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Lifestyle and Molecular Biomarkers as Predictors of Depression Outcomes: The Interplay between Physical Activity, Diet and Gut Microbiota

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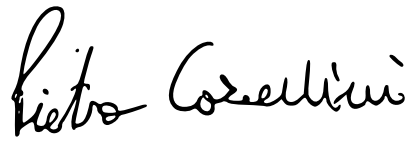

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1. INTRODUCTION

1.1 Depressive disorders: burden and clinical relevance and Anhedonia in Major Depressive Disorder: Neurobiological Basis, Clinical Relevance, and Therapeutic Challenges

Depressive disorders, including major depressive disorder (MDD), are among the most prevalent and disabling psychiatric conditions worldwide, with the World Health Organization identifying depression as a leading contributor to years lived with disability and estimating that it affects more than 280 million people globally. The global burden of MDD is considerable, accounting for an estimated 65.5 million disability-adjusted life years and ranking among the leading contributors to worldwide health burden [1]. Prevalence varies substantially, with lifetime estimates ranging from 3.7% to 6.7% for MDD and up to 20% for significant depressive symptoms [2, 3] while 12-month prevalence rates range from 0.3% to 10.2% across countries [4]. Beyond its high prevalence, MDD is associated with substantial morbidity, increased risk of suicide, and high rates of medical comorbidity; it frequently co-occurs with other psychiatric conditions and chronic physical illnesses such as cardiovascular disease, diabetes, and obesity[5-8] which not only exacerbate the overall health burden but also complicate treatment [4-6]. The disorder further impairs daily functioning, reduces productivity, increases suicide risk, and contributes to physical health decline [9], with its detrimental impact on quality of life comparable to, or exceeding, that of many chronic physical illnesses[10]. Despite advances in pharmacological and psychotherapeutic interventions, treatment outcomes remain suboptimal, as a significant proportion of patients fail to achieve full remission or experience recurrent episodes, underscoring the urgent need for a deeper understanding of the biological and environmental determinants of treatment response.

Anhedonia—defined as the diminished ability to experience pleasure, interest, or motivation in response to normally rewarding stimuli, is widely recognized as one of the most specific and clinically disabling symptoms of Major Depressive Disorder (MDD). It is one of the two core diagnostic criteria required by the DSM-5 for MDD diagnosis [11] and has been increasingly acknowledged as a potential endophenotype of depression, particularly in its melancholic subtype [11-13]

Anhedonia encompasses multiple dimensions, including anticipatory anhedonia (reduced ability to anticipate future pleasure), consummatory anhedonia (blunted pleasure during the experience), and decisional anhedonia (difficulty in effort-based decision-making) [14]. This multidimensionality has important implications for both clinical practice and mechanistic research.

From a neurobiological perspective, anhedonia is associated with dysfunction across several brain regions involved in reward processing, including the nucleus accumbens, ventral striatum, ventral

tegmental area, amygdala, hippocampus, thalamus, and prefrontal cortex [15-28]. Disruptions in the mesocorticolimbic dopaminergic system, particularly within the ventromedial prefrontal cortex–ventral striatum–amygdala circuit, are especially implicated in anticipatory anhedonia [11b]. Functional magnetic resonance imaging (fMRI) studies have shown reduced activation of the nucleus accumbens during the anticipation of pleasurable stimuli in patients with high levels of anhedonia, compared to healthy controls [29]. These findings suggest a specific impairment in encoding incentive salience and reward prediction [29].

In contrast, consummatory anhedonia is less frequently studied in isolation, yet often reported by patients as the inability to feel pleasure even during enjoyable activities. Evidence suggests a potential dissociation between subjective emotional engagement and neurophysiological activation, indicating disruptions in the immediate phases of hedonic processing [23]. Moreover, altered functional connectivity between the dorsolateral prefrontal cortex and subcortical reward structures supports the hypothesis of impaired motivational modulation mechanisms [30]. Structural imaging has also revealed volume reductions in regions such as the fornix and basal forebrain in MDD patients with anhedonia [20, 24]

Clinically, anhedonia is a strong predictor of illness severity, functional disability, and chronicity, even in patients who achieve symptomatic remission [31, 32]. It has been consistently linked to treatment resistance, poorer prognosis, and an increased risk of suicide, underscoring its prognostic value [19, 33-35]. Furthermore, improvements in anhedonia have been shown to mediate the relationship between overall depressive symptom improvement and enhanced social functioning [18].

Assessment tools for anhedonia include self-report instruments such as the Snaith–Hamilton Pleasure Scale (SHAPS), as well as experimental behavioral paradigms like the monetary incentive delay task, which help characterize reward responsiveness [18, 22, 24, 36, 37]. However, current clinical scales may not fully capture the construct's neurobiological heterogeneity, indicating the need for tools that integrate neuroscientific insights [19].

Despite its clinical relevance, anhedonia is one of the symptoms least responsive to conventional antidepressant treatments, particularly selective serotonin reuptake inhibitors (SSRIs) [12, 21, 33]. This has spurred research into novel pharmacological interventions, including ketamine, which acts on NMDA receptors and induces rapid and robust improvements in hedonic tone, and agomelatine, a melatonergic and serotonergic agent with demonstrated effects on reward processing [16, 30, 35, 38]. Vortioxetine has also shown efficacy in improving anhedonia-related functioning in MDD [39]. Neuromodulation strategies, such as transcranial magnetic stimulation (TMS) and deep brain

stimulation (DBS), have been proposed to directly modulate dysfunctional reward circuits, with DBS showing promise in treatment-resistant populations [11, 24].

Additionally, psychotherapeutic, behavioral, and lifestyle-based interventions are gaining traction, including exercise programs and probiotic supplementation, which target inflammatory and neuroplastic pathways associated with reward dysfunction [12, 22, 25].

Looking forward, addressing anhedonia will require a multilevel and cross-species translational approach, incorporating novel preclinical models, biomarker-informed assessments, and large-scale clinical trials that test both pharmacological and non-pharmacological interventions [15, 19, 38]. By advancing our understanding of the neural and molecular underpinnings of anhedonia, future work may help shift depression treatment toward more personalized, circuit-specific strategies, ultimately improving outcomes and quality of life for individuals with MDD.

1.2 Mood Disorders and Metabolic Comorbidity: The Bidirectional Interface

Insulin resistance, long considered a defining feature of metabolic syndrome and a risk factor for cardiovascular disease, is increasingly recognized as a key contributor to the pathophysiology of mood disorders [40-42]. Far from being a consequence of poor lifestyle or medication side effects alone, IR has been detected in young, drug-naïve, and non-obese psychiatric patients, suggesting it may represent an intrinsic biological vulnerability in affective illness [43, 44].

Numerous studies have documented a high prevalence of IR in individuals with major depressive disorder and bipolar disorder, even in the absence of obesity or overt metabolic disease [45, 46]. A large meta-analysis of over 240,000 participants found elevated insulin levels and HOMA-IR indices in those with acute depression, independent of antidepressant use and body weight [47]. Similarly, cross-sectional studies in drug-naïve BD patients have shown a significantly higher risk of IR compared to healthy controls, with no correlation to BMI or comorbidities [43, 48]. These findings challenge the view of IR as merely a metabolic byproduct and instead position it as a potential core component of mood pathology [42].

This has led to the identification of a "metabolic subtype" of depression, marked by IR and clinical features such as anhedonia, low energy, cognitive slowing, and poor response to first-line antidepressant treatments[41, 49]. These symptoms persist even after controlling for physical activity and BMI, pointing to an independent role for metabolic dysfunction in shaping affective symptomatology. IR has also been identified as a predictor of non-response to selective serotonin and noradrenaline reuptake inhibitors (SNRIs), further reinforcing its association with treatment-resistant depression [44].

Neurobiological studies support this link, showing that IR is associated with structural and functional brain changes, including reduced volumes in regions involved in mood regulation and cognition. These alterations may underlie the clinical features of cognitive slowing and diminished reward sensitivity observed in patients with IR [50, 51]. Additionally, higher levels of IR predict slower improvement in symptoms such as anhedonia during antidepressant treatment and are associated with worse longitudinal outcomes, including increased relapse risk and hospitalization [44, 52, 53]. For example, Watson et al. found that even mild increases in IR over a nine-year period were associated with an 89% greater risk of developing MDD.

Prospective epidemiological studies reinforce the relationship between metabolic dysfunction and mood disorders[19,39]. A large Dutch cohort study showed that moderate elevations in IR markers, such as increased waist circumference, fasting glucose, and elevated triglyceride-to-HDL ratios, were linked to a significantly higher risk of developing MDD over time [54] . Every 5 cm increase in waist circumference, for instance, was associated with an 11% greater risk of incident depression, and higher fasting glucose conferred a 37% increased risk. In that study, 14% of participants without prior depression developed MDD over nine years, with those exhibiting higher baseline IR measures at significantly higher risk [54].

IR appears to be particularly relevant in bipolar disorder, where its prevalence exceeds 50% in some samples[46, 55, 56]. Patients with BD and comorbid IR or type 2 diabetes are more likely to experience rapid cycling, chronic course of illness, and poor response to lithium or other mood stabilizers [42, 57]. IR in BD has been associated with neuroprogression, cognitive decline, and treatment resistance, independent of age, BMI, or exposure to antipsychotics[53, 55].

Mechanistically, IR represents a critical biological interface between peripheral metabolic dysfunction and central nervous system changes. It contributes to low-grade inflammation, mitochondrial dysfunction, altered energy metabolism, impaired neuroplasticity, and dysregulation of the HPA axis[58, 59]. Peripheral IR may also exacerbate central insulin resistance, disrupting dopaminergic signaling and further impairing brain function [60] .

Compounding the issue, many commonly prescribed psychotropic medications, including second-generation antipsychotics, mood stabilizers, and certain antidepressants, are known to worsen metabolic parameters [61-64]. This creates a vicious cycle in which psychiatric symptoms and metabolic dysfunction reinforce one another, further complicating treatment outcomes [53].

Taken together, the evidence positions insulin resistance not only as a metabolic concern but as a central player in the onset, severity, and treatment resistance of mood disorders. Understanding IR as a shared pathophysiological mechanism opens the door to more targeted, integrated approaches to

treatment, particularly in patients with atypical features, poor therapeutic response, or evidence of metabolic dysregulation[65].

1.3 Mediterranean Diet and Mental Health

Emerging evidence from both epidemiological and interventional studies has increasingly recognized dietary habits as modifiable determinants of mental health, particularly in relation to depressive disorders [66] Among the various dietary models explored, the Mediterranean diet (MD) has attracted significant scientific interest due to its potential protective role against Major Depressive Disorder (MDD). This dietary pattern, characterized by high consumption of fruits, vegetables, legumes, whole grains, nuts, and olive oil; moderate intake of fish and red wine; and low consumption of red and processed meats, is known for its anti-inflammatory and neuroprotective properties [67].

Several large-scale observational studies have identified an inverse association between MD adherence and depressive symptomatology. A meta-analysis of 42 studies reported that individuals with high adherence to the Mediterranean diet had a 33% lower risk of developing depressive symptoms compared to those with low adherence [68]. Supporting this, a prospective study in Swedish women found that greater adherence to MD during midlife was associated with a lower risk of depression later in life [69].

Similarly, Fan et al. (2022) reported a non-linear relationship between MD adherence and depression severity in a representative U.S. adult population, with the strongest benefits observed in individuals with high insulin resistance, suggesting a possible interaction between dietary patterns and metabolic status [70]. Prospective cohort studies have further supported this association. Oddo et al. (2022) showed that individuals who consistently adhered to the MD over time had a significantly lower risk of developing depression, even after adjusting for sociodemographic, lifestyle, and clinical confounders [71].

Meta-analytic evidence reinforces these findings. Bizzozero-Peroni et al. (2025), in a meta-analysis of randomized controlled trials, observed that adherence to a Mediterranean-style diet was associated with a clinically meaningful reduction in depressive symptoms, especially in patients with a formal diagnosis of depression. The therapeutic benefit appeared to be enhanced when dietary interventions were supervised by trained nutrition professionals, highlighting the relevance of structured support in improving adherence and clinical outcomes [72]

The beneficial effects of the Mediterranean diet on mental health appear to extend across the lifespan. Higher adherence has been associated with lower depressive symptomatology in adolescents [73, 74], while in older adults it has been linked to improved emotional well-being and lower risk of depression [75] Preliminary evidence in younger adult populations suggests that even short-term interventions

may confer subjective improvements in mood and motivation, though practical barriers such as cost, preparation time, and social support remain relevant considerations [76, 77]

Beyond observational and clinical trial findings, there is a growing body of research focused on the biological mechanisms underlying the observed associations. The Mediterranean diet is believed to exert its effects via multiple pathways, including the modulation of systemic inflammation, the regulation of gut microbiota, and the enhancement of monoaminergic neurotransmission [78] Nutritional components such as polyunsaturated fatty acids, polyphenols, fiber, magnesium, and B vitamins have been shown to reduce levels of pro-inflammatory cytokines (e.g., IL-6, TNF-α, CRP), support serotonin and dopamine synthesis, and upregulate brain-derived neurotrophic factor (BDNF), a key molecule involved in synaptic plasticity and stress resilience [79, 80].

The gut–brain axis is another critical pathway through which the MD may influence mental health. Diets rich in plant-based fiber and fermented foods can enhance the growth of beneficial gut bacteria, reduce intestinal permeability, and limit the translocation of pro-inflammatory endotoxins such as lipopolysaccharide (LPS) into systemic circulation [81]. These changes may, in turn, attenuate neuroinflammation and support central nervous system homeostasis [82]. In this context, dietary omega-3 fatty acids, particularly those derived from fish, have been shown to modulate gut microbial composition and increase short-chain fatty acid (SCFA) production, with implications for both immune and neurochemical regulation [83].

Additional physiological effects of MD components have been observed in clinical and preclinical studies. Higher vegetable intake has been associated with lower circulating IL-6 levels in older adults, independently of adiposity [84], while meta-analyses have reported reductions in C-reactive protein and TNF-α following increased fruit and vegetable consumption, along with improved immune cell function [85]. Preclinical models further suggest that whole grain consumption can reduce oxidative stress, enhance antioxidant activity, and shift gut microbiota toward more anti-inflammatory profiles [86]. Collectively, these findings position the Mediterranean diet as a promising multidimensional approach for improving mental health through integrated metabolic, immunological, and neurobiological mechanisms. This growing body of literature supports the rationale for exploring the role of dietary patterns, particularly those with anti-inflammatory and neuroprotective potential, as modifiable targets in the prevention and treatment of depression, within both clinical and subclinical populations.

1.4 Physical Activity, Depression, and Anhedonia: Neurobiological and Clinical Perspectives

Physical activity (PA) is increasingly recognized as a key modifiable lifestyle factor with robust evidence supporting its protective and therapeutic effects on mental health. Numerous randomized

controlled trials and meta-analyses have demonstrated that regular physical activity significantly reduces depressive symptoms, with effect sizes comparable to pharmacological and psychotherapeutic interventions, especially in mild to moderate depression [87]. PA is now considered not only a complementary treatment but also a preventive strategy capable of reducing the risk of depressive onset and relapse [88-90] A recent systematic review and network meta-analysis demonstrated that structured exercise modalities, including walking or jogging, yoga, and strength training, produce clinically meaningful reductions in depressive symptoms, with effect sizes comparable to those of psychotherapy and antidepressant treatment, and efficacy that increases proportionally with exercise intensity [91]. Moreover, a dose-response meta-analysis including over 190,000 participants revealed an inverse, curvilinear relationship between physical activity and incident depression: even accumulating half the recommended weekly activity (≈ 4.4 mMET-h) was associated with an 18 % lower risk of depression, while meeting the full recommendation (≈ 8.8 mMET-h) corresponded to a 25 % risk reduction; notably, additional benefits beyond this level were modest, underscoring the relevance of promoting even modest increases in activity levels [92].

The neurobiological mechanisms through which PA exerts its antidepressant effects are multifaceted. Physical activity enhances neurotransmission in several systems implicated in mood regulation, including the serotonergic, dopaminergic, noradrenergic, and glutamatergic pathways [88, 93, 94]. In particular, increased dopaminergic activity is associated with improved motivation and executive functioning, which may facilitate behavioral activation in individuals with depression [88]. In addition, PA promotes neuroplasticity and neurogenesis by upregulating brain-derived neurotrophic factor (BDNF) and other neurotrophic molecules, supporting synaptic connectivity and emotional resilience [93, 95-98].

Physical activity also plays a regulatory role in the hypothalamic-pituitary-adrenal (HPA) axis, helping to maintain stress-response homeostasis and reduce cortisol hypersecretion, commonly observed in depression [96, 97, 99]. Furthermore, PA exerts potent anti-inflammatory effects, lowering levels of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and enhancing anti-inflammatory markers such as IL-10 [93, 96, 100]. These immunomodulatory actions are particularly relevant given the strong links between systemic inflammation and depression pathophysiology [100].

Among the symptom dimensions of depression, anhedonia, the reduced ability to experience pleasure, has received growing attention for its central role and poor response to standard antidepressants. Evidence suggests that PA can directly target anhedonia by enhancing reward processing, particularly through its action on the nucleus accumbens (NAc), a core structure of the brain's reward circuit [94, 101, 102]. Preclinical and clinical studies have shown that PA improves both anticipatory and consummatory pleasure, modulates arousal in response to rewarding stimuli, and reduces negative

affect [103]. The relationship between PA and anhedonia appears to be dose-dependent, with higher frequency and intensity of PA, such as moderate-to-vigorous activity, being inversely associated with anhedonic symptoms [104, 105]. An 8-week exercise intervention significantly reduced anhedonia in individuals with depressive symptoms, partially mediated by improvements in reward processing and dopaminergic signaling.

Notably, anhedonia has been linked to reduced motivation and effort-based decision making. Individuals with depression and anhedonia often demonstrate a decreased willingness to expend effort to obtain rewards, a deficit that PA may help to reverse[106]. PA also appears to be effective in improving motivation and hedonic tone in specific clinical contexts, such as post-stroke depression, where it helps mitigate fatigue, anger, and long-term motivational impairments.

In addition to neurobiological mechanisms, PA offers psychosocial and behavioral benefits. Participation in structured, group-based physical activity has been associated with lower rates of depression compared to non-participation, underscoring the role of social engagement in the therapeutic effects of exercise [107]. Moreover, acute bouts of moderate-intensity exercise have been shown to improve both mood state and cognitive performance in patients with major depressive disorder (MDD), with effects observable shortly after activity [108].

From a clinical standpoint, PA is recommended as an adjunctive intervention to standard treatments such as antidepressant medication and psychotherapy [98, 100]. It is particularly valuable in addressing residual symptoms like fatigue, executive dysfunction, and low motivation, areas often insufficiently targeted by pharmacological therapies alone [88, 91, 109]. Given its broad range of benefits, PA should be considered a core component of comprehensive treatment plans for depression.

In summary, physical activity represents a biologically grounded, low-cost, and accessible strategy for the prevention and treatment of depression. Through its action on neurotransmitters, neurotrophic signaling, inflammation, stress regulation, and reward circuitry, PA improves mood, cognitive functioning, and particularly hedonic capacity. Different types and intensities of activity offer variable effects, but even modest increases in PA can yield clinically significant improvements in depressive symptoms and quality of life.

1.5 Gut Microbiota, Insulin Signaling, and Anhedonia: Interconnected Mechanisms in Depression

Over the past decade, the gut–brain axis has gained prominence as a key regulator of neuropsychiatric health. The human gut microbiota, comprising trillions of microorganisms, is increasingly recognized as a dynamic modulator of brain function and behavior. Microbial metabolites such as short-chain fatty acids (SCFAs), tryptophan derivatives, and secondary bile acids influence neurotransmitter

systems, immune responses, and neuroinflammatory pathways [110-113]. Clinical and preclinical studies have consistently shown that alterations in gut microbiota composition, referred to as dysbiosis, are associated with depression, anxiety, and cognitive impairment. Of particular relevance is the association between dysbiosis and anhedonia, a core symptom of major depressive disorder (MDD), characterized by reduced motivation and reward responsiveness [114-117].

The gut-brain axis (GBA) is a complex, bidirectional communication system linking the gastrointestinal tract with the central nervous system. It functions through multiple pathways, neural, endocrine, humoral, metabolic, and immune, that allow continuous exchange of signals between the gut and the brain[118-120]. By integrating information from both the microbiota and the nervous system, the GBA plays a central role in regulating mood, cognition, and overall mental health[120].

Neural communication occurs largely through the autonomic nervous system, with the vagus nerve serving as the primary channel transmitting information in both directions. This pathway influences gastrointestinal activity as well as central brain functions[118]. Endocrine signaling also contributes, particularly through the hypothalamic-pituitary-adrenal (HPA) axis, which is central to stress regulation. Gut microbiota can affect this axis, altering cortisol levels and thereby influencing mood[121]. Immune interactions represent another important mechanism: microbial activity shapes immune responses, modulating neuroinflammation and neurotransmitter production, both of which are critical for emotional regulation and cognitive processes[122]. In addition, metabolic signaling plays a role, as gut microbes produce metabolites such as SCFAs and neurotransmitters like serotonin and dopamine, which directly affect brain function and behavior [123].

Disruptions in these pathways can have profound effects on mood and cognition. Altered functioning of the GBA has been associated with mood disorders such as depression and anxiety. Because neurotransmitters like serotonin, most of which is synthesized in the gut, are strongly influenced by microbial composition, disturbances in the microbiota can significantly affect emotional well-being[123, 124]. Cognitive functions are also shaped by the GBA. Changes in microbiota composition have been linked to neurodevelopmental and neurodegenerative conditions, including autism spectrum disorder, Parkinson's disease, and Alzheimer's disease[125]. A growing body of evidence suggests that gut microbiota exerts its effects on mood through its bidirectional interactions with insulin signaling pathways. The gut microbiota plays a fundamental role in metabolic regulation, modulating glucose homeostasis, insulin sensitivity, and systemic inflammation [112, 124, 126]. Dysbiosis can lead to increased intestinal permeability, allowing the translocation of bacterial endotoxins such as lipopolysaccharides (LPS) into systemic circulation, where they trigger inflammatory cascades that impair insulin signaling [111, 113, 127]. SCFAs, such as acetate, propionate, and butyrate, produced by microbial fermentation of dietary fibers, influence insulin

sensitivity by activating G-protein-coupled receptors and stimulating GLP-1 secretion [110, 111, 114, 128]. These metabolic and endocrine effects underscore the microbiota's role in modulating both peripheral and central insulin responses [129].

Importantly, insulin signaling has a direct influence on reward circuitry in the brain, particularly in the nucleus accumbens (NAc), a key structure in hedonic processing. Preclinical studies have shown that high-fat diets (HFD) induce insulin resistance in the NAc, which in turn leads to anhedonia-like behavior in rodents [115, 116]. Elevated plasma insulin levels and disrupted insulin receptor signaling in the brain have been linked to motivational deficits, while experimental depletion of gut microbiota using antibiotics can prevent HFD-induced anhedonia and restore insulin sensitivity in the NAc [116, 130]. These effects appear to be mediated in part by adipokines such as leptin and adiponectin, which are modulated by gut microbial activity. For instance, antibiotic-induced reduction of leptin levels has been shown to prevent the emergence of anhedonic behavior in animal models [116, 117].

Mechanistically, the adiponectin/leptin ratio and the availability of microbial-derived SCFAs and other signaling molecules appear to be central in this microbiota–insulin–reward axis [115, 131]. Notably, microbially produced acetate (MPA) has been shown to increase postprandial insulin release via a sequential and integrated signaling network between the gut, brain, and pancreas, promoting energy retention but potentially contributing to altered hedonic tone [131]. These findings support the hypothesis that metabolic alterations arising from microbiota dysregulation may impact mood via central insulin resistance and impaired neuroplasticity in reward-related regions.

Given these interconnected mechanisms, interventions targeting the gut microbiota are being investigated as potential therapeutic strategies for both metabolic and psychiatric disorders. Prebiotics, probiotics, and synbiotics have shown promise in improving insulin sensitivity and glycemic control in patients with type 2 diabetes, and early evidence suggests beneficial effects on depressive symptoms, particularly anhedonia [117, 127, 132, 133]. Fecal microbiota transplantation (FMT) has also demonstrated the ability to restore eubiotic microbial profiles and reverse metabolic disturbances in diabetic patients [127]. Nonetheless, the implementation of microbiota-based therapies faces several challenges, including high interindividual variability, the complexity of host–microbe interactions, and the lack of long-term safety data [127, 133].

In summary, the evidence supports a converging model in which gut microbiota, insulin signaling, and anhedonia are intimately linked. Dysbiosis contributes to both peripheral and central insulin resistance, which may disrupt reward circuitry and promote anhedonic symptoms. Understanding and targeting this gut–metabolism–mood axis may open new avenues for integrated treatment approaches in individuals with comorbid depression and metabolic dysregulation [110, 111, 115-117, 130].

2.0 Rationale of the study, Aims and Objectives

Despite increasing recognition of the importance of lifestyle and metabolic factors in depression, few studies have comprehensively examined their combined effects on clinical outcomes, particularly anhedonia, within longitudinal clinical cohorts. Furthermore, the role of gut microbiota as a potential mediator of these associations remains poorly understood.

The overarching aim of this doctoral research is to investigate the complex interplay between lifestyle, metabolic, inflammatory, and biological factors in patients with depressive disorders, with a particular focus on the role of anhedonia as a core and treatment-resistant symptom.

This doctoral project is embedded within the framework of the European multicenter *OPADE Project* (Optimize and Predict Antidepressant Efficacy for Patient with Major Depressive Disorders using multi-omnic analysis and AI-predictive Tool), Grant Agreement: 101095436, which seeks to identify clinical, biological, and lifestyle predictors of antidepressant treatment response. Within this framework, the thesis specifically examines the contributions of adherence to the Mediterranean diet, physical activity, insulin resistance, systemic inflammation (CRP), and gut microbiota composition to depressive symptom severity and treatment outcomes over a six-month longitudinal follow-up.

By integrating clinical, sociodemographic, and molecular data, this project aims to advance a multidimensional understanding of the determinants of depression and its response to treatment, thereby contributing to the development of more targeted and personalized interventions.

Specifically, the study aims to:

1. Assess cross-sectional and longitudinal associations between adherence to the Mediterranean diet, physical activity, insulin resistance, and systemic inflammation (CRP) with depressive symptom severity and anhedonia.
2. Investigate how gut microbiota diversity and composition relate to lifestyle and metabolic variables, and evaluate whether microbial diversity indices are linked to depressive and anhedonic symptom profiles.
3. Identify clinical and behavioral predictors of early and sustained antidepressant response, integrating metabolic and microbiome-related data to delineate potential biological pathways underpinning symptom improvement.
4. Provide an integrative model highlighting the role of behavioral activation, metabolic health, and microbial balance in shaping mood outcomes, with implications for lifestyle-based and precision psychiatry interventions.

2.1 Primary Objective

To evaluate the association between adherence to the Mediterranean diet, physical activity, insulin resistance, and systemic inflammation (CRP) with anhedonia and depressive symptom severity, both at baseline and over a six-month follow-up period.

This objective focuses on understanding how lifestyle, metabolic, and inflammatory variables jointly influence the severity and course of depression and anhedonia, thereby addressing one of the most disabling and treatment-resistant dimensions of depression.

2.2 Secondary Objectives

1. To explore associations with overall depression severity, treatment response, and early versus sustained clinical improvement.
2. To assess gut microbiota composition and diversity as correlates and potential mediators of these associations.
3. To investigate correlations with sociodemographic, clinical, and pharmacological variables that may act as moderators or confounders.

3. MATERIALS AND METHODS

3.1 Study Design

This study is embedded within the broader framework of the *OPADE Project*, a European and international multicenter aimed at optimizing antidepressant treatment through the identification of clinical, biological, and lifestyle predictors of treatment response, funded to advance the development of predictive tools for optimizing antidepressant therapy [134] The overarching objective of OPADE is to identify key biomarkers that can guide clinical decision-making and improve treatment outcomes for patients with Major Depressive Disorder. The project aims to integrate clinical, biological, and digital data into an artificial intelligence (AI) and machine learning (ML)–based predictive tool that will assist clinicians in tailoring treatment strategies, thereby increasing remission rates and reducing the functional impairment associated with depression.

The scientific rationale of OPADE is strongly anchored in the gut–brain axis, which is increasingly recognized as a major player in the pathophysiology of depression. The project systematically investigates the interaction between genetics, epigenetics, microbiota composition, metabolomics, inflammatory and immune networks, and their relation to clinical symptom profiles. Through this multidimensional approach, OPADE seeks to:

- Establish patient profiles predictive of antidepressant response, thereby supporting precision psychiatry and optimizing treatment efficacy.
- Explore the correlations between neuroinflammatory indices, microbiome-related markers, metabolomic signatures, immunological profiles, and epigenetic variations.
- Identify biomarkers predictive of recurrence and chronicity in depressive illness.
- Improve diagnostic accuracy and enable early identification of individuals at high risk of developing depressive disorders.
- Retrospectively evaluate, through detailed clinical history, the onset of depressive symptoms in adolescence.
- Assess the extent to which blood biomarkers correlate with other biomarker domains (e.g., microbiota, epigenetic, metabolomic, immunologic).

The OPADE clinical study follows a prospective, observational design, recruiting 350 patients aged 14 to 50 years diagnosed with MDD across three clinical centers in three European countries and one clinical center overseas in Colombia. These centers include: UNISI (Siena, Italy), UNISANITAS (Colombia), IDIBGI (Spain), and UNIPOL (Turkey). Recruitment is stratified by age into four cohorts: 70 adolescents aged 14–17 years, 100 young adults aged 18–30 years, 90 adults aged 31–39 years, and 90 adults aged 40–50 years. This stratification enables the study of age-related differences in clinical presentation, treatment response, and biomarker expression.

Each participant is followed across six scheduled visits over a two-year period: baseline (T0), 2 months (T1), 4 months (T2), 6 months (T3), 12 months (T4), and 24 months (T5). At each visit, participants undergo a comprehensive assessment that includes validated clinical and psychometric questionnaires, as well as collection of biological samples (blood, stool, urine, saliva). These biological materials are processed and stored locally before being shipped to the consortium's analytic partners for metabolomic, transcriptomic, epigenomic, immunoprofiling, and microbiome analyses. In addition, real-time electroencephalography (EEG) recordings and cognitive assessments are conducted, providing further insights into neurophysiological markers of treatment response.

A distinctive feature of OPADE is the integration of digital health technologies. Patients are engaged through an empowerment tool that collects subjective narratives and transforms them into analyzable data, complementing traditional clinical assessments. Moreover, real-time digital monitoring tools are employed, including emotion recognition systems, which allow continuous ecological assessment of affective states outside the clinical setting. These data are ultimately incorporated into the AI/ML predictive platform, which represents the primary translational outcome of the project.

The primary aim of OPADE is to establish reproducible patient profiles that optimize the efficacy of antidepressant treatments, using multimodal biomarker integration. The secondary aim is to identify

molecular and non-molecular predictors of recurrence, which may be applied in preventive strategies and long-term patient monitoring.

The study is thus conceived as a large-scale, prospective biomarker discovery effort, with a multidimensional approach combining sociodemographic variables, clinical psychopathology, functional outcomes, digital monitoring, and multi-omics analyses. This comprehensive design is expected to generate a robust dataset enabling the training and validation of the AI/ML predictive tool, which will be the principal innovation and translational product of OPADE.

Within this framework, the present doctoral project focuses specifically on data collected from the University of Siena (UNISI) site at four timepoints (T0, T1, T2, and T3) of the OPADE study. All analyses were conducted taking into account the sample size available at each timepoint, which included 136 patients at baseline (T0), 109 at two months (T1), 89 at four months (T2), and 71 at six months (T3). This dataset was used to explore the associations between adherence to the Mediterranean diet, insulin resistance, physical activity, gut microbiota composition, and depressive symptoms, with a particular focus on anhedonia.

3.2 Participants

Participants in this study were recruited within the framework of the OPADE project. The sample was composed of patients experiencing a current major depressive episode, who were systematically assessed at baseline and re-evaluated after two, four and six months of follow-up.

Inclusion and Exclusion Criteria

Eligible individuals were required to meet diagnostic criteria for Major Depressive Disorder, established through structured clinical interviews. Specifically, diagnoses were confirmed using the Structured Clinical Interview for DSM-5 Disorders (SCID-5-CV) [135]for adults, and the Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version for DSM-5 (K-SADS-PL-DSM-5) [136] for adolescents. At the time of enrollment, patients had to be in an active depressive episode, as indicated by a Hamilton Depression Rating Scale[137] (HAM-D) score ≥18 or, alternatively, a Montgomery–Åsberg Depression Rating Scale [138] (MADRS) score ≥18.

In line with the study design, participants were included if they were about to initiate a new antidepressant treatment as part of their routine clinical care, provided that no other psychotropic medications were being newly introduced simultaneously. This criterion was essential to ensure that treatment effects could be attributed primarily to the newly initiated antidepressant, without confounding influences from concurrent pharmacological changes. The study focused on a relatively young population, and therefore included patients between 14 and 50 years of age. Additional

requirements included the ability to use mobile devices (smartphones or tablets), given the integration of digital assessments within the OPADE project, and the willingness and capacity to provide written informed consent.

Exclusion criteria were defined to minimize potential confounding factors and to ensure patient safety. Individuals with intellectual disability or significant neurological disorders (including multiple sclerosis, major neurocognitive disorder, or epilepsy) were excluded. Patients with a current or past psychotic disorder, mood disorder with psychotic features, or bipolar disorder were not eligible, as these conditions could alter illness trajectories and treatment responses in ways not aligned with the project's objectives. Similarly, those with a current diagnosis of alcohol or substance use disorder were excluded due to the well-known impact of substance use on mood, cognition, and treatment adherence.

To ensure pharmacological stability, patients who had initiated concomitant psychotropic medications less than two weeks prior to baseline assessment were excluded. From a medical standpoint, exclusion also applied to those with active inflammatory diseases (e.g., rheumatoid arthritis, polymyalgia rheumatica) or severe and unstable physical illnesses, such as a recent myocardial infarction. Furthermore, patients with a history of hepatitis B or C, human immunodeficiency virus (HIV), or active tuberculosis infection, as well as those with any systemic infection in the two weeks preceding baseline, were not eligible to participate.

Given the inclusion of microbiota analysis as a key objective of the study, individuals who had received antibiotic treatment or other medications known to alter gut microbial composition within the 30 days before baseline were excluded, in order to avoid confounding effects on microbiota data. Finally, pregnant or breastfeeding women were not included, due to both ethical considerations and the potential impact of pregnancy-related metabolic and hormonal changes on study variables.

This rigorous set of inclusion and exclusion criteria was designed to ensure a well-defined sample of patients with unipolar depression, while controlling for medical and pharmacological factors that might otherwise interfere with the study's objectives.

Sociodemographic and clinical characteristics

At baseline, the following variables are collected:

- Sociodemographic data: age, sex, education, employment status, marital status.
- Clinical data: age at onset, duration of illness, number of previous episodes, psychiatric comorbidities, history of hospitalizations, family psychiatric history, and concomitant psychotropic medications.
- Lifestyle data: smoking habits, alcohol consumption, and body mass index (BMI).

3.3 Assessments and Variables

3.3.1 Depression and Anhedonia

The assessment of depressive symptomatology was conducted using validated clinician-rated and self-report instruments to ensure a comprehensive evaluation of both global severity and specific dimensions, particularly anhedonia.

The Montgomery–Åsberg Depression Rating Scale (MADRS) is a standardized clinical instrument developed to quantify the severity of depressive symptoms and monitor treatment response over time [138]. Unlike self-administered questionnaires, the MADRS is completed by a trained clinician, typically a psychiatrist, during a structured interview that combines direct behavioral observation with the patient's subjective report.

The scale consists of 10 items, each assessing a core symptom domain of depression: apparent sadness, reported sadness, inner tension, sleep disturbances, reduced appetite, concentration difficulties, lassitude, anhedonia, pessimistic thoughts, and suicidal ideation. Each item is rated on a scale from 0 to 6, where 0 indicates absence of the symptom and 6 represents maximum severity. Anchor scores (0, 2, 4, 6) are accompanied by clinical descriptors that assist the rater in assigning the most appropriate score; intermediate values (1, 3, 5) can be used when the patient's condition falls between two levels.

The total MADRS score, derived from the sum of all item scores, ranges from 0 to 60. Based on the total score, patients can be categorized into four clinical severity levels:

- 0–6: no depression
- 7–19: mild depression
- 20–34: moderate depression
- ≥35: severe depression

MADRS is particularly valued for its sensitivity to clinical change, making it a reliable tool for tracking disease progression and evaluating the efficacy of therapeutic interventions. Psychometric studies have demonstrated high inter-rater reliability (intraclass correlation coefficient > 0.80), good internal consistency, and strong convergent validity when compared with other established depression rating scales, such as the Hamilton Depression Rating Scale (HAM-D).

Several alternative versions of the MADRS have been developed, including the MADRS-S (a self-administered version suitable for home-based follow-up) and the MADRS-Y (adapted for use in adolescents and young adults). Nonetheless, the standard version remains the most widely used in both clinical practice and adult psychiatric research. Despite its methodological robustness, the

MADRS does present some limitations, most notably, its reduced sensitivity to atypical somatic symptoms such as hypersomnia and hyperphagia, which are frequently observed in certain depressive subtypes. Nevertheless, due to its ability to detect subtle symptomatic changes over relatively short periods, the MADRS is strongly recommended for both clinical use and experimental research.

In addition, the Snaith–Hamilton Pleasure Scale (SHAPS) was used to specifically assess anhedonia, the inability to experience pleasure. The SHAPS is a 14-item self-report questionnaire that evaluates pleasure responses across various domains of everyday life, including social interaction, food, pastimes, and sensory experiences. Each item is scored dichotomously, with higher scores indicating greater anhedonic severity. The SHAPS has been validated across different populations and is considered one of the most robust tools for quantifying both consummatory and anticipatory aspects of anhedonia [137].

Together, the MADRS and SHAPS provide complementary perspectives: while the MADRS captures global depressive severity, the SHAPS isolates one of the most clinically relevant and treatment-resistant dimensions of depression.

3.3.2 Assessment of Physical Activity: The IPAQ-SF

Physical activity was assessed using the International Physical Activity Questionnaire – Short Form (IPAQ-SF), a self-report tool widely validated for use across diverse populations and international settings. Originally developed by the IPAQ research group in 1998 and validated in 12 countries by Craig et al. (2003)[139], the IPAQ-SF captures physical activity performed over the preceding seven days, offering a standardized measure of individual activity levels [140].

The questionnaire consists of seven items and evaluates three domains of physical activity based on intensity: (1) vigorous activity (e.g., running, fast cycling, competitive sports, heavy lifting), (2) moderate activity (e.g., brisk walking, cycling at moderate pace, gardening, or physically demanding household chores), and (3) walking (including commuting or recreational ambulation). For each domain, participants report the number of days per week and the average daily duration (in minutes) dedicated to that activity. In addition, the questionnaire records average daily time spent sitting, providing an estimate of sedentary behavior.

To quantify energy expenditure, the IPAQ scoring protocol converts self-reported activity into Metabolic Equivalent of Task (MET) units, a physiological indicator of activity intensity. Standard coefficients are applied:

- 8.0 METs for vigorous activity,
- 4.0 METs for moderate activity,
- 3.3 METs for walking.

The total weekly energy expenditure, expressed in MET-minutes/week, is calculated by multiplying minutes per day by days per week for each intensity level and then multiplying by the corresponding MET factor. The sum of these three components yields a comprehensive score of weekly physical activity:

- Vigorous MET = 8.0 × minutes × days
- Moderate MET = 4.0 × minutes × days
- Walking MET = 3.3 × minutes × days
- Total MET = Vigorous + Moderate + Walking METs

This metric offers an objective, comparable estimate of overall physical activity, facilitating the identification of population-level movement patterns and health-related behaviors.

For both clinical and research applications, the total IPAQ score can be analyzed as a continuous variable or categorized into three activity levels (table 1):

- Inactive (<700 MET-min/week): indicative of sedentary lifestyle and increased health risk;
- Moderately active (700–2519 MET-min/week): meeting minimum recommendations for health maintenance;
- Highly active (≥2520 MET-min/week): associated with optimal physical and metabolic health outcomes.

In the context of this study, the IPAQ-SF was employed both to investigate continuous associations between physical activity and depressive symptomatology, particularly anhedonia, and to stratify patients by activity level for group comparisons. Given the well-established bidirectional relationship between physical activity and mental health, as well as emerging links between lifestyle behaviors and metabolic dysregulation (e.g., insulin resistance), IPAQ data provide an essential behavioral dimension to the multimodal profiling of participants in the OPADE project.

Although the IPAQ-SF is inherently subject to recall and reporting biases due to its self-reported nature, its ease of administration, cross-cultural adaptability, and robust psychometric properties [140] make it a practical and widely endorsed tool in both epidemiological surveillance and clinical research.

IPAQ CATEGORY	MET-MINUTES/WEEK	CLINICAL INTERPRETATION
INACTIVE	**< 700**	Indicative of a sedentary lifestyle; associated with increased cardiometabolic and mental health risk.
MODERATELY ACTIVE	**700 – 2519**	Meets minimum WHO guidelines for physical activity; associated with general health maintenance.

HIGHLY ACTIVE	**≥ 2520**	Reflects optimal or high levels of activity; associated with enhanced physical, mental, and metabolic health outcomes.

Table 1 Classification of Physical Activity Levels According to Total MET-minutes/Week (IPAQ-SF)

3.3.3 Assessment of Adherence to the Mediterranean Diet

Adherence to the Mediterranean diet was assessed using the 14-Item Mediterranean Diet Adherence Screener (MEDAS) developed and validated within the PREDIMED trial (Prevención con Dieta Mediterránea) [141]. This brief instrument provides a reliable and widely used measure of compliance with the traditional Mediterranean dietary pattern and has been validated against comprehensive food-frequency questionnaires and biological markers of dietary intake.

The MEDAS-PREDIMED questionnaire consists of 14 items, each reflecting a core component of the Mediterranean dietary model. Thirteen items assess the frequency of consumption of specific food groups or habits, and one item assesses the primary source of culinary fat. Each item is scored 1 point if the participant meets the recommended criterion for adherence, or 0 points otherwise, resulting in a total score ranging from 0 to 14, where higher scores indicate greater adherence to the Mediterranean diet. Table 2.

The items cover the following domains:

1. Use of olive oil as the main culinary fat;
2. Daily consumption of ≥4 tablespoons of olive oil (including for cooking and dressing);
3. Intake of ≥2 daily servings of vegetables;
4. Intake of ≥3 daily servings of fruit (including natural juices);
5. Consumption of <1 serving/day of red or processed meat;
6. Consumption of <1 serving/day of butter, cream, or margarine;
7. Consumption of <1 sugar-sweetened or carbonated beverage per day;
8. Moderate wine intake (≥7 glasses per week, if not contraindicated);
9. Consumption of ≥3 servings/week of legumes;
10. Consumption of ≥3 servings/week of fish or shellfish;
11. Consumption of <3 servings/week of commercial sweets or pastries;
12. Consumption of ≥3 servings/week of nuts (including peanuts);
13. Preference for white meat (chicken, turkey, rabbit) over red or processed meat;
14. Consumption of ≥2 servings/week of traditional sofrito (a sauce made with tomato, onion, and garlic sautéed in olive oil).

Following the PREDIMED protocol, the total score was used as a continuous measure and, when necessary for group comparison, dichotomized into low adherence (≤8 points) and high adherence (≥9 points) categories, as previously applied in epidemiological and interventional studies.

The PREDIMED screener has shown strong correlations with full-length dietary questionnaires ($r \approx 0.52$; intraclass correlation = 0.51) and with objective health indicators such as waist-to-height ratio, serum lipid profile, and inflammatory markers. Its simplicity and brevity allow for efficient administration in both research and clinical settings while maintaining robust validity and reproducibility.

In the present study, the use of the PREDIMED tool provided a standardized and validated method to quantify adherence to the Mediterranean diet and to explore its potential associations with depression severity, anhedonia, insulin resistance, and inflammatory or metabolic biomarkers, as well as its possible interactions with gut microbiota composition within the OPADE project framework.

Questions	Criteria for 1 point
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	≥4 tbsp
3. How many vegetable servings do you consume per day? (1 serving : 200 g [consider side dishes as half a serving])	≥2 (≥1 portion raw or as a salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥3
5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100–150 g)	<1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)	<1
7. How many sweet or carbonated beverages do you drink per day?	<1
8. How much wine do you drink per week?	≥7 glasses
9. How many servings of legumes do you consume per week? (1 serving : 150 g)	≥3
10. How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish)	≥3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)	≥3
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?	≥2

Table 2 MEDAS-PREDIMED tool

3.3.4 Insulin Resistance and the HOMA-IR Index: Relevance for Depression and Anhedonia

Insulin resistance (IR) represents a key metabolic disturbance increasingly recognized for its role not only in type 2 diabetes and cardiovascular disease, but also in the pathophysiology of mood disorders, particularly in relation to anhedonia and treatment-resistant depression. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is one of the most widely used surrogate indices for estimating insulin resistance in both clinical and research settings. Developed by Matthews and colleagues in 1985 [142], the HOMA-IR index is calculated from fasting plasma glucose and insulin levels using the following formula:

- HOMA-IR = (Fasting insulin [μU/mL] × Fasting glucose [mg/dL]) / 405
  or alternatively, when glucose is expressed in mmol/L:
- HOMA-IR = (Fasting insulin [μU/mL] × Fasting glucose [mmol/L]) / 22.5

This index reflects the dynamic balance between insulin secretion and insulin sensitivity, providing an indirect yet reliable estimation of metabolic efficiency. While it does not serve as a definitive diagnostic tool, it is considered indicative of insulin resistance, especially when interpreted alongside other metabolic markers [143].

Commonly used cut-off values, although variable depending on population characteristics and laboratory assays, include:

- HOMA-IR < 1.0: typically indicates normal insulin sensitivity
- HOMA-IR 1.0–2.5: suggests intermediate or borderline insulin resistance
- HOMA-IR > 2.5: generally considered consistent with insulin resistance [144]

In the context of psychiatric research, HOMA-IR has emerged as a useful biomarker for identifying metabolic vulnerabilities that may contribute to mood dysregulation. Growing evidence suggests that IR may disrupt central insulin signaling, modulate reward circuitry, and exacerbate inflammatory processes, all of which are implicated in the neurobiology of anhedonia [145, 146]. Therefore, the integration of HOMA-IR within multidomain psychiatric assessments is gaining scientific relevance.

In the OPADE study, fasting blood samples were obtained from participants following overnight fasting, and glucose and insulin concentrations were measured using standardized protocols. In this study HOMA-IR was calculated only at baseline (T0) and was not repeated at subsequent follow-up assessments. The HOMA-IR index was calculated as the primary measure of insulin resistance, complemented by the separate evaluation of fasting glucose and insulin levels. These individual parameters not only support the interpretation of HOMA-IR but also allow for the detection of related metabolic alterations, such as impaired fasting glucose or hyperinsulinemia, that may not be fully captured by composite indices.

Beyond their immediate relevance for evaluating the relationship between insulin resistance and depressive symptomatology, particularly anhedonia, these metabolic markers contribute to the broader biological profiling within the OPADE project. They enable cross-domain integration with metabolomics, immune profiling, and gut microbiota data, offering a more comprehensive understanding of the biological underpinnings of treatment resistance and symptom dimensions in major depression.

3.3.5 Inflammatory Marker: C-Reactive Protein

Inflammation is increasingly recognized as a key biological pathway contributing to the pathophysiology of major depressive disorder and its treatment outcomes. Within the OPADE project, systemic inflammation is comprehensively assessed through a wide panel of circulating cytokines and growth factors, including G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8/CXCL8, IL-10, IL-12p40, IL-15, MCP-1/CCL2, TNF-α, and TNF-β/lymphotoxin-α. The quantification of these inflammatory mediators is performed using multiplexed immunoassays based on magnetic bead technology, allowing simultaneous measurement of multiple analytes from a minimal plasma volume, thereby improving sensitivity and reducing plate-to-plate variability.

For the purposes of the present doctoral study, only C-reactive protein (CRP) was analyzed, as it was the sole inflammatory biomarker available at baseline (T0) at the time of data extraction. CRP is an acute-phase protein synthesized primarily by the liver in response to proinflammatory cytokines, particularly interleukin-6 (IL-6). It serves as a reliable and widely used systemic marker of low-grade inflammation and metabolic dysregulation. In psychiatric research, elevated CRP levels have been repeatedly associated with depressive symptom severity, treatment resistance, and metabolic comorbidities, suggesting a shared inflammatory substrate across somatic and affective domains.

In this study, fasting blood samples were collected at baseline under standardized conditions, and plasma CRP concentrations were quantified using high-sensitivity immunoassays (hs-CRP), expressed in milligrams per deciliter (mg/dL). Given its stability and reproducibility, CRP was chosen as a representative inflammatory biomarker to explore cross-sectional associations with depressive severity, anhedonia, lifestyle behaviors, and metabolic markers (HOMA-IR). Although CRP alone cannot capture the full complexity of immune activation, it provides an informative index of systemic inflammation within the multidomain framework of the OPADE project. Future analyses, incorporating the broader cytokine panel available in subsequent phases, will allow for a more comprehensive characterization of immune–metabolic interactions underlying treatment response in major depression.

3.3.6 Gut microbiota

The gut microbiota constitutes a central focus of the present study, given the accumulating evidence linking alterations in microbial communities with depressive disorders, particularly with symptom dimensions such as anhedonia. Within the OPADE framework, whole gut microbiome analysis is conducted at multiple study timepoints, including baseline (T0) four (T2) and six-month follow-up (T3).

Sample collection and storage

Fecal samples are collected using standardized collection kits provided to participants, accompanied by detailed written instructions to ensure reproducibility and minimize contamination. Once collected, samples are immediately stored at –80 °C in local biobanks until shipment to the analytical laboratory. The standardized handling procedures are designed to preserve microbial DNA integrity and maintain comparability across different clinical sites.

DNA extraction and sequencing

Microbial DNA is extracted following validated laboratory protocols, ensuring high-quality nucleic acid yields suitable for metagenomic profiling. Sequencing is performed using two complementary approaches:

16S rRNA gene sequencing, employed to characterize taxonomic composition at different phylogenetic levels (from kingdom to species).

Shotgun metagenomic sequencing, applied to achieve higher-resolution taxonomic classification and to profile the functional potential of microbial communities.

Analyses are conducted using the Perseus Biomics DynaMAP platform, which enables simultaneous profiling of bacterial and fungal components of the gut microbiome. This dual approach is particularly relevant in light of recent findings suggesting that both bacterial and fungal populations may contribute to the pathophysiology of depression.

Bioinformatics and diversity metrics

Sequencing data are processed through established bioinformatics pipelines, which include quality control, taxonomic assignment, and functional annotation. Microbial community composition is characterized at multiple taxonomic levels (L0: kingdom, L1: phylum, L2: class, L3: order, L4: family, L5: genus, L6: species, L7: strain). To provide summary measures of microbial complexity within samples, a range of alpha diversity indices are computed, including species richness, Chao1, ACE, Shannon entropy, Pielou's evenness, and Simpson's index. These indices capture both qualitative (richness) and quantitative (evenness, distribution of abundances) aspects of microbial communities.

3.3.7 Other Clinical Covariates

Several additional clinical and lifestyle variables are systematically collected to account for potential confounding effects and to enrich the multidimensional characterization of the patient cohort.

- Body mass index (BMI): calculated as weight in kilograms divided by height in meters squared (kg/m^2), measured using standardized procedures at baseline and follow-up.
- Smoking status and alcohol consumption: recorded through self-report questionnaires, with categorical classification (current, former, never) for smoking, and average weekly consumption for alcohol.
- Psychiatric comorbidities: systematically assessed using structured diagnostic interviews and clinical records, including anxiety disorders, post-traumatic stress disorder, and personality disorders.
- Concomitant psychotropic medications: type, dosage, and duration of ongoing pharmacological treatments are recorded at each timepoint. Particular attention is given to antidepressants, antipsychotics, mood stabilizers, and benzodiazepines, given their potential influence on both clinical outcomes and biological measures (e.g., inflammatory markers, gut microbiota).

These covariates are included in subsequent analyses as adjustment factors to ensure that observed associations between lifestyle variables, metabolic markers, and microbiota indices are not confounded by demographic or pharmacological influences.

4. STATISICAL ANALYSIS

Descriptive statistics were conducted to summarize the characteristics of the study sample. Qualitative variables were reported as absolute frequencies and percentages, whereas quantitative variables were reported as medians with interquartile ranges (IQR). All analyses were performed separately for baseline and subsequent follow-up data to assess the temporal stability of the observed associations. Continuous variables of interest were correlated with α-diversity indices using Spearman's rank correlation coefficients (ρ), to assess non-parametric monotonic relationships while accounting for potential deviations from normality and the presence of outliers. Comparisons between two groups were performed using the Mann-Whitney U test, while comparisons across multiple groups were evaluated using the Kruskal-Wallis test, followed by Dunn's post-hoc test. Associations between quantitative variables were examined using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

All analyses were performed using R (version 4.4.2).

5. RESULTS

5.1 Sociodemographic and clinical characteristics of the study sample at baseline

The study included 136 participants, with a relatively balanced sex distribution: 57 males (41.9%) and 79 females (58.1%). The majority of participants identified as Caucasian, comprising 97.1 percent of the cohort. Anthropometric data indicated a mean height of 170.4 cm (SD = 9.7), a mean weight of 73.9 kg (SD = 19.2), and an average body mass index of 25.3 kg/m² (SD = 5.5), suggesting that the group was generally within the borderline normal weight to overweight range.

Occupationally, most participants were either students (42.6%) or self-employed (39.7 percent), which made up the two largest categories. In terms of education, the greatest proportion had completed high school (35.3%), followed by those with some university experience (22.1%), indicating that the cohort was generally well-educated. Socioeconomic conditions were mostly stable, with 69.8% reporting a steady financial status, while 25.6% described their situation as “enough” and 4.7% as “difficult.” Housing conditions were predominantly stable as well: 50.4% were homeowners, 23.6% tenants, and 26.0% reported other arrangements (e.g., living with family). With regard to marital status, the majority of the sample was single (67.7%), while a smaller percentage reported being married (15.4%). The use of psychotropic medication was common among participants. Antidepressants were the most frequently reported form of pharmacological therapy (51.5%), followed by antipsychotics (38.2%), mood stabilizers (28.7%), and anxiolytics or hypnotics (26.5%). The median waist circumference was 87.5 cm (IQR 78.75–106.25). Regarding metabolic biomarkers, HOMA-IR was available for 51 out of 137 (37.2%). Among them, 41 (80.4%) met criteria for insulin resistance, corresponding to 29.9% of the total sample. The median HOMA-IR value was 2.11 (IQR 1.16–3.51), consistent with insulin resistance in approximately 80.4% of participants. Median CRP levels were low (0.11 mg/dL, IQR 0.06–0.27), indicating minimal systemic inflammation.

Lifestyle indicators revealed suboptimal engagement in health-promoting behaviors. The median total IPAQ score was 918 MET-min/week (IQR 185.6–2403.0), placing the cohort overall within the “sufficiently active” category (700–2519 MET-min/week) but close to the lower boundary, with 44.4% classified as inactive. Only 39.1% of participants regularly practiced sport, and most reported consuming three main meals per day (61.8%), although 27.2% reported more than three daily meals and 8.1% only two. Eating irregularities were present in a subgroup: 51.4% reported binge-eating episodes (≥1/week), 10.8% restrictive or anorexic behaviors, and 13.5% insufficient nutrition due to factors such as low income or poor habits.

The median PREDIMED score was 6.6 (IQR 5–8), and only 20.4% of participants met the criteria for good adherence to the Mediterranean diet (score ≥9). Smoking habits were common, with 45.2% being regular smokers and 46.8% reporting never having smoked. Among smokers, 37.7% reported 4–5 cigarettes per day, 34.0% smoked around 20 per day, and 22.6% approximately 10 per day.

Clinically, the median MADRS score was 28.7 (IQR 24.7–31.2), with the vast majority classified as having moderate depression (84.6%), while 14.0% presented severe and only 1.5% mild symptoms. The median SHAPS score was 6.3 (IQR 4–10), indicating a moderate degree of anhedonia. The median age at depression onset was 20 years (IQR 16–28), and 75.0% of the participants had experienced at least one relapse in the past year.

Positive history of comorbid medical conditions was common: 26.4% reported a positive history of diabetes, 45.8% reported a positive history of hypertension, and 37.2% reported a positive history of hypercholesterolemia, while 15.8% reported a positive history of stroke. Cardiovascular history was slightly more frequent among males (62.5%) than females (37.5%). Overall, 59.7% were receiving pharmacological treatment for other medical conditions.

Approximately one-third of the sample (36.6%) had undergone previous psychiatric rehabilitation, suggesting a relevant degree of illness chronicity. Regarding pharmacotherapy at study entry, the vast majority (78.7%) initiated treatment with a selective serotonin reuptake inhibitor (SSRI), 7.4% with a serotonin–norepinephrine reuptake inhibitor (SNRI), and 14.0% with other antidepressants (e.g., bupropion, vortioxetine, mirtazapine, trazodone, tricyclics).

Taken together, these baseline data depict a relatively young and predominantly female population with moderate depression and moderate anhedonia, frequent insulin resistance but low systemic inflammation, and generally suboptimal lifestyle profiles, characterized by low adherence to the Mediterranean diet, limited physical activity, and prevalent smoking habits. The sample also displayed a high rate of prior illness relapses and psychiatric rehabilitation, reflecting a clinically complex and metabolically vulnerable cohort. These characteristics provide a robust framework for investigating the relationships between mood symptoms, behavioral factors, and metabolic dysfunction over the course of treatment. The key Sociodemographic and clinical characteristics of the study sample are summarized in Table 1.

Variable	**Mean (SD) or N (%)**
Gender	
Male	57 (41.9%)
Female	79 (28.1%)
Ethnicity	

Caucasian	132 (97.1%)
Other	3 (2.2%)
missing	1 (0.7%)
Height	170.4 (9.7)
Weight	73.9 (19.2)
BMI	25.3 (5.5)
Occupation	
Student	58 (42.6%)
Self-emplyed	54 (39.7%)
Looking for a job	6 (4.5%)
Unemployed	11 (8.1%)
Other	7 (5.1%)
Education	
Primary school	1 (0.7%)
Intermediate school	10 (7.4%)
2/3 years of high school	25 (18.4%)
High school diploma	48 (35.3%)
Some university years	30 (22.1%)
Bachelor's degree	16 (11.7%)
Post bachelor (master or PhD)	5 (3.7%)
missing	1 (0.7%)
Marital Status	
Single	92 (67.7%)
Married	21 (15.4%)
Other	12 (8.8%)
missing	11 (8.1%)
Antipsychotic (*Yes*)	52 (38.2%)
Antidepressant (*Yes*)	70 (51.5%)
Mood Stabiliser (*Yes*)	39 (28.7%)
Anxiolytic/Hypnotics (*Yes*)	36 (26.5%)

Table 1 Sociodemographic and clinical characteristics of the study sample.

5.2 Cross Sectional Analysis at T0 baseline

A statistically significant negative correlation was observed between adherence to the Mediterranean diet and depressive symptoms (p = 0.013), indicating that lower adherence was associated with more severe depressive symptoms. Similarly, adherence to the Mediterranean diet showed a significant negative correlation with anhedonia (p = 0.0034).

Physical activity levels were negatively correlated with anhedonia symptoms (p < 0.001); however, no significant correlation was identified between physical activity and depressive symptoms (p = 0.67). Sleep quality was not statistically significantly correlated with depressive (p=0.261) or anhedonia (p=0.068) symptoms.

A logistic regression model was constructed to evaluate the effect of Mediterranean diet adherence and physical activity levels on the prevalence of moderate/severe depressive symptoms. The results showed that low adherence to the Mediterranean diet significantly predicted moderate/severe depression, with an odds ratio (OR) of 4.72 (95% CI: 1.37–16.34, p = 0.014). However, the low level of physical activity was not statistically significant.

A Kruskal-Wallis test was performed to assess the combined effect of physical activity levels and adherence to the Mediterranean diet on depressive symptoms. No statistically significant differences were found (p = 0.117).

A negative binomial regression analysis was performed to assess the association between anhedonia, physical activity levels, and adherence to the Mediterranean diet. The analysis showed that low physical activity was associated with significantly higher anhedonia scores, with a rate ratio (RR) of 2.10 (95% CI: 1.31–3.38, p = 0.002). Similarly, low adherence to the Mediterranean diet was associated with a higher rate ratio for anhedonia (RR = 1.85, 95% CI: 1.18–2.91, p = 0.007), see Table number 2.

Analysis	Variables Examined	Statistical Test	Main Findings	p-value / OR / RR (95% CI)
Correlation analyses	Mediterranean diet adherence vs. depressive symptoms	Spearman's rho	Negative correlation: lower adherence associated with higher MADRS scores	$p = 0.013$
	Mediterranean diet adherence vs. anhedonia (SHAPS)	Spearman's rho	Negative correlation: lower adherence associated with higher SHAPS scores	$p = 0.0034$
	Physical activity (IPAQ) vs. anhedonia (SHAPS)	Spearman's rho	Negative correlation: lower activity associated with higher anhedonia	$p < 0.001$
	Physical activity (IPAQ) vs. depressive symptoms (MADRS)	Spearman's rho	No significant correlation	$p = 0.67$

	Sleep quality vs. depressive symptoms (MADRS)	Spearman's rho	No significant correlation	$p = 0.261$
	Sleep quality vs. anhedonia (SHAPS)	Spearman's rho	No significant correlation (trend)	$p = 0.068$
Logistic regression	Mediterranean diet adherence (low vs. high) predicting moderate/severe depression	Binary logistic regression	Low adherence significantly predicts moderate/severe depression	OR = 4.72 (95% CI: 1.37–16.34), $p = 0.014$
	Physical activity level (low vs. high) predicting moderate/severe depression	Binary logistic regression	Not statistically significant	$p > 0.05$
Negative binomial regression	Anhedonia (SHAPS) predicted by physical activity and Mediterranean diet adherence	Negative binomial regression	Low physical activity associated with higher anhedonia (RR = 2.10, 95% CI: 1.31–3.38, $p = 0.002$); low diet adherence associated with higher anhedonia (RR = 1.85, 95% CI: 1.18–2.91, $p = 0.007$)	—
Combined effect	Physical activity × Mediterranean diet on depressive symptoms	Kruskal–Wallis	No significant combined effect	$p = 0.117$

Table 2. Associations between Mediterranean diet adherence, physical activity, sleep quality, and depressive or anhedonic symptoms

Further evaluation of the combined effects of physical activity and adherence to the Mediterranean diet on anhedonia was performed using a Kruskal-Wallis test across four groups based on these two factors. This analysis showed a significant overall difference ($p = 0.0027$). Post-hoc comparisons showed:

- Individuals with both low adherence to the Mediterranean diet and low physical activity levels ("0 0" group) had significantly higher anhedonia scores compared to those who adhered to both behaviors ("1 1" group).
- Significant differences were also observed between the "0 0" group and the "0 1" group ($p < 0.05$), as well as between the "0 0" group and the "1 0" group.

As shown in the box plot below, the "0 0" group had the highest anhedonia scores, while the "1 1" group had the lowest scores. Figure number 1.

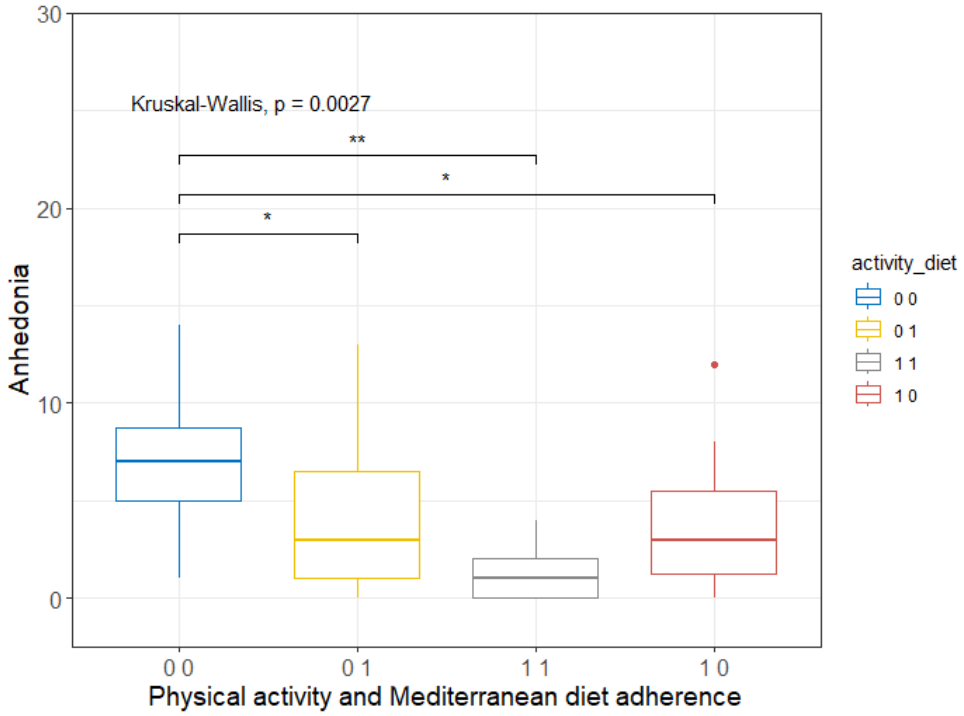

Figure 1

5.2.1 Insulin Resistance

In the sample, 35 patients (70%) had some degree of insulin resistance. The Wilcoxon test showed no statistically significant difference in adherence to the Mediterranean diet between those with and without insulin resistance ($p = 0.1915$). However, patients with insulin resistance had significantly higher anhedonia scores than those without insulin resistance ($p < 0.001$).

To examine the combined effect of adherence to the Mediterranean diet and insulin resistance on anhedonia, a Kruskal-Wallis test was performed across the four groups based on these two factors. The analysis showed a significant overall difference ($p = 0.002$). Post-hoc comparisons showed significant differences in anhedonia scores between:

- Non-adherents to the Mediterranean diet with insulin resistance versus non-adherents without insulin resistance.
- Non-adherents to the Mediterranean diet with insulin resistance versus adherents without insulin resistance.

As shown in the box plot below, people with low adherence to the Mediterranean diet and insulin resistance had the highest anhedonia scores. Conversely, those without insulin resistance and high adherence to the Mediterranean diet had the lowest anhedonia scores. Figure number 2.

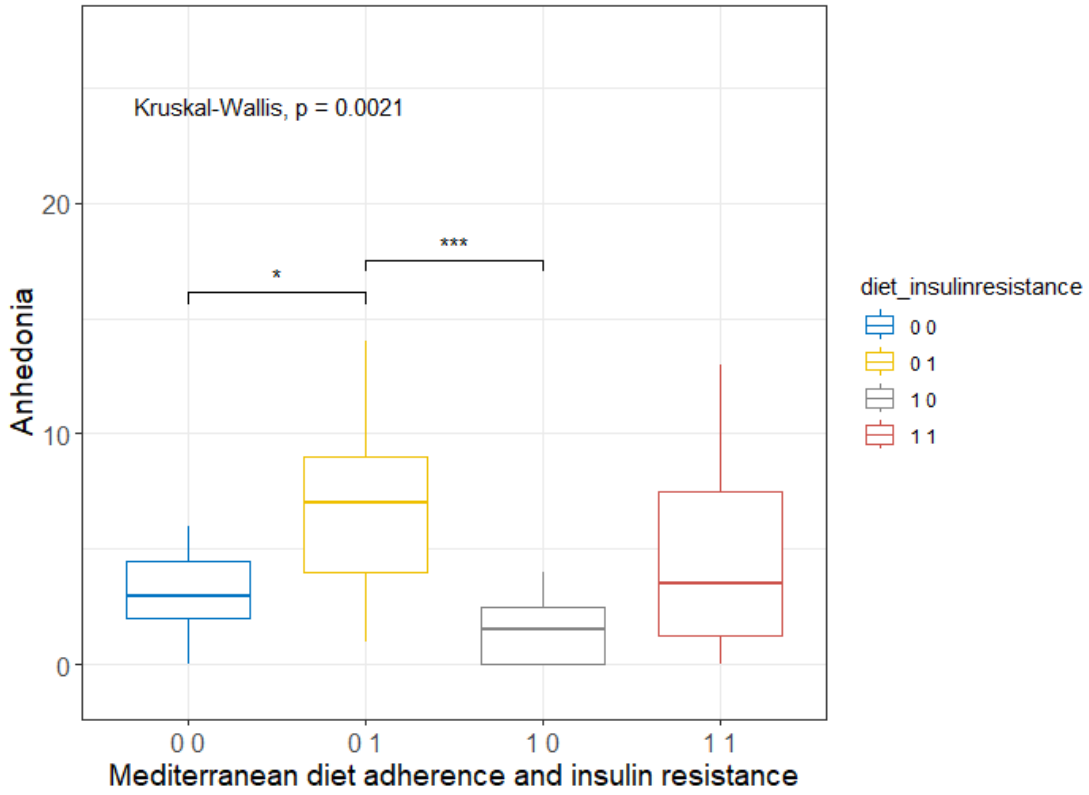

Figure 2

5.2.2 CRP

Analysis of CRP levels showed a statistically significant difference in CRP levels between non-adherents to the Mediterranean diet and adherents ($p = 0.0242$). However, no significant differences in CRP levels were found between individuals with low versus moderate/high physical activity levels ($p = 0.1896$).

The combined effects of physical activity and Mediterranean diet adherence on CRP levels were evaluated using a Kruskal-Wallis test, which revealed a significant difference ($p = 0.036$). Post-hoc comparisons showed:

- Individuals with low adherence to both the Mediterranean diet and physical activity levels ("0 0" group) had significantly higher CRP levels compared to those with high adherence to both behaviors ("1 1" group).
- Significant differences were also observed between the "0 0" group and the "0 1" group.

As shown in the box plot below, individuals with low adherence to both behaviors had the highest CRP levels, while those with high adherence to both behaviors had the lowest CRP levels. Figure number 3.

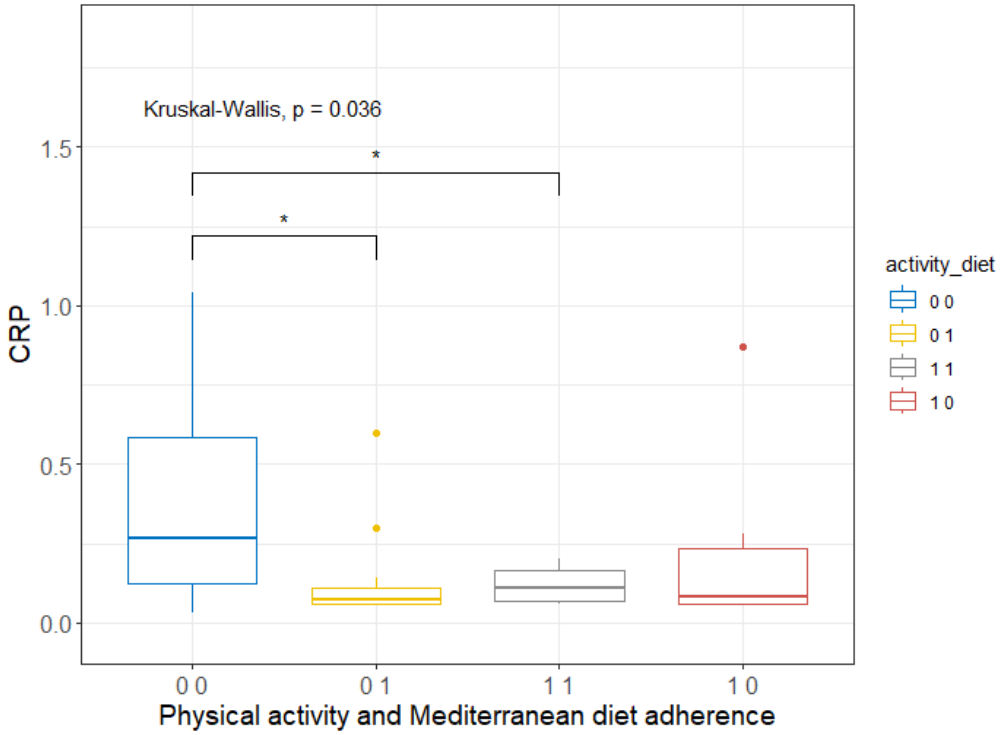

Figure 3

5.3 Associations between depressive severity, anhedonia, lifestyle factors, and metabolic parameters

Variable	T0	T1	T2	T3
MADRS total score	28.7 (4.9)	16.8 (8.2)	14.9 (7.2)	14.7 (9.2)
SHAPS total score	6.3 (3.9)	2.8 (3.5)	2.2 (2.8)	2.4 (3.0)
Total PREDIMED diet score	6.6 (2.5)	6.8 (2.1)	6.7 (1.9)	7.2 (2.5)

Table 2 Trends in variables over time

Table 2 outlines consistent improvements in mood-related symptoms and anhedonia across the four assessment time points. These improvements are accompanied by positive, though less pronounced, changes in physical activity and dietary adherence.

The total score on the MADRS showed a significant decrease from the baseline (T0 = 28.7) to the first assessment (T1 = 16.8), with further small reductions at the second (T2 = 14.9) and third assessments (T3 = 14.7). This trend indicates a substantial and sustained improvement in depressive symptoms over time.

Similarly, the total score on the SHAPS showed a steady decline from 6.3 at baseline to values ranging from 2.2 to 2.8 in the later assessments, which reflects a reduction in anhedonia and increased pleasure responsiveness.

Lastly, adherence to the Mediterranean diet improves gradually over the study period. The Total Diet Score rises from 6.6 at T0 to 7.2 at T3, indicating enhanced dietary quality and greater adherence to Mediterranean nutritional principles.

Overall, these results suggest clinically meaningful improvements in both psychological health and health-related lifestyle behaviors throughout the follow-up period.

At baseline, a statistically significant negative correlation was observed between adherence to the Mediterranean diet and both depressive (MADRS; $p = 0.013$) and anhedonic (SHAPS; $p = 0.0034$) symptoms, indicating that lower adherence was associated with greater clinical severity. However, these associations were not maintained at subsequent timepoints, as no significant correlations emerged between Mediterranean diet scores and either MADRS or SHAPS at two or four months (all $p > 0.05$). Although binary measures of adherence suggested modest positive trends (e.g., MADRS–adherence at T2 = 0.615), these did not reach statistical significance. Therefore, adherence to the Mediterranean diet did not show a direct linear association with depressive or anhedonic severity, though it may exert synergistic or long-term effects, particularly in combination with physical activity.

Conversely, physical activity was the behavioural factor most consistently associated with clinical improvement. At two months (T1), MADRS scores were inversely correlated with total IPAQ ($\rho = –0.340$; $p = 0.002$), and the same pattern was observed for SHAPS ($\rho = –0.323$; $p = 0.003$), indicating that higher activity levels were linked to lower depressive and anhedonic symptoms. By four months (T2), the correlations remained negative but were no longer statistically significant ($\rho = –0.284$; $p = 0.08$). When applying established cut-offs (<700 MET-min/week = inactive; 700–2519 = sufficiently active; ≥2520 = highly active), participants who were at least “sufficiently active” consistently exhibited lower MADRS scores.

At baseline, 70% of the evaluated patients presented some degree of insulin resistance. Cross-sectional analyses revealed that insulin-resistant individuals had significantly higher anhedonia scores compared to those without insulin resistance ($p < 0.001$), while no significant differences were observed in adherence to the Mediterranean diet ($p = 0.1915$). However, these associations were not sustained longitudinally. In the follow-up analyses, no significant differences in anhedonia were found between insulin-resistant and non–insulin-resistant participants across timepoints. Non–insulin-resistant individuals continued to show a trend toward higher physical activity levels and slightly better dietary adherence, particularly at 8 and 16 weeks (borderline significance, $p \approx 0.1$).

Overall, these findings suggest that while insulin resistance was initially associated with greater anhedonic burden, its impact on affective and behavioural variables diminished over time, possibly reflecting the moderating influence of treatment and lifestyle adjustments.

Collectively, these findings emphasize the interconnection between metabolic health, behavioural factors, and affective symptoms in depression, supporting the role of physical activity and metabolic regulation as potential targets for integrated therapeutic strategies.

5.4 Predictors of early and sustained clinical response

At baseline (T0), most participants presented with at least moderate depressive symptoms, with a median MADRS score of 28.7, above the established threshold (≥20) for moderate depression, and approximately 85% falling within this category. Physical activity levels were highly variable, with a median total IPAQ score of 918 MET-min/week, placing the cohort overall within the "sufficiently active" range (700–2519 MET-min/week) but with a wide inter-individual distribution. The clinical cut-offs used to define treatment outcomes were a ≥50% reduction in MADRS scores for clinical response and a MADRS score <7 for remission.

At the two-month follow-up (T1), responders, defined by a ≥50% reduction in MADRS, showed significantly higher levels of physical activity compared to non-responders (median IPAQ = 1,619 vs 396 MET-min/week; $p = 0.011$). According to established cut-offs, responders were predominantly "sufficiently active" (700–2519 MET-min/week), while non-responders were mostly "inactive" (<700 MET-min/week). Consistent with these findings, a larger proportion of responders fell within the higher IPAQ categories (69% vs 36%; $p = 0.007$). Responders also reported substantially lower rates of previous psychiatric rehabilitation (14% vs 49%; $p = 0.001$) and were more likely to experience financial instability (60% vs 82% with stable finances; $p = 0.036$). A non-significant trend toward lower SHAPS scores (i.e., less anhedonia) was observed among responders. No significant differences were found in diet adherence, smoking status, or medical comorbidities. These findings indicate that being at least "sufficiently active" at baseline was associated with a higher likelihood of achieving a clinically meaningful antidepressant response at two months, independent of other sociodemographic or metabolic variables.

At the four-month follow-up (T2), the profile of sustained responders showed both overlapping and distinct characteristics. Although differences in physical activity were no longer statistically significant (median IPAQ = 1,506 vs 609 MET-min/week), responders continued to display higher activity levels on average, consistent with the early response pattern. Sustained responders were characterized by a later age at illness onset (22 vs 19 years; $p = 0.041$), higher body weight and BMI (74 kg vs 61 kg; BMI = 23.8 vs 21.8; $p = 0.039$–0.045), fewer concomitant psychotropic medications

(41% vs 71%; p = 0.012), and a lower frequency of psychiatric rehabilitation (18% vs 41%; p = 0.048). As at T1, financial instability remained more common among responders (61% vs 86%; p = 0.013).

Overall, early clinical response (at 2 months-T1) was associated primarily with greater physical activity, absence of prior psychiatric rehabilitation, and less financial stability, while sustained response (at 4 months-T2) was predicted by later illness onset, higher BMI, fewer concomitant treatments, lower psychiatric rehabilitation history, and similarly less stable financial conditions. Across both timepoints, participants with less chronic illness trajectories (i.e., fewer prior rehabilitative interventions) and greater behavioural activation (higher IPAQ scores) showed the highest probability of achieving a ≥50% MADRS reduction.

Although remission (MADRS <7) was not directly quantified in these analyses, future studies should assess remission rates and employ ROC-based approaches to determine optimal IPAQ thresholds predictive of both response and remission. Together, these findings suggest that lifestyle and clinical factors, particularly physical activity and illness chronicity, may serve as early indicators of antidepressant response, whereas sustained outcomes at four months appear further influenced by metabolic and sociodemographic variables.

With regard to metabolic markers, HOMA-IR values at baseline indicated a high prevalence of insulin resistance across the cohort (~80%). Although responders at both T1 and T2 consistently showed lower median HOMA-IR values compared to non-responders (1.48 vs 2.30 at T1; 1.64 vs 3.02 at T2), these differences did not reach statistical significance. This suggests that while better insulin sensitivity may be weakly associated with a greater likelihood of clinical response, insulin resistance per se did not emerge as a robust discriminator of antidepressant treatment outcomes within this sample.

5.5 Microbiome analysis

At both baseline and follow-up, significant associations emerged between physical activity, gut microbiota diversity, and depressive symptomatology. At baseline, participants with higher levels of physical activity displayed increased microbial diversity (higher Shannon index, p=0.007) and greater evenness (Pielou's index, p=0.022), indicating a more balanced microbial ecosystem. Conversely, individuals with insulin resistance showed reduced microbial diversity (Simpson's index, p = 0.036), suggestive of a community dominated by a limited number of taxa. Spearman's correlations further highlighted that higher microbial evenness (Pielou's index, ρ=–0.289, p=0.008; Simpson's index, ρ=–0.229, p=0.038) was inversely related to depressive severity (MADRS scores), while physical activity was positively associated with microbial diversity (Shannon index, ρ=0.260, p=0.036). At six

months, these findings were corroborated: participants with higher physical activity continued to exhibit greater richness (Species richness, Chao1, and ACE indices; all $p<0.05$), with robust positive correlations between physical activity and diversity indices ($\rho\approx0.45$, $p\approx0.01$). Although correlations between microbial diversity and depressive symptoms at follow-up did not reach statistical significance, a consistent negative trend was observed ($\rho\approx-0.25$ to -0.31). Collectively, these results suggest that physical activity exerts a protective role by promoting both higher biodiversity and more balanced microbial communities, while reduced microbial evenness is associated with greater depressive symptom severity. Figure number 4.

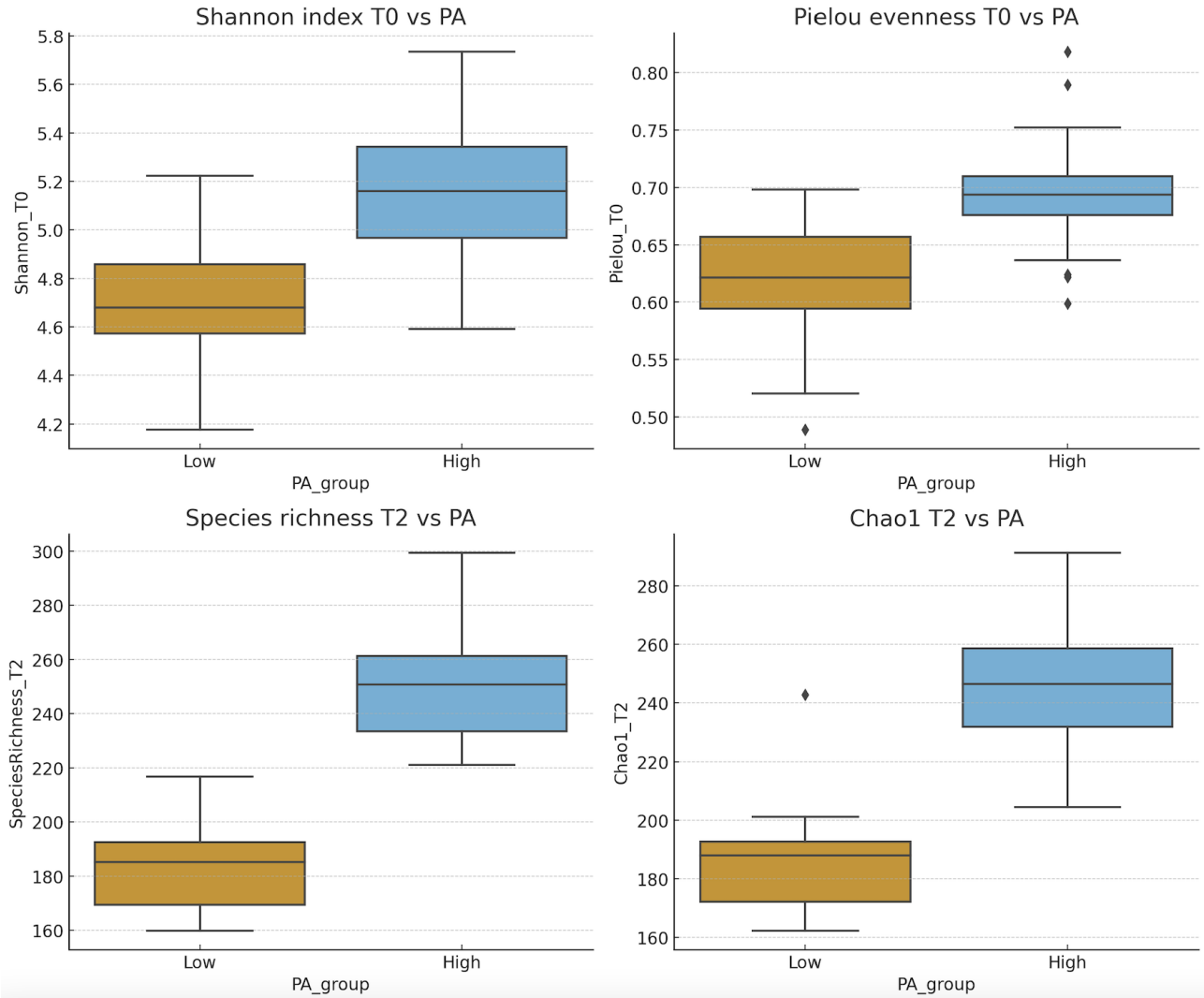

Figure 4

Interestingly, insulin resistance (as indexed by higher HOMA-IR values) was positively correlated with the ACE index, which estimates species richness ($\rho=0.402$, $p=0.043$). At first sight, this may

appear counterintuitive, since higher microbial diversity is typically associated with metabolic health. However, the ACE index is particularly sensitive to the presence of rare taxa, and therefore elevated values may not necessarily reflect a more functional ecosystem. Rather, this pattern may indicate a state of dysbiosis characterized by the proliferation of low-abundance or opportunistic species, coupled with the loss of balance in community structure, as also suggested by the reduced evenness observed in insulin-resistant individuals. Thus, the association between insulin resistance and higher richness may represent a shift toward a more heterogeneous yet functionally imbalanced microbiota, rather than a marker of improved microbial health.

5.6 Interaction between physical activity, Mediterranean diet, metabolic status, and gut microbiota indices

To further explore the combined influence of lifestyle and metabolic variables on gut microbial diversity, additional analyses were performed considering both physical activity levels (IPAQ) and adherence to the Mediterranean diet (PREDIMED). Participants were stratified into four groups according to activity (inactive vs. active/sufficiently active) and dietary adherence (low vs. high; PREDIMED ≥9), resulting in the following categories: inactive/low adherence (0–0), active/low adherence (1–0), inactive/high adherence (0–1), and active/high adherence (1–1).

Significant between-group differences emerged across multiple alpha-diversity indices. For the Shannon index, overall microbial diversity differed significantly between groups (p = 0.015), with the highest median value observed among participants who were physically active but not adherent to the Mediterranean diet (median = 5.42), followed by the active/adherent group (median = 5.31). The lowest diversity was found in inactive individuals with low dietary adherence (median = 4.86). Similarly, the Simpson index showed significant group differences (p = 0.046), again indicating higher diversity and evenness among physically active participants. Figure number 5.

Analysis of the Pielou evenness index confirmed this pattern (p = 0.010), with the most balanced microbial communities found among physically active participants, irrespective of diet adherence, and lower evenness in inactive individuals. Figure number 6. Collectively, these results indicate that physical activity was more strongly associated with microbial richness and balance than dietary adherence alone, although the combination of both behaviours was associated with the most stable microbial profiles. When comparing participants with and without insulin resistance, those without metabolic impairment exhibited higher microbial diversity as measured by the Simpson index (median = 0.70 vs. 0.62; p = 0.040), suggesting a more homogeneous and resilient microbial community structure among metabolically healthy individuals. Figure 7.

These findings collectively support the link between behavioural and metabolic factors and the diversity of the gut microbial ecosystem, highlighting the positive associations of physical activity, and, to a lesser extent, dietary quality, with microbial diversity and evenness at baseline.

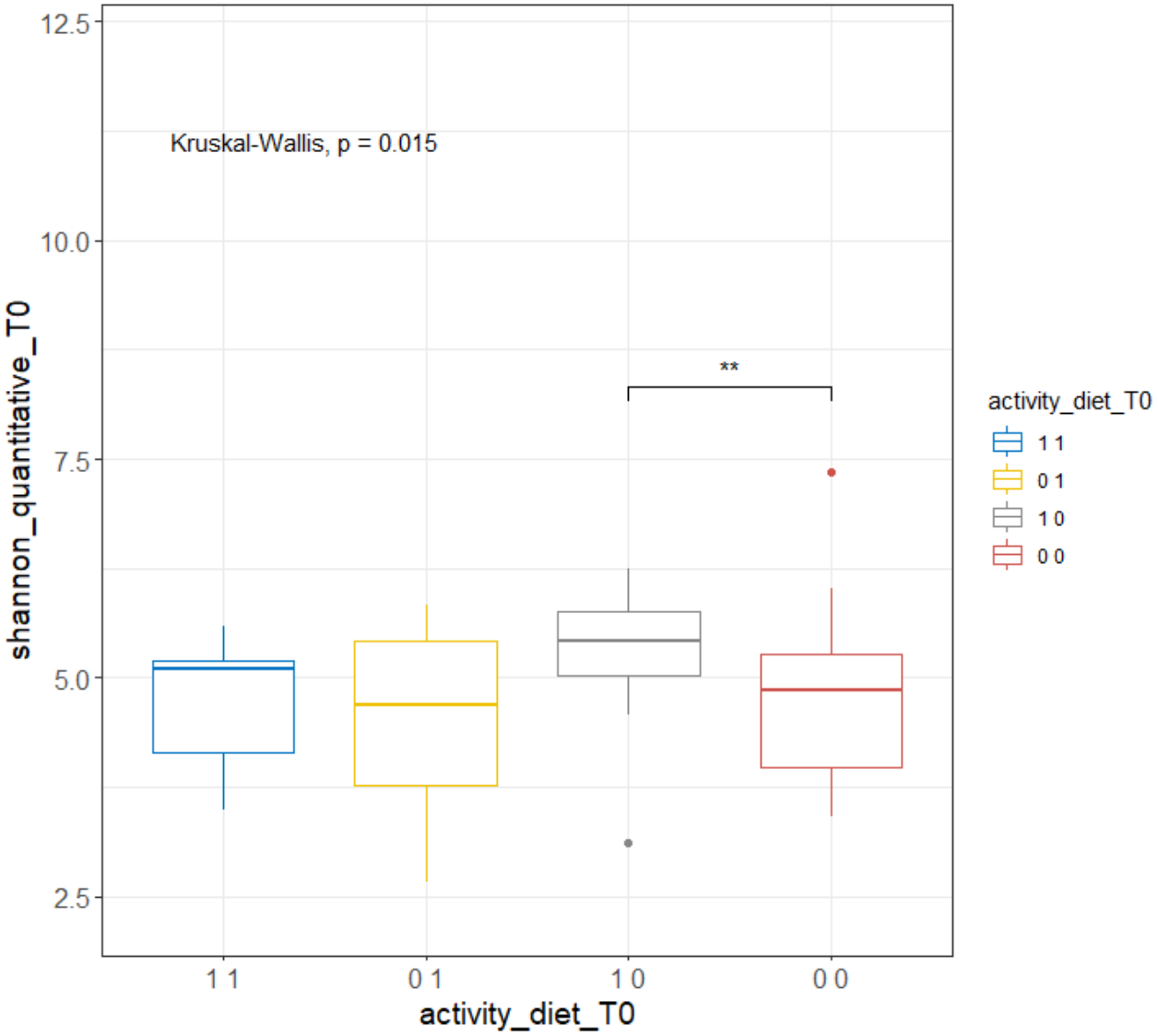

Figure 5

Combined interpretation: physical activity remains the dominant variable, but the Mediterranean diet amplifies the effects on microbial balance. It is therefore the combination of active behaviour and healthy eating that ensures maximum evenness.

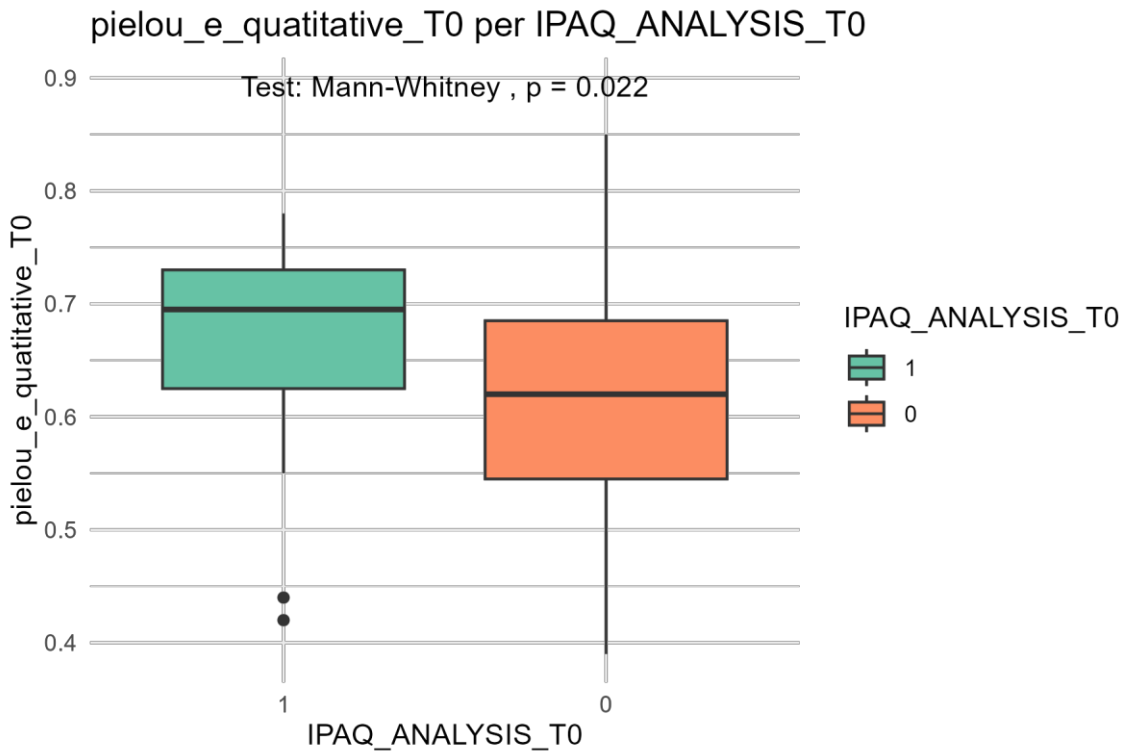

Figure 6

Consistent with the idea that physical activity not only increases diversity, but also the stability and balance of microbial composition.

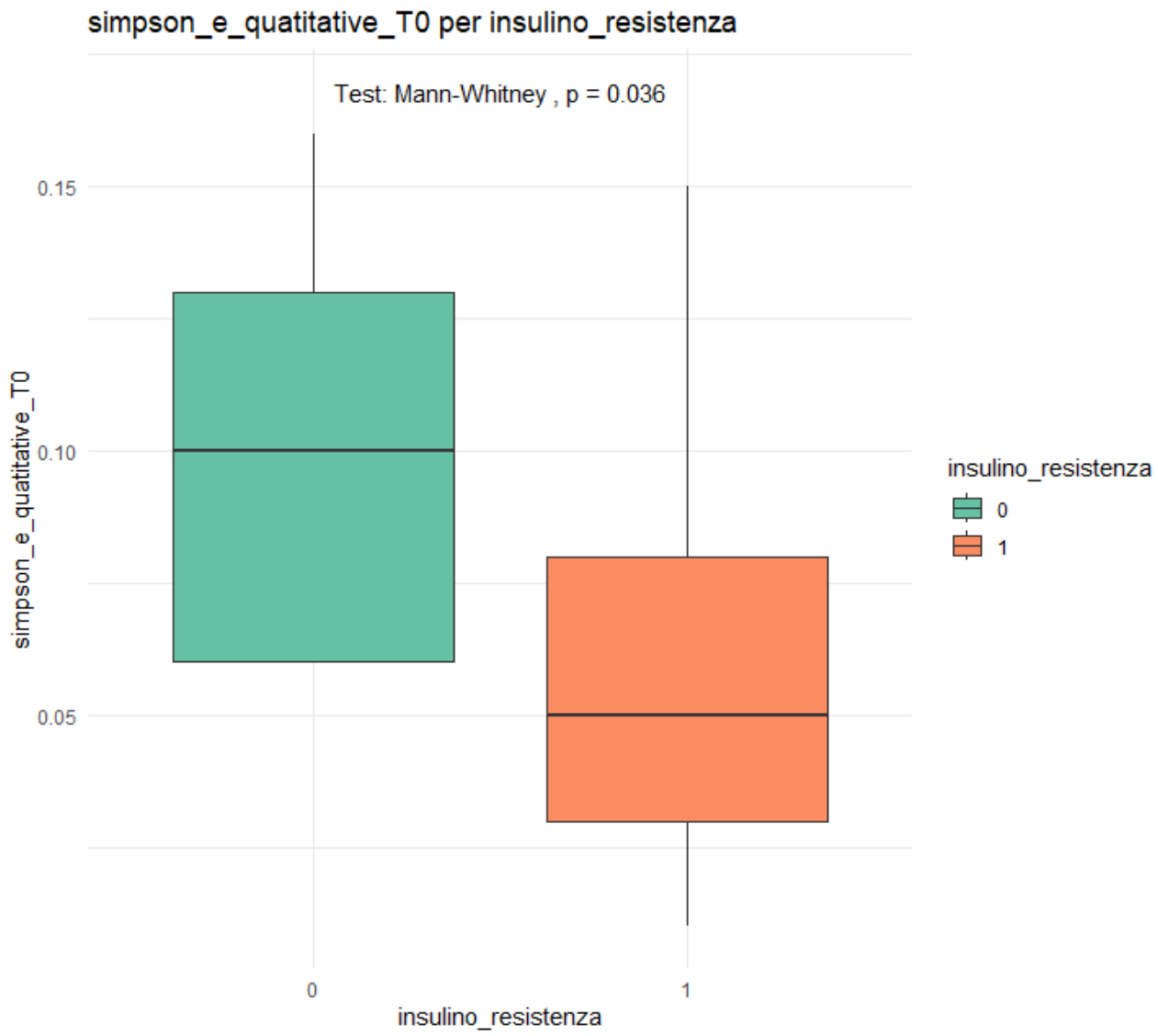

Figure 7

This suggests that insulin resistance is associated with a less balanced microbiota, consistent with a state of functional dysbiosis and metabolic vulnerability.

6. DISCUSSIONS

This thesis examined how lifestyle habits, metabolic functioning, inflammation, and depressive symptoms interact in individuals with Major Depressive Disorder, with particular attention to anhedonia. By combining cross-sectional and longitudinal analyses, it offers a multidimensional view of how behavioral and biological factors jointly influence clinical trajectories in depression.

The cross-sectional findings provided an initial overview of how adherence to the Mediterranean diet, physical activity, and insulin resistance relate to depressive and anhedonic symptoms. The longitudinal analyses then explored how these same factors, along with gut microbiota diversity, predicted symptom improvement and treatment response over time. Altogether, these results support the increasingly accepted view that depression is a systemic condition, where behavioral, metabolic, and microbiological factors interact within a broader biopsychosocial framework.

Clinical and Sociodemographic Profile

At baseline, the sample consisted mostly of young women with moderate depressive symptoms, moderate anhedonia, and a high rate of metabolic abnormalities, particularly insulin resistance, found in around 80% of participants. The clinical picture, characterized by early-onset depression, recurrent episodes, and frequent psychiatric rehabilitation, suggests significant chronicity and psychosocial burden. Interestingly, while insulin resistance was widespread, systemic inflammation, as measured by CRP, was relatively low. This pattern aligns with previous studies suggesting that metabolic disruption may precede overt inflammatory activation [147, 148]. It may also reflect a distinct vulnerability profile relevant to younger adults with mood disorders.

Physical Activity and Depressive Symptoms

At baseline, lower levels of physical activity were linked to more severe anhedonia. Those with both low physical activity and poor dietary habits showed the greatest burden of anhedonic symptoms. Among behavioral factors, physical activity stood out as the strongest predictor of symptom improvement. Higher IPAQ scores were associated with lower MADRS and SHAPS scores, particularly during the first two months, supporting the antidepressant and pro-hedonic effects of exercise. These findings are in line with large-scale meta-analyses showing that moderate-to-vigorous physical activity yields antidepressant effects comparable to medication, especially in early treatment phases [149, 150]. Mechanistically, exercise is known to enhance neuroplasticity, boost BDNF levels, reduce inflammation, and regulate HPA axis activity, all processes implicated in both depression and anhedonia [151-153].

From a clinical perspective, these results highlight the importance of early behavioral activation. Participants who met the "sufficient activity" threshold (at least 700 MET-min/week) were

significantly more likely to achieve a 50% reduction in depressive symptoms within two months. Physical activity may therefore serve as a marker of treatment responsiveness, reflecting both neurobiological resilience and behavioral adaptability.

Metabolic Factors and Treatment Response

The high prevalence of insulin resistance reinforces its strong association with depressive disorders [148, 154]. Cross-sectionally, insulin resistance correlated with higher anhedonia, even after accounting for dietary quality. Individuals with both insulin resistance and poor diet reported the most severe anhedonic symptoms. This supports emerging models linking disrupted insulin signaling to impaired reward processing [155, 156]. While group differences in HOMA-IR between responders and non-responders were not statistically significant, responders consistently showed lower median values at both follow-up points. This suggests that while insulin sensitivity may support treatment response, insulin resistance alone is unlikely to predict it. Rather, it may act as a modifiable vulnerability factor influencing neuroplasticity and motivation. Accumulating evidence indicates that insulin resistance is associated with blunted antidepressant response, cognitive impairment, and residual anhedonia [157-159]. The absence of a strong effect in the present study may also reflect limited statistical power due to the small subgroup of non–insulin-resistant participants. Interestingly, patients who maintained response at four months had higher BMI and body weight. Though counterintuitive, this may reflect a metabolic paradox, where mild to moderate adiposity confers resilience, possibly via nutritional or endocrine pathways. Alternatively, higher BMI may indicate lower illness chronicity or better nutritional status, in line with evidence linking underweight status to poorer antidepressant outcomes [160]. These observations suggest that both metabolic overload and malnutrition can negatively impact recovery.

Sociodemographic and Clinical Correlates of Response

Participants with fewer past psychiatric rehabilitation interventions and less financial stability were more likely to improve. While financial hardship may serve as a stressor, it could also reflect less chronicity or greater readiness for change. In contrast, prior rehabilitation may signal a more entrenched or treatment-resistant clinical course. These findings resonate with the concept of functional reserve: patients with less chronic, less disengaged profiles may retain greater neurobiological flexibility and behavioral responsiveness [161, 162].

Sustained responders were also taking fewer psychotropic medications, suggesting lower treatment complexity and possibly fewer pharmacological interferences with neurobiological mechanisms of recovery [163, 164]. A later age of onset was another feature linked to better outcomes, echoing studies showing that early-onset depression is associated with chronicity, recurrence, and higher metabolic risk [165, 166].

Mediterranean Diet and Lifestyle Interactions

The Mediterranean diet showed a multifaceted role in mood regulation. At baseline, low adherence was associated with greater depressive severity and more pronounced anhedonia. Regression models confirmed that poor diet independently predicted moderate-to-severe depression. These results support evidence linking Mediterranean-style diets to better mental health, likely through anti-inflammatory and neuroprotective pathways [167, 168]. Moreover, studies have demonstrated that moderate-to-high adherence to the Mediterranean diet is associated with lower risk of depression and improved treatment outcomes, particularly when combined with regular physical activity [169].

Longitudinally, however, dietary adherence did not show a strong linear association with symptom improvement. While trends were favorable, they did not reach statistical significance. This may be due to limitations in how adherence was measured, using a dichotomous cut-off in the PREDIMED score likely reduced sensitivity to subtle changes. Re-analyses using continuous or tiered scoring may yield clearer insights. It's also plausible that diet influences mood through indirect mechanisms, like reducing inflammation or improving insulin sensitivity, rather than by producing rapid symptomatic relief.

In this context, the cross-sectional findings may reflect stable, trait-level associations, whereas the longitudinal data point to the limited short-term impact of diet alone. Nonetheless, when combined with physical activity, dietary adherence appears to have additive benefits, reinforcing the idea of lifestyle synergy in mood and metabolic regulation.

Inflammation and Behavioral-Metabolic Interactions

CRP levels were higher in participants with both poor diet and low physical activity, suggesting additive effects of unhealthy behaviors on systemic inflammation. Although CRP was not significantly linked to symptom severity, there was a trend toward lower CRP among more active individuals. This supports prior findings that exercise reduces inflammation mainly by improving insulin sensitivity and altering cytokine profiles, rather than through CRP reduction alone [170, 171]. In this relatively young cohort, the overall low inflammation levels may have limited statistical power.

Overall, the behavioral and biological data support a bidirectional model in which depressive symptoms reinforce behavioral disengagement and metabolic dysfunction, which in turn sustain inflammation and HPA axis dysregulation. Targeting both lifestyle and metabolic factors may help interrupt this cycle and promote clinical recovery.

Gut Microbiota, Physical Activity, and Metabolic Health

Microbiota analysis revealed that participants who were more physically active had greater microbial diversity and evenness, indicating a healthier and more stable gut environment. In contrast, those with insulin resistance showed reduced evenness but higher species richness, often interpreted as a marker

of dysbiosis driven by low-abundance, potentially pathogenic taxa. This combination, sometimes referred to as "false richness," has been associated with poorer metabolic outcomes [172-176].

Microbial evenness also correlated inversely with depressive severity, suggesting that imbalance in the gut ecosystem may play a role in depressive pathophysiology [177, 178]. These findings align with the literature showing that physical activity supports microbial health, likely through anti-inflammatory and metabolic pathways [179, 180]. The relationship between microbiota and depression may involve altered production of short-chain fatty acids, immune signaling, and tryptophan metabolism [181, 182].

Additional analyses showed that even in the absence of ideal diet adherence, physically active individuals maintained healthier microbiota profiles. While dietary adherence alone had limited impact on diversity, its combination with physical activity improved microbial evenness. This supports evidence suggesting that exercise may exert a stronger influence than diet on microbiome composition [180]. Notably, the link between greater microbial evenness and lower depressive severity lends support to the idea that a balanced gut microbiota may play a role in mood regulation—possibly through its influence on immune, metabolic, and neuroendocrine pathways. In a similar vein, the finding that insulin resistance is associated with reduced microbial evenness suggests that metabolic disturbances could disrupt gut homeostasis, fueling low-grade inflammation and increasing vulnerability to depression [183].

Taken together, these findings point toward a coherent model: physical activity promotes both metabolic and microbial resilience, which in turn support better mood outcomes. Clinically, these insights suggest that structured behavioral interventions, especially those promoting regular exercise, may be effective adjuncts to pharmacotherapy in depression, particularly in patients with metabolic vulnerability.

Clinical Implications and Future Directions

From a clinical perspective, several key implications emerge. First, physical activity appears not only as an effective adjunctive treatment but also as a predictor of antidepressant response, emphasizing the importance of assessing and promoting movement in routine care. Second, the high prevalence of insulin resistance, even in a relatively young and non-obese sample, suggests that metabolic screening should become standard in psychiatric assessments. Third, the connections observed between microbiota composition, metabolic health, and depressive symptoms support a multisystemic understanding of depression, highlighting the potential of interventions targeting gut-brain-metabolism pathways to improve both psychological and physical outcomes.

Future research should aim to replicate these findings in larger samples, using longitudinal designs capable of examining causal and mediating mechanisms linking metabolic, behavioral, and microbial

variables. More nuanced assessments of lifestyle, such as continuous or categorical modeling of diet adherence, could enhance sensitivity to subtle interactions between diet, exercise, and metabolic function. Integrating multi-omics data, inflammatory biomarkers, and neuroimaging will be critical to clarify the pathways underlying differential treatment responses. These efforts align with the goals of precision psychiatry, where behavioral, biological, and social factors are combined to guide individualized care in mood disorders.

7. LIMITATIONS

Several limitations should be acknowledged. The cross-sectional phase cannot support causal conclusions, and the longitudinal analysis was constrained by relatively short follow-up and attrition. Self-report measures of diet and physical activity may have introduced recall bias. Some null findings, such as the non-significant associations with diet adherence and CRP, may reflect limited statistical power or restricted variability in the sample.

Future studies should adopt continuous or multi-level models of diet adherence, explore mediation pathways linking insulin resistance, inflammation, and microbiota, and extend follow-up to capture long-term treatment effects. Incorporating omics technologies (e.g., metabolomics, transcriptomics, microbiomics) alongside neuroimaging and behavioral assessments will help clarify the interplay between lifestyle, metabolism, and affective symptoms.

8. CONCLUSIONS

This research provides a comprehensive view of how behavioral, metabolic, and microbiological factors interact to shape depressive symptoms and treatment outcomes. Across multiple analyses, a consistent pattern emerged in which physical activity, metabolic health, and gut microbiota diversity were closely linked with improvements in mood and anhedonia. At baseline, participants were mostly young adults with significant metabolic vulnerability, marked by high rates of insulin resistance, low engagement in healthy behaviors, and moderate-to-severe depressive symptoms. These characteristics reflect the broader recognition of depression as a systemic disorder with interconnected biological and psychological roots.

Physical activity stood out as the most consistent behavioral predictor of clinical response and microbial balance. Individuals who were physically active at baseline experienced faster and more substantial improvements in both depressive and anhedonic symptoms, particularly in the early treatment phase. Microbiota analyses showed that greater physical activity was associated with higher

microbial diversity and evenness, suggesting a more resilient gut ecosystem. Conversely, insulin resistance was linked to reduced microbial evenness and greater anhedonia, though these associations weakened over time, possibly indicating that lifestyle changes or treatment effects can partially reverse these patterns. While the Mediterranean diet was initially associated with reduced depressive and inflammatory markers, these effects did not persist longitudinally, underscoring the complex and dynamic nature of diet-mood relationships.

Together, these results reinforce the value of approaching depression within a multidimensional biopsychosocial–metabolic framework. The convergence of behavioral, clinical, and biological data points to the potential of interventions that promote physical activity, metabolic balance, and gut health. From a translational standpoint, integrating lifestyle and metabolic assessments into routine psychiatric care could support more personalized and proactive treatment strategies. Future studies should build on these insights using mechanistic and longitudinal approaches, such as multi-omics and neuroimaging, to clarify the causal links between lifestyle, metabolism, microbiota, and mood regulation. Ultimately, this integrative approach may help shift the treatment of depression toward a precision psychiatry model, where behavioral and metabolic targets complement pharmacotherapy to optimize outcomes and promote long-term recovery.

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