly enter, and our body's internal defense system directly interacts with the external environment. This oral environment contains the microbiota we previously mentioned. In a healthy mouth, the immune system doesn't try to completely eliminate these microbes; instead, it keeps them under control. The microbes also train the immune system, preventing it from overreacting, thus maintaining mucosal homeostasis. Saliva has a two-pronged defense mechanism; it physically eliminates microorganisms and also provides biochemical defense by neutralizing them with proteins such as lysozyme, lactoferrin, and most importantly, sIgA. Salivary immunoglobulin A (sIgA), the most common mucosal antibody, binds to both commensal and pathogenic bacteria, preventing their adhesion to epithelial surfaces and agglutinating them for removal—that is, forming large groups by binding multiple bacteria together making it much easier for the body to swallow and expel a large cluster of bacteria from the mouth rather than a single bacterium acting alone. Saliva is also rich in antimicrobial proteins such as lysozyme, which breaks down bacterial cell walls, lactoferrin, which chelates iron to prevent biofilm formation, and peroxidases, which inhibit microbial glycolysis. These proteins provide a biochemical barrier against infection and influence the composition of oral microbes (Nagakubo & Kaibori, 2023). These salivary components help protect against local dysbiosis as well as assist in systemic immune surveillance by eliminating potential pathogens before they penetrate epithelial barriers.

The structure and diversity of the oral microbiota are significantly altered by obesity, which can disrupt the immunological balance of the oral cavity. In a cohort of adults with type 2 diabetes, obese participants showed significant changes in the relative abundance of major bacterial taxa in both subgingival plaque and salivary microbial communities compared to non-obese individuals (Tam et al., 2018). Reduced microbial diversity is a hallmark of weakened mucosal immunity, which can increase the risk of chronic inflammation and inadequate removal of pathogens, coinciding with this shift towards a dysbiotic oral microbiome. This imbalance is further exacerbated by chronic low-grade inflammation in obesity because pro-inflammatory mediators produced by adipose tissue can alter cytokine signaling pathways in mucosal tissues, thereby reducing immune surveillance and promoting the overgrowth of opportunistic bacteria (Tam et al., 2018). Therefore, oral immune systems may be weakened and vulnerable to microbial challenges increased due to obesity-related microbial changes.

Ketogenic diet therapies, such as very low-calorie ketogenic diets (VLCKD), have been shown to alter inflammatory pathways that can affect oral immune function and are effective in reducing systemic inflammation. In response to sterile and metabolic stressors, the ketone body β -hydroxybutyrate (BHB) has been shown to inhibit the activation of the NLRP3 inflammasome, a key driver of pro-inflammatory cytokine production, including IL-1 β and IL-18; that is, normally in conditions such as obesity or diabetes, there is a constant metabolic stress in the body, this stress keeps the NLRP3 alarm constantly on, which causes chronic inflammation, but when BHB is activated, it shuts off the NLRP3 alarm, so inflammation-triggering cytokines cannot be produced and tissue damage is prevented (Youm et al., 2015). Therefore, high circulating BHB can reduce chronic low-grade inflammation in the context of a ketogenic diet by reducing innate immune activation. This may have downward effects on oral mucosal immunity, reduce inflammatory load and possibly re-establish a more balanced host-microbiota relationship (Youm et al., 2015).

4. Type 2 Diabetes Mellitus

Diabetes mellitus is a collection of long-term metabolic diseases marked by persistent hyperglycemia brought on by deficiencies in insulin secretion, action, or both. These conditions cause anomalies in the metabolism of proteins, fats, and carbohydrates as well as long-term harm to many organs and tissues (ElSayed et al., 2023). Diabetes mellitus is categorized based on etiological criteria rather than just clinical presentation due to its diverse pathophysiology. This allows for the reflection of underlying variations in disease mechanisms and treatment needs (Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus et al., 2010). Type 1 diabetes mellitus and type 2 diabetes mellitus are the two main types, and they are mainly differentiated by the pathogenic processes that underlie them. The autoimmune or idiopathic loss of pancreatic β -cells causes type 1 diabetes mellitus, which ends in a complete lack of insulin and necessitates lifelong insulin replacement therapy. On the other hand, rather than total β -cell failure, type 2 diabetes mellitus is a more diverse range of conditions marked by an imbalance between insulin sensitivity and insulin production. The argument for classifying diabetes mellitus into multiple categories is based on this etiological distinction, which highlights the possibility that identical hyperglycemic symptoms could result from essentially differing biological causes. The present classification system include not only these basic kinds but also gestational diabetes mellitus and other specialized types of diabetes associated with endocrinopathies, pancreatic illnesses, genetic disorders, or drug-induced conditions (Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus et al., 2010).

The complex, long-term metabolic disease known as type 2 diabetes mellitus (T2DM) is typified by persistent hyperglycemia that arises from a dynamic interaction between peripheral tissue insulin resistance and a progressive impairment of pancreatic β -cell function, which ultimately results in insufficient insulin secretion to meet metabolic demands (Lu et al., 2024; Vybhavi et al., 2025). T2DM is regarded as a highly heterogeneous disease entity rather than a single uniform condition because, in contrast to autoimmune-mediated diabetes, it develops through multiple mechanisms involving genetic vulnerability, environmental effects, and metabolic stresses. Clinically, type 2 diabetes (T2DM) is the most frequent type of diabetes diagnosed in adults and accounts for the great majority of diabetes cases globally. Its prevalence is rising in tandem with rising rates of obesity, sedentary lifestyles, and population aging (Lu et al., 2024). Biologically speaking, type 2 diabetes is becoming more widely acknowledged as a systemic illness that goes beyond glucose metabolism. It includes dysregulation of lipid handling, oxidative stress, chronic low-grade inflammation, and changes in cellular signaling pathways in various organs, such as the immune system, adipose tissue, liver, skeletal muscle, and pancreas (Vybhavi et al., 2025).

4.1 Epidemiology

With an anticipated 506 million adults affected in 2021, type 2 diabetes mellitus (T2DM) is one of the most serious and quickly rising chronic disease burdens in the world. Since 1990, its absolute prevalence has increased by around 290% over the course of three decades (Huang et al., 2025). The increasing number of people diagnosed with type 2 diabetes (T2DM) is a result of a number of factors, including changing lifestyle patterns marked by increased consumption of high-calorie diets and sedentary behavior, as well as demographic changes like population aging and growth. By the late 2010s, hundreds of millions of individuals worldwide had type 2 diabetes (T2DM), according to earlier epidemiological evaluations, which also revealed a significant global disease burden and predicted further rises based on demographic and health transition models (Khan et al., 2020).

There is significant geographic and sociodemographic variation in the distribution of T2DM burden: areas with higher sociodemographic index (SDI) tend to have higher age-standardized incidence and prevalence rates, probably as a result of dietary and lifestyle exposures, while areas with lower SDI frequently have higher age-standardized mortality and disability-adjusted life year (DALY) rates, which reflect differences in access to effective disease management and preventive care (Huang et al., 2025). Age stratification shows that while the burden of T2DM is still largest in older persons, the relative impact in younger groups has grown, partly due to rising obesity rates and urbanization. High body mass index, elevated fasting plasma glucose, particulate air pollution, low physical activity, and dietary components like excessive consumption of sugar-sweetened beverages are important modifiable risk factors that have been identified across global analyses. These factors all significantly contribute to mortality and DALY rates attributable to the burden of type 2 diabetes (Huang et al., 2025). Forecasts until 2050 indicate that the prevalence of type 2 diabetes will continue to increase worldwide, underscoring the necessity of focused treatments that address these significant risk factors in addition to continued surveillance and modifications to the healthcare system (Huang et al., 2025).

4.2 Pathophysiology and Insulin Resistance

According to Galicia-Garcia et al. (2020), type 2 diabetes mellitus (T2DM) is a complex metabolic illness marked by a gradual loss in pancreatic β -cell activity and the increasing development of insulin resistance in peripheral tissues. Over time, these two fundamental disorders interact dynamically to cause extensive metabolic dysregulation and persistent hyperglycemia. A key early event in the pathophysiology of type 2 diabetes is insulin resistance, which is defined as a decreased biological response of target tissues to normal or higher circulating insulin concentrations. Insulin resistance frequently occurs years before the clinical onset of hyperglycemia (Saini, 2010). Pancreatic β -cells first increase insulin secretion to make up for decreased insulin sensitivity; however, prolonged

metabolic stress gradually depletes this compensatory mechanism, leading to β -cell malfunction and ultimately failure (Galicia-Garcia et al., 2020).

Impairments in insulin signaling pathways inside important metabolic tissues, such as skeletal muscle, adipose tissue, and the liver, are the main biological cause of insulin resistance (Saini, 2010). Insulin binding to its receptor under physiological conditions triggers a signaling cascade that includes protein kinase B (Akt), phosphatidylinositol 3-kinase (PI3K), and insulin receptor substrates (IRS). This cascade promotes the uptake of glucose, the synthesis of glycogen, and the suppression of hepatic glucose production. Reduced tyrosine kinase activity of the insulin receptor, inhibitory serine phosphorylation of IRS proteins, and impaired downstream PI3K/Akt signaling all interfere with this signaling pathway in type 2 diabetes, which results in decreased translocation of glucose transporter type 4 (GLUT4) to the cell membrane in muscle and adipose tissue (Saini, 2010).

Through both metabolic and inflammatory pathways, adipose tissue dysfunction is essential to the onset and maintenance of insulin resistance. Increased circulating free fatty acids that build up ectopically in the liver and skeletal muscle are a consequence of insulin-resistant adipocytes' increased lipolysis (Galicia-Garcia et al., 2020). Stress-related kinases, including as protein kinase C and c-Jun N-terminal kinase, are activated by the intracellular buildup of lipid intermediates such diacylglycerols and ceramides. These kinases further impede insulin signaling by encouraging serine phosphorylation of IRS proteins (Saini, 2010). Concurrently, defective adipose tissue modifies adipokine secretion, which is typified by elevated synthesis of pro-inflammatory mediators and decreased levels of insulin-sensitizing adiponectin.

One of the main characteristics of insulin resistance and the pathogenesis of type 2 diabetes is persistent low-grade inflammation. Through the release of cytokines like tumor necrosis factor- α and interleukin-6, which disrupt insulin signaling pathways and worsen metabolic dysfunction, enlarged adipocytes and infiltrating immune cells, especially macrophages, contribute to a pro-inflammatory environment (Galicia-Garcia et al., 2020). These inflammatory mechanisms reinforce insulin resistance and hasten the course of the disease by interacting intimately with metabolic stress responses.

Another important aspect of type 2 diabetes is hepatic insulin resistance. Hepatic gluconeogenesis and glycogenolysis are suppressed by insulin in healthy persons, but this regulatory impact is weakened in insulin-resistant conditions, resulting in increased endogenous glucose synthesis despite hyperinsulinemia (Saini, 2010). Simultaneously, hepatic insulin resistance increases hepatic steatosis and de novo lipogenesis, exacerbating metabolic abnormalities and causing fasting hyperglycemia and dyslipidemia (Galicia-Garcia et al., 2020).

While insulin resistance plays a role in the early stages of the disease, the transition from compensated metabolic impairment to overt diabetes is ultimately determined by growing β -cell failure. Long-term exposure to high levels of free fatty acids and hyperglycemia causes lipotoxicity and glucotoxicity, which affect insulin gene expression, interfere with mitochondrial function, and increase oxidative and endoplasmic reticulum stress in β -cells (Galicia-Garcia et al., 2020). Insulin resistance manifests clinically as prolonged hyperglycemia as a result of these processes, which eventually lead to decreased insulin secretory capability and β -cell death.

4.3 Type 2 Diabetes and Immune Dysfunction

Chronic hyperglycemia, a hallmark of type 2 diabetes mellitus (T2DM), impairs immune system homeostasis through inflammatory and metabolic pathways, resulting in immunological dysfunction and heightened vulnerability to infections. Adipose tissue inflammation and systemic release of inflammatory mediators like tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and other cytokines create a proinflammatory background that modifies immune cell behavior in the diabetic milieu due to persistently high blood glucose and insulin resistance (Berbudi et al., 2020). In addition to impairing traditional metabolic pathways, elevated glucose levels also increase the generation of reactive oxygen species and advanced glycation end products, which exacerbate oxidative stress and inflammatory signaling and contribute to immunological dysregulation (Berbudi et al., 2020).

The immunological response is negatively impacted by hyperglycemia in a number of ways. Under normal circumstances, pathogen penetration is effectively prevented by innate immune mechanisms and physical barriers such as intact skin and mucosa. However, the combined effects of hyperglycemia and metabolic stress weaken these defensive systems in people with type 2 diabetes, resulting in abnormalities in cytokine generation, decreased phagocytosis, and impaired immune cell killing of microorganisms (Berbudi et al., 2020). It has also been demonstrated that persistently elevated glucose levels directly impair leukocyte function, which increases the risk of infection and decreases pathogen clearance (Berbudi et al., 2020). Diabetic individuals are therefore more vulnerable to a wide range of illnesses, and the immune system is unable to create a strong defense against invasive bacteria.

Both the innate and adaptive immune systems are impacted by type 2 diabetes, which is indicative of extensive functional alterations in immune cell populations. In the innate arm, neutrophil counts and function are reduced, further undermining the first line of defense, and proinflammatory macrophages are more prevalent in adipose tissue, aggravating both local and systemic inflammation (Zhou et al., 2018). In type 2 diabetes, natural killer (NK) cells also perform poorly, making it harder for them to identify and destroy diseased or altered cells. Dysfunctional adaptive responses and extended inflammation are caused by changes in T lymphocyte populations and cytokine profiles in the adaptive immune system, including modifications in T helper cell activation and signaling (Zhou et al., 2018).

The inflammatory state is sustained and antigen-specific responses are weakened by similarly altered B cell responses, which show signs of abnormal antibody production and changed interactions with T cell subsets.

In T2DM, adipose tissue is crucial in the connection between immunological dysfunction and metabolism. In addition to being a source of energy, adipose tissue is a rich immune organ that contains T cells, B cells, macrophages, and other immune components that interact with stromal cells and adipocytes (Zhou et al., 2018). Immune cells with proinflammatory phenotypes penetrate enlarged adipose tissue in obese and diabetic conditions, releasing adipokines and cytokines that disrupt insulin signaling pathways in peripheral organs and contribute to systemic low-grade inflammation. A vicious loop is created by this process of metaflammation, or metabolically driven inflammation, whereby immune activation worsens insulin resistance and insulin resistance further disrupts immune control.

Changes in immune cell signaling and functional ability highlight the altered immune responses in type 2 diabetes. For instance, T2DM frequently results in increased proinflammatory cytokine release and macrophage polarization toward a proinflammatory (M1) phenotype, while anti-inflammatory regulatory systems are weakened (Zhou et al., 2018). Furthermore, diabetes alters the expression and activity of intracellular signaling molecules and surface receptors involved in immune cell migration and activation, which impairs immune surveillance and causes dysregulated responses to infections (Zhou et al., 2018). Due to both innate and adaptive immunological failure, people with type 2 diabetes have a higher incidence and severity of infections than people without the disease, which can be explained by these extensive immune changes (Ferlita et al., 2019).

4.4 Type 2 Diabetes and Susceptibility to Infections

One of the main causes of morbidity and mortality in diabetic populations is the significantly elevated sensitivity to infections that is linked to type 2 diabetes mellitus (T2DM). This increased risk is caused by a complicated interaction between immune system dysfunction, chronic metabolic dysregulation, and pathogen-related variables rather than just hyperglycemia. Insulin resistance, low-grade systemic inflammation, and persistent hyperglycemia all work together to weaken host defenses, making people with type 2 diabetes more susceptible to a variety of bacterial and viral infections (Ferlita et al., 2019).

Immune dysfunction brought on by type 2 diabetes is a major factor in infection susceptibility at the molecular level. Hyperglycemia causes oxidative stress and the build-up of advanced glycation end products, which impede the antibacterial and immunological cell signaling processes. Key elements of both innate and adaptive immunity, including as cytokine-mediated communication, antigen presentation, and macrophage activation, are compromised by these metabolic changes. As a result,

pathogen recognition and clearance are delayed or ineffective, allowing infections to develop more readily and progress more severely in individuals with T2DM (Ferlita et al., 2019).

A significant contributing factor to the elevated risk of infection seen in type 2 diabetes is innate immune deficiencies. Neutrophil dysfunction, characterized by reduced chemotaxis, impaired phagocytosis, and diminished intracellular killing capacity, compromises the first line of defense against invading pathogens. Concurrently, macrophages have modified profiles of activation and cytokine production, which results in ineffective germ control even in an environment that promotes inflammation. Hyperglycemia directly inhibits immune cell function and fosters an environment that is conducive to microbial growth, aggravating these innate immune abnormalities (Carrillo-Larco et al., 2022).

Adaptive immune responses are also adversely affected in T2DM, further contributing to infection susceptibility. The host's capacity to produce enduring and efficient immune responses against intracellular pathogens is diminished by changes in T lymphocyte function, such as dysregulated cytokine responses and compromised cell-mediated immunity. Acute and chronic infection susceptibility is increased and adaptive immunity is weakened when antigen-presenting cell dysfunction restricts appropriate T cell activation (Ferlita et al., 2019). These immune alterations are particularly relevant for pathogens that require robust cell-mediated immune responses for effective control.

Clinically, individuals with T2DM exhibit a higher incidence and severity of bacterial infections, including respiratory tract infections, urinary tract infections, and skin and soft tissue infections. Furthermore, infections in diabetic patients are frequently linked to longer hospital stays, higher rates of complications, and delayed resolution. Additionally, a growing amount of data suggests that T2DM is linked to a higher risk of infections brought on by organisms resistant to antibiotics, which makes clinical management and treatment outcomes even more challenging (Carrillo-Larco et al., 2022). These findings underscore the broader public health implications of diabetes-related immune dysfunction.

Another important factor that increases susceptibility in people with type 2 diabetes is viral infections. T2DM-related metabolic and immunological dysregulation compromises antiviral defense systems, such as cytotoxic immune cell activity and interferon responses. In patients with diabetes, viral infections are therefore frequently more severe and linked to worse outcomes. According to recent data, viral infections and type 2 diabetes have a reciprocal interaction in which the former can worsen metabolic dysregulation and glycemic instability while the latter makes people more vulnerable to viral infections (Turk Wensveen et al., 2021). The susceptibility of diabetics to viral outbreaks and pandemics is further reinforced by this mutual interaction.

4.5 Type 2 Diabetes and the Oral Microbiota

One of the most intricate microbial ecosystems in the human body is found in the oral cavity, where hundreds of different bacterial species live side by side in a dynamic equilibrium with the host immune system. It is becoming more well acknowledged that oral microbial populations play a part in systemic inflammation and metabolic control in addition to their local function in mouth health. More and more data in recent years has shown that changes in the oral microbiota are closely linked to metabolic diseases, especially type 2 diabetic mellitus (T2DM). In people with type 2 diabetes, this new field of study has identified the oral microbiota as a possible mediator between systemic inflammatory load, immunological dysregulation, and chronic hyperglycemia (Huang et al., 2025).

Although the precise patterns of modification differ throughout investigations, systematic reviews of the literature show that T2DM is consistently linked to changes in the composition of oral microbes. Oral microbial diversity and the relative abundance of certain taxa were shown to differ between people with type 2 diabetes and normoglycemic controls, according to a recent comprehensive review that summarized the information that was already available (Huang et al., 2025). Significant variation in research design, sampling techniques, and analytical methodologies is highlighted by the fact that some studies find decreased microbial diversity in type 2 diabetes, while others report changes in community structure without consistent changes in richness. Notwithstanding these discrepancies, the majority of data points to the existence of oral microbial dysbiosis in type 2 diabetes, indicating that long-term metabolic dysfunction affects the ecological equilibrium of oral microbial communities (Huang et al., 2025).

In addition to global compositional shifts, a number of studies have linked certain oral bacterial taxa to the risk of type 2 diabetes. Different microbial profiles were shown to correspond with the prevalence of the illness and metabolic parameters in a population-based research investigating the relationship between the oral microbiome and type 2 diabetes (Long et al., 2017). The fact that certain genera were decreased and others were enriched in T2DM patients suggests that metabolic state may be reflected in oral microbial fingerprints. These results provide credence to the idea that the oral microbiota might contribute to metabolic dysregulation in addition to acting as a disease marker. Crucially, the discovery of taxa linked to the risk of type 2 diabetes increases the potential for the use of oral microbiome profiles as non-invasive biomarkers for disease monitoring and prediction (Long et al., 2017).

In addition to taxonomic changes, oral microbiota variations in type 2 diabetes are also associated with functional shifts seen in modified metabolic outputs. Oral dysbiosis in type 2 diabetes is linked to alterations in metabolite profiles obtained from microbes, according to evidence from integrated microbiome–metabolome investigations (Li et al., 2023). Both the systemic inflammatory and

metabolic pathways as well as the local oral environment may be impacted by these metabolic changes. Increased synthesis of pro-inflammatory metabolites and disruption of host-microbe interactions have been associated to dysregulated microbial metabolism, which may exacerbate insulin resistance and low-grade inflammation that are hallmarks of type 2 diabetes (T2DM) (Li et al., 2023). These results emphasize how crucial it is to take into account the functional effects of oral microbiota dysbiosis rather of concentrating just on compositional variations.

A number of molecular theories have been put out to explain the connection between changes in oral microbiome and type 2 diabetes. By altering the salivary composition, pH, and nutritional availability, chronic hyperglycemia may have a direct impact on the oral environment and promote the proliferation of particular microbial taxa. Parallel to this, immunological dysfunction linked to diabetes may make it more difficult for the host to regulate microbial populations, which might lead to dysbiosis. Oral cavity-derived microbiological products have the potential to alter inflammatory signaling or reach the bloodstream, which might lead to immunological and metabolic dysregulation. Current data suggests a bidirectional link in which T2DM both impacts and is impacted by changes in the oral microbiota, even if causation has not yet been conclusively demonstrated (Huang et al., 2025; Li et al., 2023).

There are still a lot of methodological issues in this topic, despite its increasing popularity. Inconsistencies across studies are caused by variations in sequencing platforms, bioinformatic pipelines, clinical characterisation of research participants, and collection locations (saliva versus dental plaque). In order to increase repeatability and elucidate causal links, a recent review highlighted the pressing need for standardized procedures and longitudinal study designs (Guan et al., 2024). To completely understand the function of the oral microbiota in the pathogenesis of type 2 diabetes and to investigate its potential as a target for metabolic disease diagnosis or treatment, these limitations must be addressed.

5. Aim of the Study

The primary objective of the SIDERALE study is to analyze differences in the composition of the oral 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.

6. Materials and Methods

6.1 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 oral microbiota, oral cavity samples were collected in the morning under fasting conditions using a salivary swab.

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 39 patients, involved baseline monitoring through saliva analysis. The total amount of samples was 78.

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.

6.2 DNA Extraction

Using the QIAamp DNA Microbiome Kit (Qiagen, Milan, Italy), a patented technique created especially for the extraction of high-quality microbial genomic DNA from body fluids like saliva.

The QIAamp DNA Microbiome Kit uses the spin column technology with a modified protocol designed to evenly enrich the bacterial microbiome DNA content of a sample. Host cells are gently lysed first, leaving bacterial cells intact. The released host DNA is then enzymatically degraded. An optimized combination of mechanical and chemical lysis is used to subsequently disrupt bacterial cells and carefully designed buffer chemistry promotes the binding of the released DNA to the silica membrane. Finally, after washing away unwanted components, the bacterial microbiome DNA is eluted from the column

For downstream molecular processes like PCR amplification and next-generation sequencing, the resultant DNA was sufficiently pure and high-quality.

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

6.3 Library Production

The Microbiota Solution A kit (Arrow Diagnostics Srl, Genoa, Italy) was used to amplify the DNA of bacteria. In order to characterize bacterial populations, this kit makes it possible to amplify the hypervariable regions V1–V2–V3 of the bacterial 16S rDNA gene using PCR.

Two separate PCR reactions made up the amplification protocol:

- (i) a target PCR for amplifying the chosen 16S rDNA gene regions;
- (ii) an index PCR for sample barcoding by including index sequences.

A fluorescent double-stranded DNA intercalating dye (SYBR Safe®), which allows DNA bands to be seen under UV light, was used in agarose gel electrophoresis to assess the amplification results after each PCR process.

Agencourt® AMPure® beads were then used to purify the amplified DNA in order to eliminate any remaining primers and amplification byproducts.

6.4 Next-Generation Sequencing

Using the MiSeq platform (Illumina Inc., San Diego, CA, USA) and the MiSeq Reagent Nano Kit v2 (500 cycles), as well as PhiX as an internal sequencing control, next-generation sequencing was carried out.

Each sample's purified and quantified libraries were combined at equimolar quantities, denatured, diluted to the proper loading concentration, and mixed with a denatured PhiX control at the time of sequencing. Illumina's standard automated approach was used to perform sequencing once the final library pool was placed into the MiSeq reagent cartridge.

6.5 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.

7. Results

The findings pertain to 39 patients who were part of the SIDERALE experiment at the University of Eastern Piedmont's Endocrinology Unit, as well as SS. Antonio and Biagio and Cesare Arrigo Hospital in Alessandria. For the purpose of analyzing their oral flora, all participants had baseline saliva samples taken.

Patients were divided into two groups depending on whether they had concurrent chronic bacterial and/or viral illnesses, in accordance with the predetermined research criteria. Patients with a history of chronic infections (such as asymptomatic bacteriuria, urinary tract infections, pyelonephritis, periodontitis, herpes infection, or varicella-zoster virus infection) were included in the case group, while patients without such a history were matched for age, sex, and hypoglycemic medication in the control group.

The purpose of the study was to examine any correlations between microbial signatures and clinical features, as well as variations in oral microbiota composition linked to the occurrence of chronic infections in patients with obesity and type 2 diabetic mellitus (T2DM).

The results of the microbiota analysis are presented below, including alpha diversity analysis, beta diversity analysis, and differential abundance analysis using linear discriminant analysis effect size (LEfSe).

7.1 Biodiversity of Microbial Communities

The diversity of the oral microbiota in each saliva sample was assessed using alpha diversity. This analysis provides a characterisation of the heterogeneity of the oral microbiota among the study groups by evaluating the total number of species present and the relative distribution of their abundances within each sample.

Species richness (Observed index), which is the total number of species found in each sample, and two other biodiversity indices (Shannon and Simpson) were used to analyze the samples.

Analysis of alpha diversity was done at the taxonomic level of species.

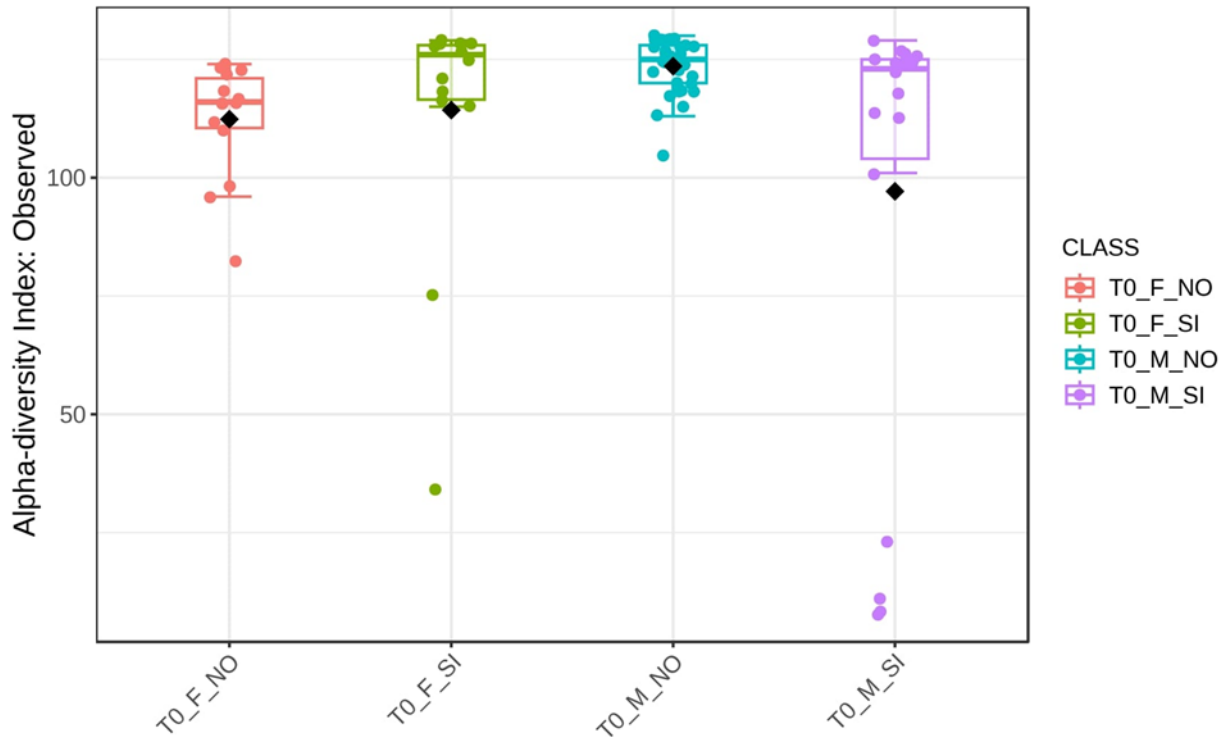


Figure 3. Baseline (T0) alpha diversity of the oral microbiota, measured by the Observed index, in patients with obesity and type 2 diabetes mellitus (T2DM), according to sex and the exposition to chronic bacterial and/or viral infections. Participants were grouped as T0_F_NO (females without chronic infections), T0_F_SI (females with chronic infections), T0_M_NO (males without chronic infections), and T0_M_SI (males with chronic infections). Individual samples are represented by dots; boxplots indicate median and interquartile ranges, while black diamonds represent mean values. $p\text{-value} < 0.0016213$.

The Observed index's measurement of alpha diversity reveals varied distributions among the four baseline categories. As shown in Figure 3, in all the analyzed cases, the Observed index is associated with a value greater than 100. The difference between categories is statistically significant; however, it is not associated with marked differences between groups. Nevertheless, it can be observed that among patients, both male and female, who were exposed to infections (T0_F_SI and T0_M_SI), there are outliers exhibiting richness values well below the mean. This finding indicates the presence of dysbiosis in subjects exposed to infection.

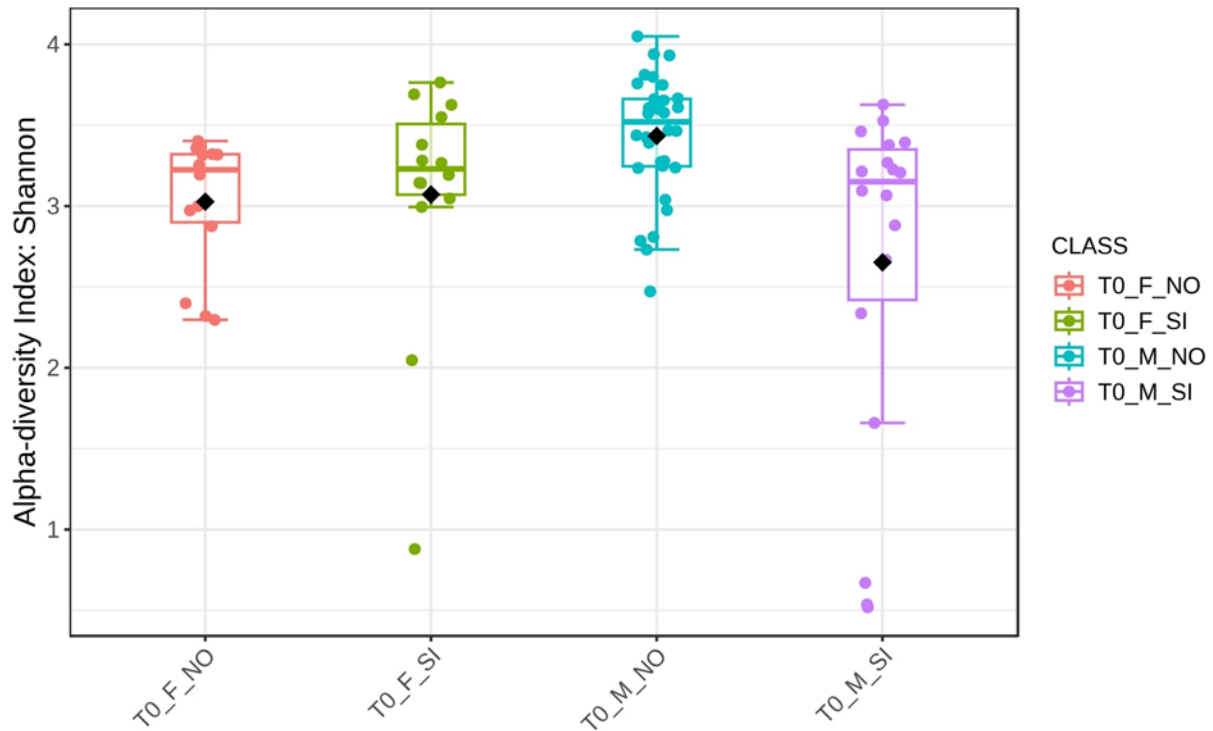


Figure 4. Alpha diversity of the oral microbiota, expressed as the Shannon index, at baseline (T0) in patients with obesity and type 2 diabetes mellitus (T2DM), stratified by sex and by the exposition to chronic bacterial and/or viral infections. Study groups were defined as follows: T0_F_NO, females without chronic infections; T0_F_SI, females with chronic infections; T0_M_NO, males without chronic infections; and T0_M_SI, males with chronic infections. Each dot represents an individual sample; boxplots indicate the interquartile range with median values, and black diamonds represent mean values. $p\text{-value} < 0.0012377$.

Variations in species distribution among the study subgroups are revealed by alpha diversity as measured by the Shannon index, as seen in Figure 4. The T0_M_NO group has higher median Shannon values among male individuals than the T0_M_SI group, suggesting that male patients without chronic illnesses have a more uniform distribution of bacterial species. Despite the presence of several taxa, males with chronic infections have lower median values, indicating a less balanced species distribution.

The median Shannon values between infected and non-infected people are more similar in female categories. Inter-individual variability in species evenness is highlighted by the existence of outlier samples in exposed groups, with males with chronic infections showing the most noticeable dispersion.

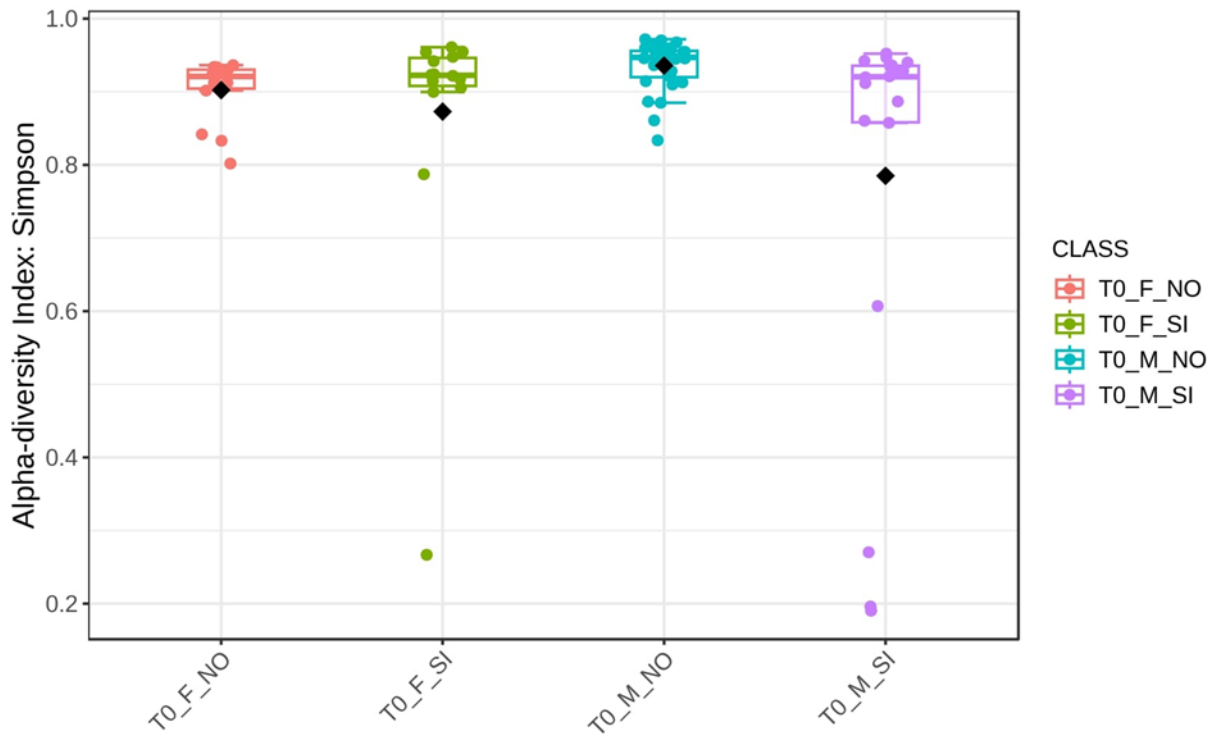


Figure 5. Alpha diversity of the oral microbiota, expressed as the Simpson index, at baseline (T0) in patients with obesity and type 2 diabetes mellitus (T2DM), stratified by sex and by the exposition to chronic bacterial and/or viral infections. Study groups were defined as follows: T0_F_NO, females without chronic infections; T0_F_SI, females with chronic infections; T0_M_NO, males without chronic infections; and T0_M_SI, males with chronic infections. Each dot represents an individual sample; boxplots indicate the interquartile range with median values, and black diamonds represent mean values. $p\text{-value} < 0.0024927$.

A typically low likelihood that two randomly chosen bacteria are members of the same species is indicated by high values for alpha diversity as measured by the Simpson index across all baseline subgroups, as seen in Figure 5. Male participants without chronic infections appear to have a more equally distributed microbial population, as indicated by the T0_M_NO group's higher median Simpson values as compared to T0_M_SI.

The Simpson index values of males with chronic infections, on the other hand, show a broader dispersion and lower median values, indicating more dominance of particular taxa in a subset of samples. The median values between infected and non-infected people are more similar in female categories. Outlier samples with lower Simpson index values are seen in exposed groups, suggesting that individual differences in bacterial species dominance exist.

The assessment of differences across microbial communities is made possible by beta diversity, which is a measure of the variance in microbial community composition among various samples or groups. In the current investigation, the exposition to chronic bacterial and/or viral infections was utilized to assess variations in the oral microbiota composition between individuals with obesity and type 2 diabetes mellitus (T2DM) using beta diversity. Principal Coordinate Analysis was used to visualize beta diversity (PCoA). To measure variations in microbial composition, a distance matrix was created for every pair of samples. The primary coordinate axes that resulted show the proportion of variance that could be explained by each sample.

To identify compositional variations in the oral microbiota linked to the state of chronic infections, beta diversity analysis was carried out at the species taxonomic level.

Beta diversity analysis highlights differences in the composition of microbial communities across the study groups, reflecting variations in the overall oral microbiota structure between groups.

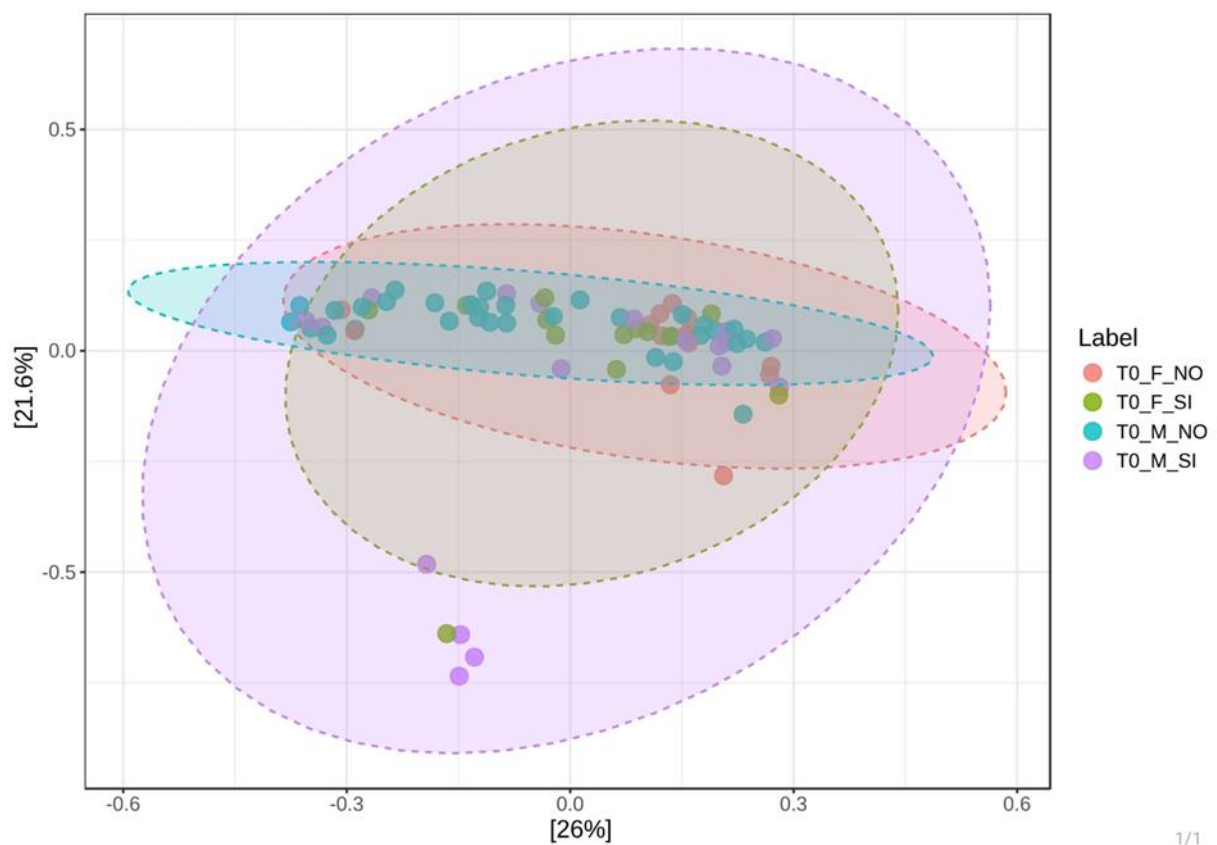


Figure 6. Beta diversity of the oral microbiota at baseline (T0) in patients with obesity and type 2 diabetes mellitus (T2DM), visualized by Principal Coordinate Analysis (PCoA). Samples are colored according to sex and the exposition to chronic bacterial and/or viral infections: T0_F_NO, females without chronic infections; T0_F_SI, females with chronic infections; T0_M_NO, males without chronic infections; and T0_M_SI, males with chronic infections. Each point represents an individual

sample. Ellipses indicate the dispersion of samples within each group. The percentage of variance explained by each principal coordinate is reported on the corresponding axes. $p\text{-value} < 0.002$.

7.2 Signature Definition

Several bacterial species were found to be differently represented across the research subgroups at baseline using LDA-LEfSe analysis. Only taxa demonstrating statistical significance following multiple testing correction were taken into consideration in the species taxonomic level study.

With an LDA score of 3.44 ($p < 0.05$, $FDR < 0.05$), *Alloprevotella* sp. was much more abundant in two groups (T0_M_NO and T0_F_NO), particularly in male participants, according to sex. Similar to this, *Mogibacterium vescum* (LDA score = 2.87, $p < 0.001$, $FDR < 0.05$) varied in abundance between groups, with greater values shown in the T0_M_NO subgroup and in the T0_F_NO subgroup, which included people free of chronic infections. The greater abundance was mainly related to female participants.

Additionally, there were notable group differences for *Aggregatibacter segnis* (LDA score = 2.63, $p < 0.01$, $FDR < 0.05$), with the T0_M_NO subgroup and the T0_F_NO subgroup showing higher relative abundance. Furthermore, it was shown that *Prevotella jejuni* was a discriminant species, with greater abundance in people who did not have chronic infections, particularly in male participants (LDA score = 2.92, $p < 0.01$, $FDR < 0.05$).

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
<i>Alloprevotella</i> sp.	0.00025416	0.033041	3526.9	1630.4	5934.8	445.09	3.44
<i>Mogibacterium vescum</i>	0.00068805	0.044724	1987.5	503.51	1692.4	544.8	2.87
<i>Aggregatibacter segnis</i>	0.0014749	0.048978	36.777	439.64	896.87	247.56	2.63
<i>Prevotella jejuni</i>	0.001507	0.048978	694.78	672.59	1741.9	87.53	2.92

Table 3. Differentially abundant bacterial species identified by LDA-LEfSe analysis.

8. Discussion

One of the most varied microbial communities in the human body is found in the oral cavity, and its makeup is essential for both systemic homeostasis and local oral health. This intricate ecosystem affects human physiology overall by regulating the immune system, metabolic processes, and inflammatory responses. Recent evidence indicates, there is a clear correlation between the makeup of the oral microbiota and long-term metabolic diseases including obesity and type 2 diabetes mellitus (T2DM), which are also associated with altered microbial diversity and an increased risk of infection. Several taxa exhibit distinct abundance patterns according to metabolic state, and systematic studies have shown that people with type 2 diabetes have alterations in oral microbial diversity and community structure when compared to non-diabetic controls (Huang et al., 2025).

These findings suggest that oral microbiota dysbiosis may be a factor entangled with immunological disruptions and disease pathogenesis rather than only a result of metabolic disorders. For instance, several cross-sectional investigations in T2DM patients have shown consistent compositional alterations at the phylum and genus levels, including enrichment of *Streptococcus* and *Porphyrromonas* species in diabetic cohorts, as well as varied changes in alpha diversity indices (Huang et al., 2025). Furthermore, unique changes in the diversity and composition of oral microbiota that are unrelated to glycemic control have been associated with obesity, a significant risk factor for type 2 diabetes (Tam et al., 2018)..

This research provides the significance of microbial community structure in systemic metabolic and immune disorders and offers a basis for investigating how baseline oral microbiota profiles may vary in people with obesity and type 2 diabetes based on their history of persistent bacterial and viral infections.

To describe baseline variations in community structure among individuals with obesity and type 2 diabetes mellitus (T2DM), stratified by chronic infection status and sex, an initial assessment of oral microbial biodiversity was carried out. Microbial richness and evenness differed among the research groups, according to alpha diversity analysis. However the Observed, Shannon, and Simpson indices showed consistent trends. In particular, oral microbial alpha diversity was lower in male patients with a history of chronic bacterial and/or viral infections than in male patients without such illnesses. This suggests that there were fewer taxa identified and that the species distribution within samples was less uniform.

In research investigating oral microbiota in metabolic illnesses, including as obesity and type 2 diabetes, where changes in community composition and diversity metrics have been connected to systemic metabolic and inflammatory abnormalities, patterns of decreased alpha diversity have been documented (Tam et al., 2018). Additionally, salivary microbiota analyses in T2DM and overweight

cohorts have shown reduced richness and unique microbial community signatures when compared to metabolically healthy controls, which lends more credence to the idea that metabolic disease state may influence oral microbial diversity (Shaalan et al., 2022). According to a recent systematic review of oral microbiota in diabetes, a number of included studies observed that patients with diabetes had different alpha diversity measurements than people without the disease, indicating diverse but physiologically significant changes in diversity profiles (Huang et al., 2025).

However, the median alpha diversity values across the infected and non-infected groups were more similar in female research participants, indicating a relative retention of microbial richness and evenness in spite of the chronic infection load. Furthermore, there were low-diversity outliers and increased inter-individual variability in infected men across all parameters, which is consistent with oral microbial instability when metabolic and pathogenic stresses are coupled. Together, our results show that patients with obesity and type 2 diabetes have lower oral microbial diversity due to chronic infections; this impact is sex-specific and seems to be more noticeable in men.

Principal Coordinate Analysis (PCoA) displayed the variations in oral microbial community structure across the research groups, which were identified by beta diversity analysis. In comparison to other categories, the ordination plot shows that samples from male patients with obesity and type 2 diabetes who have a history of chronic infections typically inhabit a larger and more dispersed compositional space, indicating a higher degree of community heterogeneity. The distributions of the non-infected male and female categories, on the other hand, were more concentrated, suggesting that the community profiles of those groups were comparatively similar. These findings suggest that changes in the overall architecture of the oral microbiota, which go beyond simple variations in richness or evenness to include changes in the relative abundances of constituent taxa, are linked to chronic infections.

Studies of oral microbiota in metabolic and inflammatory diseases have shown differences in beta diversity, with different community architectures correlated with illness aspects including glycemic management and local inflammatory load (Tam et al., 2018). A destabilized microbial environment impacted by both metabolic failure and immunological stress may be the cause of the increased dispersion seen in sick males, which might make them more vulnerable to dysbiotic conditions. Overall, our findings underscore the significance of beta diversity as a measure of community difference and support the idea that chronic infection histories are associated with compositional changes in oral microbial communities in individuals with obesity and type 2 diabetes.

Four bacterial taxa with substantially different relative abundances across study groups were found using differential abundance analysis using LEfSe; all of these taxa were considerably lower in male and female patients with chronic infections than in men and female without illnesses. As a member of the core oral anaerobic microbiota, the species *Alloprevotella* is commonly found in healthy oral

communities and is believed to support ecological balance rather than overt pathogenicity (Könönen & Gursoy, 2022). *Prevotella jejuni* and other members of the *Prevotella* spp. are also known to be common commensals in the oral cavity; species in this genus are frequently found in stable and diversified oral microbial ecosystems (Könönen & Gursoy, 2022).

Despite being less commonly investigated at the species level, *Aggregatibacter segnis* and *Mogibacterium* spp. have been identified as resident elements of the oral microbial ecology. *Aggregatibacter* spp. are prevalent, native components of the oral microbiota, as evidenced by metapangenomic investigations of the human oral cavity, which show that they occupy various ecological niches throughout oral habitats, including plaque and mucosal surfaces (Giacomini et al., 2024). Because these species are found in a variety of oral habitats, their decline may be a sign of the instability of long-standing microbial networks rather than the just disappearance of individual diseases.

Anaerobic bacterial community members, such as *Mogibacterium* spp., have also been shown to form part of complex anaerobic consortia in healthy persons and are commonly detected in investigations of balanced oral microbiota (Oogai et al., 2025). Therefore, a shift away from microbial configurations associated with ecological equilibrium toward community structures more characteristic of dysbiotic or disturbed oral states may be reflected in the decreased abundance of *Aggregatibacter segnis* and *Mogibacterium vescum* in chronically infected male patients.

In individuals with obesity and type 2 diabetes mellitus (T2DM), changes in the makeup of the oral microbiota may be a reflection of the interaction between immunological dysregulation, metabolic dysfunction, and chronic viral load. It has been demonstrated that obesity and type 2 diabetes-related chronic low-grade inflammation and compromised innate immune responses affect oral epithelial barrier function and microbial community stability, perhaps promoting changes toward dysbiotic oral ecosystems (Shalan et al., 2022). Because sex-related changes in immune modulation and inflammatory responses have been shown to impact microbiota composition and resilience across mucosal locations, including the oral cavity, they may contribute to the more noticeable microbiota abnormalities seen in male patients. These variations could make men more vulnerable to microbiota instability when they have ongoing illnesses (Elderman et al., 2018).

Further long-term studies integrating functional microbiome analyses with clinical outcomes are needed to clarify the role of oral microbiota in infection susceptibility and metabolic disease progression.

9. Conclusion

In conclusion, the present thesis explored the baseline oral microbiota profiles in patients with type 2 diabetes mellitus (T2DM) and obesity, in relation to a history of chronic bacterial and/or viral infections.

The results reveal that oral microbial diversity and community composition among individuals show observable changes depending on their history of chronic infections, and these changes are more pronounced in male patients. The idea that the combined effects of obesity, type 2 diabetes mellitus, and chronic inflammation influence host-microbiota interactions is supported by our findings, which demonstrate that the oral microbiota structure changes not only in relation to metabolic status but also to infection susceptibility.

This study focuses on the basic oral microbiota signatures the initial, unique structure of the microbial community in an individual's mouth and offers new insights into the potential role of oral microbial profiles as indicators of immunometabolic imbalance resulting from disruptions in the harmony between the immune system and metabolism within this population. While causality cannot be established whether oral disorders cause disease or disease disrupts the mouth the observed relationships reveal a strong link between oral microbiota and disease, suggesting that oral microbiota could be a promising, non-invasive biomarker of infection-related susceptibility in patients with metabolic disease.

Oral microbiota analysis is a very promising way to monitor health without drawing blood samples or performing complicated tests; however, to better understand the role of oral microbiota in infection susceptibility and metabolic disease development, more long-term research integrating functional microbiome analysis with clinical outcomes is needed.

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