Universitat de Girona
Facultat de Medicina

FINAL DEGREE PROJECT

THE BACTEROIDETES/FIRMICUTES RATIO: A RELEVANT BIOMARKER OF GUT DYSBIOSIS IN MAJOR DEPRESSION DISORDER?

A pilot cohort study

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1. ABSTRACT

BACKGROUND: Major Depressive Disorder (MDD) is one of the most prevalent conditions in the psychiatry and primary care services. It is considered the second leading cause of disability worldwide and is one of the most significant risk factors for suicide. Despite numerous treatments on the market, they are effective in less than half of the patients, and the high prevalence of this disorder remains unchanged. Many efforts have been made to understand this disorder; nevertheless, the exact pathophysiology remains unknown. In the last decade, the inflammatory theory has emerged, suggesting an intimate relationship between the alteration of gut microbiota and the development of depressive symptoms. Current literature reveals inconsistent results and indicates a long path for further research.

OBJECTIVE: The aim of the study is to determine if young people with and increased *Bacteroidetes/Firmicutes* ratio in gut microbiota are more susceptible to develop a MDD over a five-year period compared to those who do not have an increased ratio. Secondary objectives include assessing this susceptibility in relation to bacterial diversity and the observed differences in gut microbiota between depressive and non-depressive participants.

STUDY DESIGN: The study is designed as a multicenter observational, analytic, prospective cohort study that will be carried out in the four basic health areas of Girona with a follow-up of five years.

PARTICIPANTS AND METHODS: The study population will be formed by young adults between 18 and 30 years that meet the inclusion and none of the exclusion criteria. A total of 1452 participants, 363 subjects in the exposed group and 1089 in the non-exposed group, will be probabilistically and randomly selected from the reference population, in proportion to each basic health area. To achieve the outlined objectives, their microbiota composition, relative abundance, and diversity will be analyzed in faecal samples using 16S rRNA gene sequencing.

KEY WORDS: Major Depression Disorder (MDD), ratio *Bacteroidetes/Firmicutes*, gut microbiota.

2. ABBREVIATION AND ACRONYMS

ACTH	Adrenocorticotropic Hormone
BHA	Basic Health Area
CFS	Cerebrospinal fluid
CNS	Central Nervous System
CRH	Corticotropin Releasing Hormone
CT	Computed Tomography
DSM-5-TR	Diagnostic and Statistical Manual of Mental Disorders 5th edition Text Revision
ECT	Electroconvulsive Therapy
HPA	Hypothalamic-Pituitary-Adrenal
Hs-CRP	High sensitivity C-Reactive Protein
IdibGi	Institut d'investigació biomédica de Girona
IL-1	Interleukine-1
IL-6	Interleukine-6
LPS	Lipopolysaccharides
MDD	Major Depression Disorder
MGB	Microbiota-Gut-Brain
MRI	Magnetic Resonance Imaging
n-3	Omega-3
n-6	Omega-6
PET	Positron Emission Tomography
PHQ-9	Patient Health Questionnaire-9
PUFAs	Polyunsaturated Fatty Acids
SPECT	Single Photon Emission Computed Tomography
SSRIs	Selective Serotonin Reuptake Inhibitors

3. INTRODUCTION

3.1. MAJOR DEPRESSION DISORDER

3.1.1. Preface

'*Depression is a disorder characterized by a persistent decline in mood, negative thoughts, and, in some cases, somatic symptoms'* (1). According to World Health Organization (WHO) data depression is very different from usual mood changes. It represents a high risk to life and an alteration in the life quality, affecting many areas such as work and school (2). It is a challenge for health systems and for society since it is one of the main risk factors for suicide, being four times higher in people with depression compared to the general population (3). Moreover, it is considered the second cause of disability in the world (1).

Major depressive disorder (MDD) is the most classic type of mood disorders, but in addition to MDD, there is a wide range of subtypes: disruptive mood dysregulation disorder, persistent depressive disorder, substance/medication-induced depressive disorder, premenstrual dysphoric disorder, depressive disorder due to another medical condition, other specified depressive disorder, and unspecified depressive disorder. These subtypes share symptoms, but differ in duration, temporal presentation and presumed etiology (4).

Despite numerous psychotherapeutic treatments and drugs, the prevalence of depressive disorders remains unchanged. Furthermore, there is a great variety in the response to these treatments and they are effective in less than half of diagnosed patients (5).

3.1.2. Epidemiology

MDD is one of the most treated diseases in psychiatry and primary health care (1). In terms of prevalence, 3.8% of the world's population experiences depression, 5% of adults (4% men and 6% women) and 5.7% of adults over their 60s. These data on a global scale translate into about 280 million people suffering from depression (2).

Women are more susceptible to depression than men (2,4,6), which is largely explained by the theory of biological and hormonal differences, but other relevant factors have also been found to be associated, such as greater exposure of women to sexual abuse, domestic violence, lower-status jobs and the caregiver role (4,6,7). It has been observed that these figures rise to 11.9% in the perinatal period (8). As for the onset, it typically occurs around mid-20s (9). The recent pandemic has added an increased depressive burden according to the 2020 Global Burden Disease data from 204 countries, which indicates that COVID-19 pandemic led to a 27.67% increase in MDD cases (10).

In Spain, according to the latest European Health Survey in 2020, of a total of 39,974,000 respondents, 976,700 had symptoms of MDD (2.46%), with an increasing percentage in correlation with age. Of the women surveyed, 3.42% presented symptoms of MDD while 1.45% of men (11,12). However, although in Spain and global scale the prevalence increases with age, in Western a lower prevalence was found with age (13).

3.1.3. Pathophysiology

Numerous hypotheses are postulated about the pathophysiology of depression. These will be described below.

- **MONOAMINERGIC HYPOTHESIS**

The monoamine theory defends the existence of a defect in the functioning of the monoamine neurotransmission of serotonin, noradrenaline and in a third-place dopamine in certain areas of the brain. This hypothesis was largely based on the use of classical antidepressants since their mechanism of action consisted of the inhibition of monoamioxidases or the reuptake of monoamines (14).

The serotonergic hypothesis is supported for several reasons. Firstly, many of the antidepressants used produce an increase in serotonergic neurotransmission, such as SSRIs (Selective Serotonin Reuptake Inhibitors). Furthermore, a decrease in 5HIAA (5-hydroxy-indoleacetic acid), the main metabolite of serotonin, has been found in the cerebrospinal fluid (CFS) and less uptake of serotonin by the platelets of patients with

suicidal behavior. A decrease in the plasma concentration of tryptophan, an amino acid precursor of serotonin, has also been detected.

The noradrenergic hypothesis is supported by the fact that some antidepressants are based exclusively on norepinephrine, such as desipramine and reboxetine. Moreover, MHPH (methoxy-hydroxy-phenylglycol) levels in the urine are low in depressive episodes.

Other neurotransmission systems may be involved, such as dopamine. Low levels of HVA (homovalinic acid), the main metabolite of dopamine, have been found in depressed patients (15). Additionally, treatments such as MAOIs or bupropion increase dopamine levels. Likewise, dopamine plays a very important role in motivation and the perception of pleasure, which are one of the most important signs of depression (14).

The alteration of central cholinergic activity is also postulated as a possible theory, since depressive syndromes produced by drugs that increase cholinergic activity have been described (15).

- **NEUROTROPHIC HYPOTHESIS AND NEURONAL PLASTICITY**

The neurotrophic hypothesis is based on the idea that sustained stress is capable of producing modifications in the central nervous system (CNS), especially in the limbic system (15). Chronic stress leads to an increase in the production of glutamate and cortisol, and a decrease in BDNF (brain-derived neurotrophic factor), a neuroprotective factor. These changes result in injuries that manifest primarily in the hippocampus, causing atrophy. Neurons in this area lose the ability to detect excess cortisol and do not provide negative feedback to the adrenal glands to stop cortisol production, leading to hypercortisolemia. All these factors contribute to a reduction in neuroplasticity and neurodegeneration, consequently reducing the volume of the hippocampus (14,15).

- **NEUROANATOMIC THEORY**

Based on the anatomical changes visualized in CT and MRI, consistent with dilation of the brain ventricles in patients with severe depression, especially when psychotic symptoms are present and in bipolar disorder. A decrease in the size of the frontal lobe and the caudate nucleus has also been observed, along with lesions in the subcortical white matter, particularly in bipolar disorder. Additionally, studies using SPECT and PET show reduced blood flow in the prefrontal cortex, basal ganglia, and thalamic nuclei, as well as an excess of activity in the amygdala (15).

- **NEUROENDOCRINE ALTERATIONS**

It posits that there are alterations in the hypothalamic-pituitary axis that are related to affective disorders. The adrenal axis in patients with depression is activated, as evidenced by elevated cortisol levels, although such elevation can be found in other pathologies. Hypothyroidism, and to a lesser extent hyperthyroidism, have also been associated. Additionally, in depressed patients, a decrease in growth hormone during sleep and a blunting in the response to clonidine administration have been described (15).

- **PSYCHOLOGICAL THEORIES**

Psychoanalytic Theory: According to Freud, melancholic depression is a consequence of a significant loss for which the patient feels responsible.

Behavioral and cognitive theories: These theories defend that the patient holds distorted beliefs about oneself and the surrounding environment, leading to the misinterpretation of situations and excessive attention to negative events. Beck's theory suggests the existence of a cognitive triad based on a negative conception of oneself, one's experiences, and a pessimistic view of the future. According to the learned helplessness theory, the repetition of negative experiences without the ability to defend oneself eventually leads to a passive reaction similar to feelings of worthlessness and hopelessness.

Evolutionary Theories: Psychologists supporting these theories consider that depression may be a final syndrome resulting from a loss of social rank, exposure to chronic stress, loss of an attachment bond, or anticipation of dangers (15).

- **INFLAMMATION HYPOTHESIS OF DEPRESSION**

It suggests that a low degree of chronic systemic inflammation is associated with MDD, although not all cases can be supported by this theory. Elevated inflammatory markers in blood and CFS have been implicated such as hs-CRP (high-sensitivity C-reactive protein), IL-1, IL-6, and TNF-alpha (16–18). High levels of hs-CRP have been associated with functional alterations developing symptoms of depression, such as a decrease in the corticostriatal reward pathway (19), increased basal ganglia glutamate (20), and elevated CRP in CSF (21), therefore, hs-CRP is a potential useful biomarker in peripheral blood for inflammatory depression (22). Other evidence that supports this theory is that the therapeutic administration of INF-alpha (Interferon-alpha), an inflammatory substance, has been associated with the induction of depression and its recurrence (23). The circuit is bidirectional, depression facilitates inflammatory responses while inflammation promotes depression, so joint treatment of both could improve recovery and the risk of recurrence (24).

This subtype of depression has been associated with resistance to treatment with SSRIs, obesity, medical comorbidities as well as specific symptoms such as drowsiness, fatigue, tiredness, reduced appetite, anxiety and lack of sleep (25).

The causes of chronic low-grade degradation are not completely known, but the most explored factors are medical illness such as autoimmune disorders and infections, genetic predisposition, physical inactivity, obesity, diet and stress in childhood and adulthood (24,26). These factors lead to chronic inflammation that causes monoaminergic depletion, glutamate excess, activation of the Kynurenine pathway and the neuroendocrine system, causing depressive symptoms such as anhedonia, anxiety and sleep disturbances among others (26).

There are upstream mechanisms that explain how inflammation is generated, such as intestinal dysbiosis and increased dietary omega-6/omega-3, and downstream mechanisms that explain how inflammation leads to depressive symptoms, such as the kynurenine pathway of tryptophan metabolism and dopaminergic neurotransmission (22).

Upstream mechanisms

Dysbiosis refers to an imbalance in the gut microbiota characterized by reduced microbial diversity and fewer beneficial microorganisms (27). Factors such as diet habits and physical activity can modify the composition of gut microbiota (28,29). Dysbiosis can lead to disruptions in intestinal permeability, known as 'leaky gut,' resulting in the translocation of bacteria from the intestinal barrier into the circulation (30,31). This triggers the activation of an immune response that contributes to systemic inflammation through the activation of Toll-like receptor 4 (TLR4) upon the binding of lipopolysaccharides (LPS). LPS are present in many gram-negative bacteria, and as they enter the bloodstream, they bind to LPS-binding protein (LBP), initiating an immune response that releases cytokines (32).

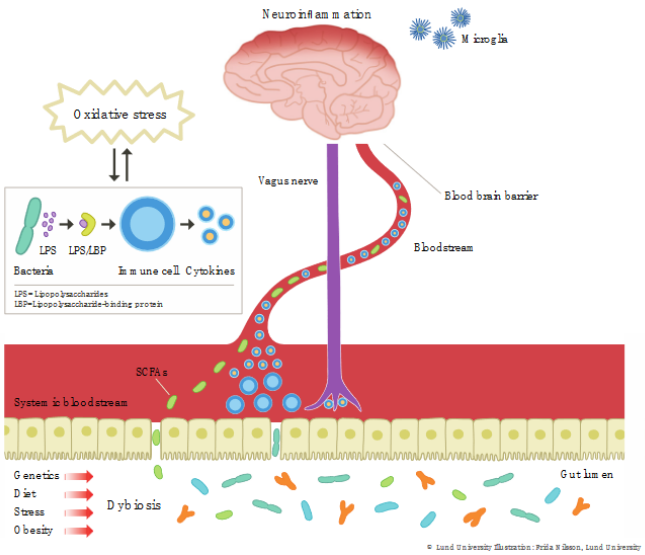

Figure 1. *Numerous factors such as diet, stress, sedentary lifestyle, and genetics predispose to an altered microbiota. This promotes a leaky gut that may lead to bacterial translocation of LPS from the lumen to*

the circulation, producing cytokines locally or through activation of the vagus nerve. This all leads to neuroinflammation and activation of microglia. Abbreviations: LPS (lipopolysaccharides), SCFAs (short-chain fatty acids), LBP (lipopolysaccharide-binding protein, BBB (blood-brain barrier). Extracted from (22).

Cytokines act directly by crossing the blood-brain barrier (BBB) or by stimulating the vagus nerve, contributing to neuroinflammation and microglial activation. On the other hand short-chain fatty acids (SCFAs) have an anti-inflammatory effect as they contribute to the epithelial barrier (22).

Increased Dietary Omega-6/Omega-3 Ratio. Omega-3 (n-3) and omega-6 (n-6) are polyunsaturated fatty acids (PUFAs) that can be obtained through diet. N-3 has an anti-inflammatory effect, while n-6 is proinflammatory. N-3 polyunsaturated fatty acids (n-3 PUFAs) encompass eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while among n-6 PUFAs, linoleic acid (LA), alpha-linolenic acid (ALA), and arachidonic acid (ARA) are the most recognized (33). According to a study, the intake of n-3 seemed to be related with an increase in *Lactobacillus*, while n-3 led to an increase in *Bifidobacterium* (34). Excessive intake of n-6 PUFAs and low amounts of n-3 PUFAs is commonly seen in western diets which may promote a systemic low-grade inflammation (29). For humans, the main source of n-3 is fish (35) while vegetable oils and animal-based are the major sources of n-6 (33).

Downstream mechanisms

The Kynurenine Pathway of Tryptophan Metabolism. Inflammatory cytokines produced by upstream mechanisms can modify serotonergic, glutamatergic, and dopaminergic systems through changes in the metabolism of tryptophan, which is a precursor of serotonin and is metabolized in the kyneurine pathway. This alteration is due to the activation of the enzyme indoleamine 2,3-dioxygenase (IDO) by cytokines, which converts tryptophan into kyneurine and reduces the formation of tryptophan into serotonin (22).

Dopaminergic Neurotransmission. It has been shown that the symptoms of depression, such as anhedonia, arise from the impact of cytokines on the mesolimbic

dopamine signaling system (36). This is explained because the oxidative stress produced by cytokines reduces BH4 (tetrahydrobiopterin), resulting in a lower capacity for dopamine synthesis (22).

3.1.4. Risk factors

Numerous risk factors are involved in MDD according to various sources. These are divided into personal, environmental, psychological, genetic, and sociodemographic factors. All of them are found in *Table 1*.

***Table 1**: Risk factors for MDD divided into personal, environmental, psychological, genetic, and sociodemographic areas.*

PERSONAL FACTORS	ENVIRON MENTAL FACTORS	PSYCHOLOGICAL FACTORS	GENETIC FACTORS	SOCIO-DEMOGRAPHIC FACTORS
Female (4,6,7)[1]	Major negative life changes (37)	High levels of negative affectivity (Neuroticism) (4)	First-degree and second-degree family members (3,4)[4]	Single, divorced or widowed. (3,13)
Adulthood (12,37)[2]	Trauma (37)	Automatic thoughts (3)	Polymorphisms in serotonin transporters(3)[5]	Country(13,38)[6]
Medical diseases: chronic or disabling medical conditions, morbid obesity (4) cancer, Parkinson, heart disease, hypothyroidism **(**37**)** insomnia, AIDs, respiratory diseases **(**38**)**, migraine (3), endocrine diseases: diabetes (3,4), Cushing's syndrome, Addison's disease and amenorrhea hyperprolactinemia (3)	Chronic stress (37)	Cognitive distortions (3)		Low income (4,11)[7]
Psychiatric disorders (anxiety, substance use, borderline personality, obsessive-compulsive disorder, feeding and eating…)(4)	Adverse childhood experiences ; sexual abuse (4)	Dysfunctional beliefs (3)		Limited formal education (4)
Perinatal period (4,8)		Ruminative thought (3)		Unemployment (11)
Western Diet based on high calories, saturated fats and low phytochemicals, vitamins and minerals (39,40)		Attentional biases (3)		Reduced role functioning (13)
Unhealthy lifestyle: smoking and alcohol (3)				Discrimination (4)
Medication (37)[3]				

[1] MDD is 50% higher in woman than men (4). [2] Depression can happen at any age, but it often begins in adulthood (37) [3] Some medication used for certain illness can cause side effects that contribute to depression (37). [4] First-degree family members have two-fourfold higher risk than general population. Second-degree family members are also related to a higher risk but not as important as first-degree (3).[5] The polymorphism in the gene that encodes the serotonin transporter could decrease the transport of the neurotransmitter (3). [6] Geographic location has been associated with depression. It has been found that the cities with the highest rates of depression are those with the lowest income. Moreover, some studies estimate that the differences could also be influenced by the fact that each country uses its own symptom rating interview and therefore the thresholds are different (13) [7] Having low income is associated with the risk of suffering from depression 2.5 times more than high income (11).

3.1.5. Clinical presentation

The main symptoms are outlined in the diagnostic *Table 2* of the DSM-V-TR (Diagnostic and Statistical Manual of Mental Disorders 5th edition Text Revision).

The clinical manifestations can be collected in four fundamental areas: alterations in affectivity, cognitive dysfunction, alterations in circadian rhythms and motor deficits.

Affective disturbances are observed, such as a decrease in mood, loss of interest or enjoyment, hopelessness, and dysphoria. Cognitive alterations manifest themselves as difficulties in attention, slowing of speech, slow thinking, poor motivation, alteration of executive functions, alterations in memory, apathy, rumination, and excessive guilt. Regarding circadian rhythms, the sleep pattern is altered, also, there is decreased libido, lack of energy, poor vital drive, changes in weight and alterations in appetite. Finally, psychomotor deficits manifest themselves as slow movement, restlessness or agitation (15).

Appetite disturbances can manifest as an increase or decrease in intake. In severe cases, there may be a significant alteration in weight. Regarding sleep, it may manifest as difficulty sleeping, either as middle insomnia (waking up during the night and having difficulty returning to sleep) or as terminal insomnia. Initial insomnia can also occur but

is less common. Despite most of the patients manifest some type of insomnia, there are others who may experience hypersomnia. Decreased energy and fatigue are associated with even the simplest everyday activities, such as showering or dressing, which are considered a significant effort. The feeling of guilt is an irrational and disproportionate emotion. Patients magnify their flaws and feel a great responsibility for day-to-day events (4).

3.1.6. Diagnosis

Diagnosis of MDD based on a single episode is possible, although the disorder is a recurrent one in most cases. For the diagnosis, all DSM-V-TR criteria must be present (*Table 2*)(4). The extended criteria table is found in *Annex 1.*

Table 2. *Diagnostic criteria for MDD according to DSM-V-TR. Extracted and adapted from (4)*

A	Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. 1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). 2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation). 3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month) or decrease or increase in appetite nearly every day. 4. Insomnia or hypersomnia nearly every day. 5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down). 6. Fatigue or loss of energy nearly every day. 7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick). 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others). 9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.
B	The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C	The episode is not attributable to the physiological effects of a substance or another medical condition.
D	At least one major depressive episode is not better explained by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.
E	There has never been a manic episode or a hypomanic episode.

There are different types of specifiers that provide more specific details about the type of depression: with anxious distress, with mixed features, with melancholic features, with atypical features, with mood-congruent psychotic features, with mood-incongruent psychotic features, with catatonia, with peripartum onset, and with seasonal pattern (4).

3.1.7. Prognosis and evolution

MDD can occur at any age, it tends to start more often during puberty. The course is quite variable, as some patients experience remission while others remain with few or no symptoms for years between episodes. If depressive symptoms persist for a long time, there's a higher chance of having underlying personality, anxiety, or substance use issues. In such cases, the likelihood of treatment fully resolving the symptoms decreases compared to those who recently developed symptoms. Recovery begins within 3 months from the onset in 40%, and within a year in 80%. Traits associated with poorer recovery rates include personality disorders, severe symptoms, psychotic features, and a high level of anxiety. The risk of a diagnosis of mania or hypomania increases if the 'with mixed features' specifier is present (4).

3.1.8. Treatment

Currently, the range of therapeutic possibilities for the treatment of depression is highly broad *(Figure 2),* although not all have the same level of recommendation (3). However, the response is very variable and appears to be effective in less than half of the cases (5). Approximately 30% of treated individuals do not achieve remission after 2 or more first-line treatments and are considered to have treatment-resistant depression (41). Moreover, despite of the numerous treatments, more than 75% of affected people in low socioeconomic stratum countries do not receive treatment (2).

The stepped-care model has been proposed to maximize treatment efficiency based on the severity of the patient's depression and thus avoid unnecessary adverse effects. In this way, all necessary psychotherapeutic, psychosocial, and pharmacological interventions are encompassed. The specific interventions in each type of depression are detailed in the *Figure 3.*

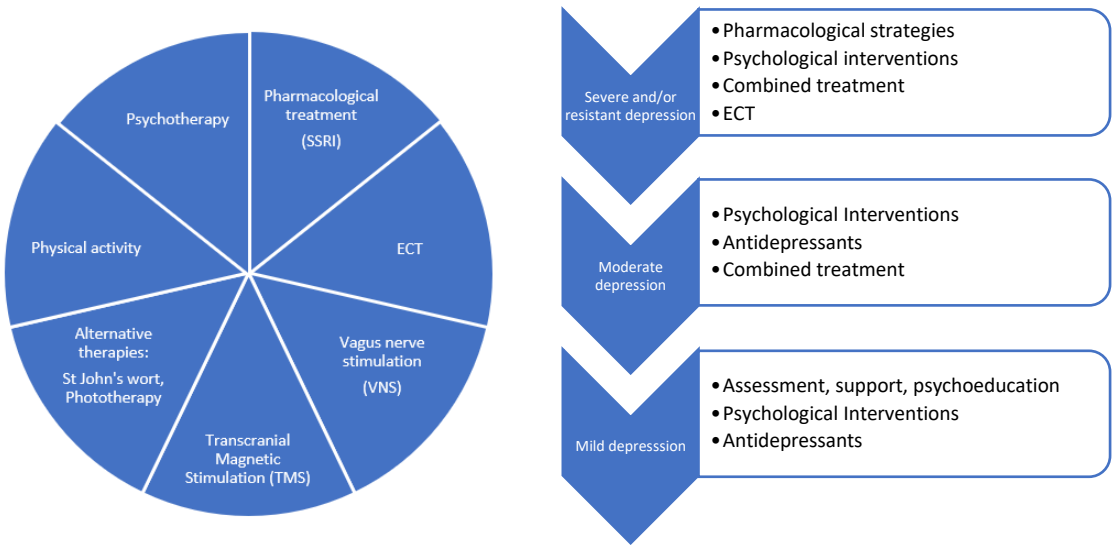

***Figure 2**: The treatment options for depression. Adapted from (1,3). Abbreviations: SSRI, Selective Serotonin Reuptake Inhibitor; ECT, Electroconvulsive Therapy; VNS, Vagus Nerve Stimulation, TMS: Transcranial magnetic stimulation.*

***Figure 3**. Stepped-Care Model for depression treatment. ECT, Electroconvulsive therapy. Extracted and adapted from:* (3).

Regarding psychotherapeutic treatment, there are different models that vary in theory and application of their techniques, but the most widely used so far is cognitive-behavioral therapy. It aims to modify dysfunctional behaviors through patient education and collaboration by creating new behaviors (3). Additionally, in a study that analyzed seven types of psychotherapeutic interventions, improvement was observed compared to the waitlist group, although there were no consistent differences between the interventions themselves (42).

A crucial pillar of MDD treatment is pharmacological intervention. There are various types based on their mechanism of action and structure. Currently, the most used are the new generation antidepressants comparing to classical antidepressants, as the latter exhibit poorer tolerance and more pharmacological interactions. All of them require a period of 2 to 4 weeks to take effect and it is recommended to maintain the treatment for at least 6 months after the remission of the episode. Patients who discontinue the treatment earlier are at a higher risk of recurrence, especially if there are risk factors such as advanced age, recurrent episodes, chronicity, associated

psychotic symptoms, residual symptoms, and severe episodes (3). The most used among the new generation antidepressants are the SSRIs due to their good tolerance and broad spectrum of indications. However, they have lower efficacy in severe depressions compared to tricyclic antidepressants. Their most frequent side effects are at the gastrointestinal level and sexual dysfunction (1). The remaining antidepressant treatments are outlined in the *Table 3*.

Table 3. *Classification of various antidepressants marketed in Spain. Adapted from: (3).* Abbreviations: MAOI, Monoamine oxidase inhibitors; SSRIs, Selective serotonin reuptake inhibitors; NRIs, Norepinephrine reuptake inhibitors; SNRIs, Serotonin and norepinephrine reuptake inhibitors; ASARI, Antagonists of serotonin receptors 5-HT2 and weak inhibitors of 5-HT uptake; NASSA, Noradrenergic and specific serotonergic antidepressants; NRAs, Norepinephrine reuptake inhibitors.

CLASSIC ANTIDEPRESSANTS	**Non-selective MAOI**	Tranylcypromine
	Selective MAOI MAO-A	Moclobemide
	Heterocyclics	Tricyclics: Amitriptyline, Imipramine, Clomipramine, Trimipramine, Nortriptyline, Doxepin
NEW GENERATION	**SSRIs**	Citalopram, Fluoxetine, Fluvoxamine, Paroxetine, Sertraline, Escitalopram
	NRIs	Bupropion
	SNRIs	Venlafaxine, Desvenlafaxine, Duloxetine
	ASARI	Trazodone
	NASSA	Mirtazapine
	NRAs	Reboxetine
	Melatonergic Agonist	Agomelatine

If the patient does not respond to pharmacological treatment or responds only partially, it is considered treatment-resistant depression. Regarding pharmacological treatment, strategies include dose escalation, switching to another antidepressant, combination of antidepressants, or augmentation with antipsychotics such as quetiapine, lithium, anticonvulsants, zinc, or benzodiazepines. Non-pharmacological options include psychotherapy and electroconvulsive therapy.

Electroconvulsive therapy (ECT) involves inducing generalized seizure episodes through electrical stimulation of the CNS. Indications include severe depressive episodes, treatment-resistant depression, and first-line treatment in cases of suicide risk or patients with significant organic impairment.

Vagus nerve stimulation and transcranial magnetic stimulation are among the therapeutic possibilities, but they are not recommended due to uncertainties about their effectiveness. Vagus nerve stimulation is also discouraged because it involves an invasive intervention. St. John's Wort is not recommended either due to interactions with certain drugs, lack of dose standardization, and uncertainty about the active ingredient (3).

Another treatment currently used in treatment-resistant depression is intranasal esketamine. Its efficacy has been tested against a placebo (43). According to a systematic review, all studies showed that it is an effective and safe treatment in combination with an oral antidepressant for resistant depression (44).

3.2. GUT MICROBIOTA

3.2.1. Introduction

The microbiota is defined as the set of microorganisms that inhabit the intestines and coexist peacefully with the host, contributing to their health and well-being (45). It has trillions of microbes that coexist with the human (46). The modification of its composition depends on various genetic, nutritional and environmental factors. Its balance is fundamental, since an alteration of this can lead to the appearance of diseases, especially gastrointestinal, metabolic, immunological and psychiatric (47).

Among the known composition, the majority is inhabited by bacterial communities, while a small fraction consists of fungi, protozoa, and viruses (45,48).

Previously, vitro cultivation techniques were employed but it wasn't an optimal method since not all species grow in the culture medium, so they did not represent the entire microbiota. The knowledge of new metagenomic sequencing techniques of the 16S ribosomal RNA gene has been highly relevant in understanding, as we do today, the composition of the microbiota (49,50). Currently there are different sequencing techniques; 16S rRNA gene sequencing, shotgun metagenomic sequencing, and RNA sequencing, each with their advantages and disadvantages. One of the most common is amplicon sequencing, where we amplify a region of DNA using PCR, specifically the bacterial 16 S ribosomal RNA gene (50).

3.2.2. Gut microbiota composition

The configuration and multiplication of the microbiome begins from birth until the age of three when the microbiota matures (47,51) Newborns delivered through vaginal birth have a microbiota similar to the mother's vagina, whereas those born via cesarean section have a microbiota similar to the skin (52).

The microbiota consists of over 1500 species, distributed across more than 50 different phyla (47). According to biological classification, microorganisms are categorized into different hierarchical taxonomic ranks: phylum, family, genus, species from broadest to narrowest spectrum *(Figure 4).*

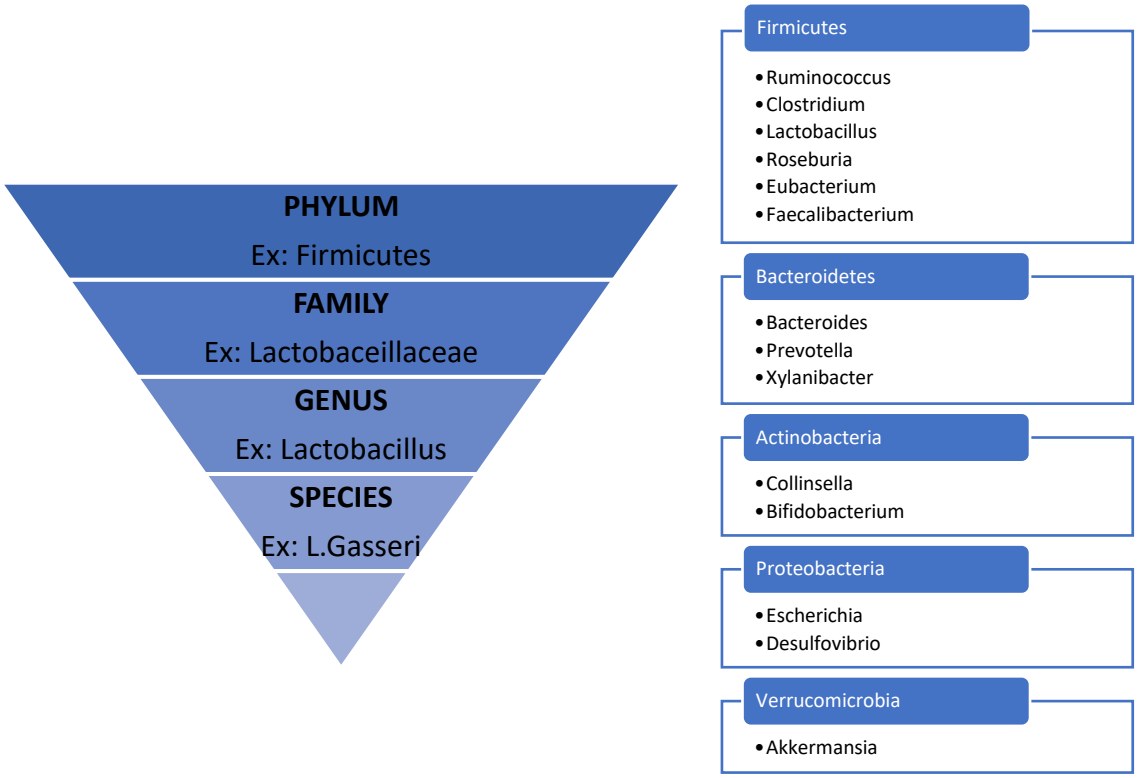

Figure 4. *The taxonomic classification of microorganisms in the intestinal microbiota witch examples of each group.*

Figure 5. *Composition of the microbiota; main phyla and genera. The phyla are: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. The most frequent genera are in the boxes. Source:* (45)

The predominant bacterial groups are classified into five phyla: *Firmicutes* (mainly from the genera *Lactobacillus*, *Streptococci*, and *Enterobacter*), *Bacteroidetes* (primarily *Bacteroides* followed by *Bifidobacterium*, *Faecalibacterium*, and *Roseburia*), *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, with the first two being the most dominant. Among these phyla, *Firmicutes* is the predominant one, constituting almost 50% of the total bacterial population (45).

According to the dominance of the genus, which is usually *Bacteroides*, *Prevotella*, or *Ruminococcus*, three enterotypes are established; enterotype 1 is characterized by the predominance of *Bacteroides*, enterotype 2 is characterized by the predominance of *Prevotella*, and enterotype 3 is characterized by the predominance of *Ruminococcus*. Diet is one of the main determinants of the enterotype. The *Bacteroides* enterotype is

the most common in industrialized countries, while *Prevotella* is more characteristic of areas with agricultural cultures featuring fibre-rich diets and low levels of animal protein and fat (53). Despite this, even individuals with the same diet exhibit different microbiota profiles. Taxonomic differences do not always imply a functional difference, as many bacteria share functions (54).

The intestinal microbiota in humans varies based on physiological and epidemiological factor such as age, gender, exercise, diet, eating habits (45), birth mode (55) and the intake of antibiotics (56).

Regarding age, *Actinobacteria* is more abundant during weaning and decreases post-weaning and with age. *Firmicutes* are more abundant in children older than 4 years compared to those under 4 years. *Proteobacteria* are more abundant in elderly individuals, especially the Enterobacteriaceae family (45). There is evidence that the *Firmicutes/Bacteroidetes* ratio increases with age (57,58). Also, microbial diversity decreases, and proinflammatory microorganisms increase while others with anti-inflammatory capacity, such as *Faecalibacterium prausnitzii* and *bifidobacteria*, decrease (53).

An abundance of *Faecalibacterium prausnitzii*, *Roseburia hominis*, and *Akkermansia muciniphila* has been observed in active women with an exercise-based lifestyle, whereas sedentary women showed an increase in *Paraprevotella* and *Desulfovibrionacea* (45).

According to the regions of the gastrointestinal tract, the composition of microorganisms varies. *Lactobacillus* and *Streptococci* species have been observed primarily in the stomach, while *Bacteroides* and *Bifidobacterium* predominate in the intestine and colon (46).

3.2.3. Role of gut microbiota in metabolism

Metabolic processes and the interaction between the microbiota and the host have a profound impact on human physiology. The microbiota plays a key role in maintaining the health of the intestinal lining, producing substances that fight against harmful microorganisms, defending the body from external invaders, and contributing to the

development of the immune system. Additionally, it provides essential nutrients like enzymes and vitamins, and influences cognitive functions through the synthesis of specific compounds. Disruptions in this delicate balance, known as dysbiosis, caused by factors such as a sedentary lifestyle or an imbalanced diet, are crucial in the onset of certain diseases (46).

An example of its functions is the production of SCFAs derived from the metabolization of indigestible dietary elements such as celluloses, pectin, and butyric acids. Additionally, it allows the synthesis of numerous vitamins such as vitamin B and K and enables the synthesis of neurochemicals like gamma-aminobutyric acid (GABA), which plays a significant role in neuropsychiatric disorders. Furthermore, it is involved in the generation of carbohydrates, amino acids, bile acids, and cholesterol.

The microbiota also plays a role in bone growth and development by being associated with the absorption of calcium and phosphorus through the fermentation of SCFAs. It also has an immunoregulatory function on osteoclasts-osteoblasts through certain bacterial strains such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus GG*, Lactobacillus reuteri, *Lactobacillus paracasei*, and *Bacillus clausii* (47).

3.2.4. Intestinal barrier

The intestinal barrier consists of three interconnected layers: the luminal mucosa, the intestinal epithelial layer, and the inner layer that constitutes the mucosal immune system. In this way, it enables defense against microorganisms, toxins, and food antigen (59,60).

The epithelial barrier is formed by a layer of columnar epithelial cells closely joined by tight junctions (TJ). The epithelium serves as a semi-permeable physical barrier that allows the passage of nutrients, protects against pathogen invasion and responds to numerous signals from the microbiota and the immune system (61).

Epithelial cells, goblet cells, and Paneth cells are the main antimicrobial cells with two key functions: ‘segregation’ and ‘mediation’. Segregation involves separating the intestinal microbiota from the host's immune cells to prevent an inflammatory

reaction. Mediation involves signaling communication between intestinal microbes and host immune cells. Epithelial cells produce mediators such as cytokines and chemokines to induce immune responses from T cells or deliver antigens to antigen-presenting cells (APCs) for a specific response (59).

Paneth cells and microfold cells are exclusive of the small intestine, whereas enterocytes, goblet cells and enteroendocrine and tuft cells are present in both the small intestine and colon (59,60).

3.2.5. The microbiota-gut-brain axis (MGB axis)

Numerous studies indicate the existence of bidirectional connections between MDD and dysbiosis. Consequently, a vicious pathological cycle ensues, wherein the pathological alterations linked to depression not only result from but also exacerbate dysbiosis. Thus, while shifts in the microbiota may manifest early in MDD and potentially play a role in its initiation, the disorder progressively contributes to the modification of the gut microbiota (24,48,62).

Microbiota and the brain can communicate with each other by several direct and indirect pathways such as the autonomic nervous system (the enteric nervous system and the vagus nerve), the hypothalamic-pituitary-adrenal (HPA) axis, the immune system and metabolic pathways (tryptophan metabolism, short-chain fatty acids, brain chain amino and peptidoglycan) (48,63).

The microbiota has the ability to generate amino acids (tryptophan, tyramines...), microbial metabolites (SCFAs) and neurotransmitters (5-HT (5-hydroxytryptamine), GABA, dopamine, norepinephrine). These metabolites can travel through portal circulation and interact with the immune system and stimulate the vagus nerve, which can signal directly to the brain or interact with local enteric neurons. Nevertheless, stress, emotions, or a disorder such as depression can activate the HPA axis (CRH is released, leading to the production of ACTH by the pituitary gland and ultimately triggering cortisol to be released by the adrenal gland) and the subsequent release of cortisol, which can alter the epithelial barrier and even the composition of the microbiota (64).

Dysbiosis promotes intestinal permeability, known as "leaky gut", allowing bacterial translocation and triggering an immune response that contributes to systemic inflammation, a significant factor in the pathophysiology of MDD (30,31). This increased intestinal permeability occurs due to enterocyte apoptosis, mucosal degradation, and disruption of tight junctions (31).

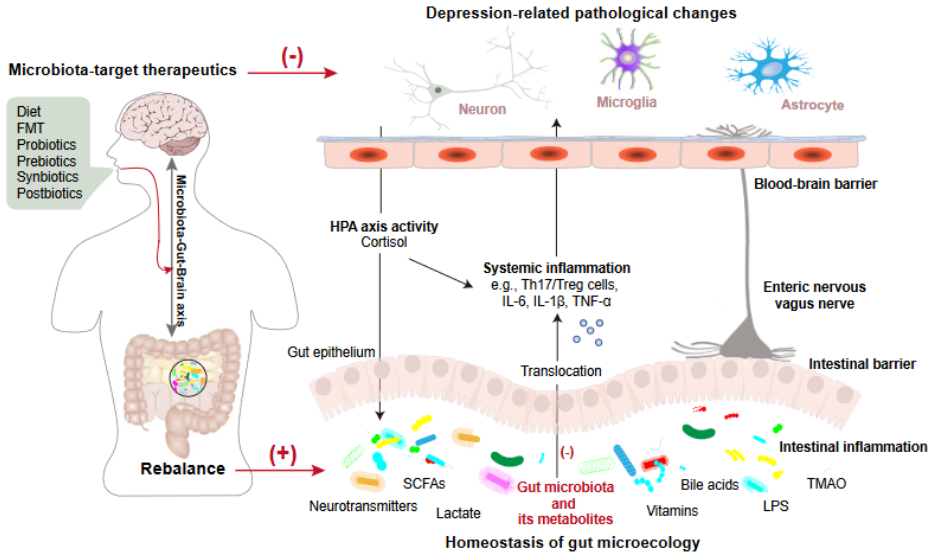

Figure 6. *The microbiota-gut axis in the pathogenesis of depression. The microbiota communicates with the brain through different pathways, including the HPA axis, the autonomic nervous system, the immune system, and metabolic pathways. Extracted from:* (65). *Abbreviations: HPA, hypothalamic-pituitary-adrenal; SCFAs, Short-Chain fatty-acids; LPS, Lipopolysaccharide; TMAO, Trimethylamine-N-oxide; IL-6, Interleukin-6, IL-1β, Interleukin-1βTNF-α, Tumor necrosis factor-alpha.*

The vagus nerve also plays a significant role in the communication of the MGB axis. The intestine is under the control of the parasympathetic efferents of the vagus nerve and the sympathetic efferents of the splanchnic nerves (66). The stimulation of the vagus nerve used as a treatment in patients with MDD resulted in a greater response than those in the usual treatment group (67).

Systemic inflammation exacerbates the pathophysiological condition of underlying illnesses (31). Some studies demonstrate a significant correlation between leaky gut and gastrointestinal diseases such as inflammatory bowel disease (IBD), irritable bowel

syndrome (IBS) and celiac disease. A strong correlation has also been observed in inflammatory, autoimmune and metabolic diseases, as well as cancer and neurodegenerative diseases (61,66,68).

Some components of bacteria that translocate induce central changes such as demyelination, synaptic defects, abnormal neurogenesis, and neurotransmitter release (64,69).

Brain disorders are linked to the impairment of the enteric nervous system, known as the ''second brain''. While it has the capacity to function autonomously from the CNS, there is a form of communication between these two systems (66).

3.2.6. Microbiota and depression

Several studies have shown an association between dysbiosis and depression by reporting changes in the gut microbial composition among healthy individuals and MDD patients, particularly relative to microbial diversity and specific bacterial taxa (5,70–72). In patients with MDD, an increase in pro-inflammatory bacteria and a reduction in anti-inflammatory bacteria have been observed, while the results regarding the enrichment of certain phyla have been inconsistent (5,65,70). These inconsistencies could be due to differences in study design, small sample sizes, unmeasured confounding factors such as diet, or the various statistical methods employed (73). Certain genera have been associated with an anti-inflammatory character, such as *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium*, leading to the pathophysiology of MDD being induced by immune and inflammatory responses derived from the composition of the microbiota. As discussed in previous sections, a disruption in the microbiota leads to bacterial translocation due to the disruption of tight junctions, triggering immune-mediated responses to bacterial LPS, creating an inflammatory state (73).

Studies have also been conducted with rodents, where it was observed that the administration of broad-spectrum antibiotics led to dysbiosis, depressive symptoms, and hippocampal alterations, which were reversed with the administration of *Lactobacillus casei* (56). Moreover, preclinical studies have demonstrated an

improvement of psychiatric conditions following the administration of faecal microbiota transplants (72).

The phyla most notably impacted are *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*(70,74); specially an increase in the *Bacteroidetes/Firmicutes* ratio was denoted in MDD patients according to recent reviews, with an increase of the genus *Bacteroides* and a decrease of *Blautia*, *Faecalibacterium* and *Coprococcus*. Consistent patterns of elevated *Eggerthella* and reduced *Sutterella* have been observed in individuals with MDD (65,73).

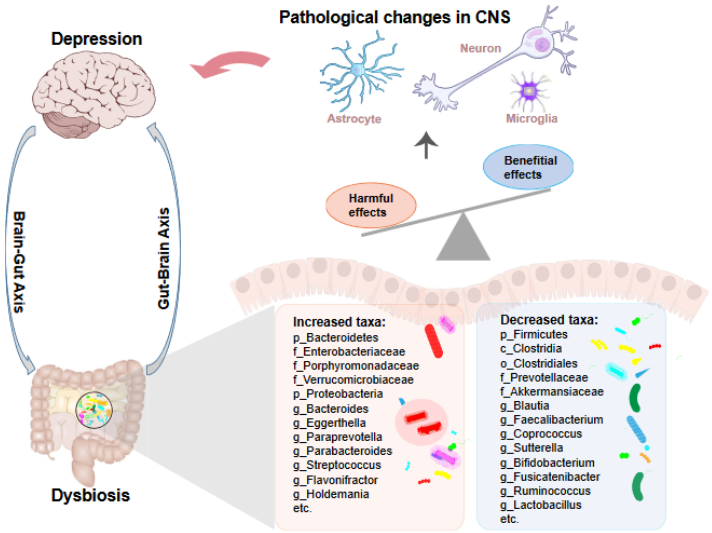

Figure 7. *Representation of the bidirectional pathway between dysbiosis and depression and the main increased and decreased taxa in gut microbiota in depression. Extracted from:* (65)

As mentioned earlier, it seems that depression and dysbiosis have a bidirectional association. The alteration of the gut microbiota induces depressive symptoms, while depression itself leads to changes in the microbiota. In a study with rodents where microbiota from MDD patients was transplanted, the emergence of anxious-depressive symptoms was observed (75). In contrast, studies have revealed that many classes of antidepressants, such as fluoxetine, enriched certain bacterial species (76). Another example is that it was observed that women suffering from depression had higher levels of cortisol and fat oxidation levels, which may have a modulating effect on the microbiota (77).

Furthermore, not only intestinal bacteria play a role in the pathogenesis of depression, but also non-bacterial microbiota has its role. Bacteriophages beneficially promote diversity, with documented changes in *Caudovirales* bacteriophages in patients with MDD. The role of fungi (the so-called 'mycobiome') has also been previously studied, such as *Candida albicans*, suggesting that its increase could trigger depressive symptoms and immune alterations (65,78).

In addition to the morphological view of the microbiota, functional approaches have been undertaken, revealing differences in metabolites between depressed and non-depressed patients. Changes in the composition of the microbiota lead to changes in the metabolome. On one hand, low levels of SCAFs such as bile acids (BAs) seem to have a protective role against depression. On the other hand, trimethylamine-N-oxide (TMAO) from choline metabolism, as well as LPS, have been associated with the pathogenesis of MDD (65).

Arginine, proline, and histidine were negatively associated with depression, and all of them converge in the glutamate pathway. The most significant finding is the association of high levels of circulating proline with high depression scores. Proline levels are heavily dependent on diet and the composition of the intestinal microbiome. Moreover, proline supplementation has been observed to exacerbate depressive behavior in mice. Additionally, it was observed that a decrease in the expression of proline transporters conferred protection against depression in Drosophila flies (79).

4. JUSTIFICATION

Major Depressive Disorder is one of the most treated conditions in the field of psychiatry and primary care (1), with a high global prevalence of 280 million people worldwide suffering from this disorder (2). It is considered the second cause of disability in the world (1,2) and it carries a high risk to life, being one of the most important risk factors for suicide (3).

Personal (3,4,6,37–40), environmental (4,37), genetic (3,4), psychological (3) and sociodemographic (3,4,11,13,38) risk factors have been described associated with this disorder and numerous hypotheses have been proposed such as the monoaminergic theory, the neurotrophic hypothesis, the neuroanatomical hypothesis and neuroendocrine alterations (14,15,22). However, the exact pathophysiology remains unknown which prevents the application of new therapeutic interventions. Despite numerous treatments on the market, they are effective in less than half of the patients and the high prevalence of this disorder remains unchanged (5).

In recent decades, from different specialties, the role of the microbiota has been a new focus, since it is known that its alteration is related to several diseases (61,66,68). In the field of psychiatry, a new hypothesis has been proposed to explain MDD that proposes a neuroinflammatory state that causes depressive symptoms as a result of an initial dysbiosis (22,73).

According to highly recent reviews, previous studies have shown that depression is associated with marked alterations in the composition of the intestinal microbiota. Nonetheless, the results regarding the enrichment of certain microbiota phyla are inconsistent. This abundance of specific bacterial taxa has been evaluated in some studies by the *Firmicutes/Bacteroidetes* ratio parameter (65,73). By critically analyzing the existing literature, significant limitations have been identified such as the lack of measurements of some relevant confounding factors, too small sample sizes and lack of data on eating habits (73).

Furthermore, there are a large number of cross-sectional studies defining the microbiota of MDD patients but a lack of large-scale prospective studies to identify characteristic microbial changes that change in parallel with symptoms (65).

We also know that the microbiota depends largely on diet, therefore, the external validity of studies carried out outside Spain is limited by the characteristics conferred by the diet itself (28,40).

For all these reasons, with this study we want to add value and open up a new field of research through the study of the *Bacteroidetes/Firmicutes* ratio as a potential biomarker for the prevention and treatment of MDD, as well as provide a prospective vision of the evolution in the composition and diversity of the microbiota starting from a healthy basal situation. Moreover, we will take into account the limitations of previously carried out studies probably associated with the inconsistencies found.

This project can provide us with the following benefits:

- To discover a **new biomarker** that is more objective than the clinical criteria established until today. The microbiota, especially the *Bacteroidetes/Firmicutes* ratio parameter, could be very useful to identify people at risk of suffering from this disorder.

- **Early detection** of people at risk. If we detect those people who will eventually develop MDD, close monitoring could be done in order to avoid or mitigate it.

- The intestinal microbiota could be used as a new **therapeutic target** for the treatment of MDD. This study could serve as a basis for future research on the effectiveness of treatments that modulate microbiota composition.

- To establish a better **association between gut microbiota alteration and MDD** by observing changes in gut microbiota parallel with the onset, persistence, or remission of depressive symptoms over a long follow-up period.

All these potential benefits could be achieved without the need for invasive testing. With just a stool sample, we could shed light on a disorder that still requires a significant amount of research.

5. HYPOTHESIS

5.1. Main hypothesis

- Young adults exposed to an increased *Bacteroidetes/Firmicutes* ratio in gut microbiota will be more susceptible to develop a Major Depressive Disorder over a five-year period compared to those who do not have an increased ratio.

5.2. Secondary hypothesis

- Reduced bacterial α-diversity will be associated with a higher risk of developing MDD compared to individuals with higher diversity.
- Participants who develop MDD during the study will have an increased *Bacteroidetes/Firmicutes* ratio compared to healthy participants.
- The microbiota composition in the same participant will change based on whether they are diagnosed with MDD or are in a healthy state.

6. OBJECTIVES

6.1. Main objective

- To determine whether is a significant difference in developing MDD between the group exposed to a high *Firmicutes/Bacteroidetes* ratio versus the non-exposed group over a five-year period.

6.2. Secondary objectives

- To study the alpha diversity of the intestinal microbiota in order to assess whether lower diversity is associated with a higher risk of experiencing MDD.
- To evaluate if the *Bacteroidetes/Firmicutes* ratio is increased in MDD participants compared to healthy participants.
- To identify changes in gut microbiota in parallel with the onset or disappearance of MDD.

7. METHODOLOGY

7.1. Study design

The investigation is designed as a multicenter observational, analytic, prospective cohort study that will be carried out in the four basic health areas of Girona (*Annex 2*) with a follow-up from December 2024 to December 2029.

7.2. Study population

The study population will be formed by young adults between 18 and 30 years old that meet the inclusion and none of the exclusion criteria. They will be selected probabilistically and randomly from each basic health area and included in the study once their informed consent is obtained.

7.3. Inclusion and exclusion criteria

7.3.1. Inclusion criteria

- Young adults aged between 18 and 30 years old who are registered as residents in the Girona region.
- Individuals without previous or current MDD diagnosis
- Individuals with family support who are able to cooperate in the study.
- Individuals who agree to participate in the study by understanding and signing the informed consent *(Annexes 3 and 9)*

7.3.2. Exclusion criteria

- Previous or current diagnosis of severe mental disorders according to DSM-V TR criteria.
- Individuals who are under active treatment with antibiotics or have been treated during the month prior to stool collection.
- Individuals with inflammatory bowel disease.
- Regular use of psychotropic medications.

7.4. Sample selection

The sample frame will be randomized and probabilistic. A family physician from each basic health area will be responsible for sending a letter requesting the participation in the study to random people from the health registry applying the inclusion and

exclusion criteria. The number of people selected will be proportional to the reference population of each basic health area. Participants will receive details regarding the study's purpose and any supplementary tests they will undergo. The study information document *(Annex 4)* and the biobank information document *(Annex 8)* will be provided. Individuals interested in joining the study will be presented with the informed consent forms *(Annexes 3 and 9)* for signature and will be scheduled for a new visit the following week for data collection *(Annex 12)*, to collect the faecal containers and to fill out the questionnaires *(Annexes 5, 6, 7, 10 and 11)*

7.5. Sample size

The sample size was estimated using GRANMO software, a sample size calculator. Accepting an alpha risk of 0,05 and a beta risk of 0,2 in a bilateral contrast, **363** subjects are needed in the exposed group and **1089** in the non-exposed group to detect a minimum relative risk of 1,5 if the disease rate in the non-exposed group is 0,15. The total of the selected population will be divided into quartiles based on the *Bacteroidetes/Firmicutes* ratio. The ratio between the number of un-exposed and exposed will be 3, since those below or equal to 25th percentile will enter as the exposed group and the rest as the unexposed group. A loss to follow-up rate of 15% has been estimated due to voluntary withdrawal of participants, loss of contact or lack of participation in scheduled evaluations.

7.5.1. Independent variable

- **Ratio *Bacteroidetes/Firmicutes* in gut microbiota**

The independent variable in this research focuses on the composition of the gut microbiota, specifically on the proportion between bacteria from the phyla *Bacteroidetes* and *Firmicutes*, both being the two most predominant phyla. The *Bacteroidetes/Firmicutes* ratio is a quantitative continuous variable, but it will be expressed as a dichotomous qualitative variable since the total sample will be divided into quartiles and those who are below or equal to the 25th percentile will become part of those exposed to an increased ratio while the rest will be the non-exposed group, with a non-increased ratio.

This proportion is calculated by analyzing stool samples from participants, using advanced next-generation sequencing techniques. To measure microbiota composition, amplicon sequencing analysis of the 16S rRNA gene will be performed. This approach will allow the identification and quantification of the different bacterial species present in faecal samples. The *Bacteroidetes/Firmicutes* ratio will be calculated for each participant, which will serve as an indicator of the relative composition of these two bacterial phyla.

Participants will be instructed to collect the faeces samples themselves in stool samples containers provided at the first scheduled visit and they will freeze the sample immediately for 4 days. Then, participants will bring their samples to their respective basic health areas and deliver them to the nursing team. The stool samples will be stored at -20ºC and labelled previously with the corresponding participant code to guarantee confidentiality. All those samples will be sent to the *Institut d'Investigació Biomèdica de Girona (IdibGi)* for storage in a freezer at -80ºC for at least 24 hours to preserve the DNA integrity.

The microbiological analysis will be carried out by IdibGi microbiologists. DNA extraction will be performed following a standardized protocol in accordance with the guidelines of the manufacturer Illumina MiSeq System (80). DNA extraction will be carried out using extraction and dilution solutions (NucleoSpin® 96 DNA Stool) and sample processing by vortex mixing and boiling water bath. The subsequent stage involves amplifying the amplicons through PCR (Polymerase Chain Reaction**)**. A PCR plate, forward primer, reverse primer, and DNA polymerase will be required. This method will be performed targeting the V3 and V4 regions of the 16s gene.

The specific primers will be forward primer 341F, 5'-CGTCGGCAGCGTCAGA TGTGTATAAGAGA CAGCCTACGGGNGGCWGCAG-3', and reverse primer 805R, 'GTCTCGTGGGCTCGGAGATGTGTATCTAATTCC-3).

The results will be visualized by electrophoresis in agarose gels.

Following the first PCR, it's necessary to purify the 16S V3 and V4 amplicons to eliminate the primers. Subsequently, a second PCR will be executed utilizing the Nextera XT Index Kit, followed by another purification step.

The library quantification will be made using the Qubit®assay Kit in the Fluorometer Qubit® 4 to measure DNA concentration. Pooling and denaturation will be performed prior to MiSeq sequencing.

The MiSeq® system (next generation sequencing) will allow an analysis of 16s rRNA amplicon sequencing data, taxonomic classification results for each sample and graphical representation with MiSeq Reporter Software. Moreover, the QIIME Pre-processing and Visualizations program will allow a more detailed analysis, including the generation of OTU (Operational Taxonomic Unit) clustering and diversity analysis.

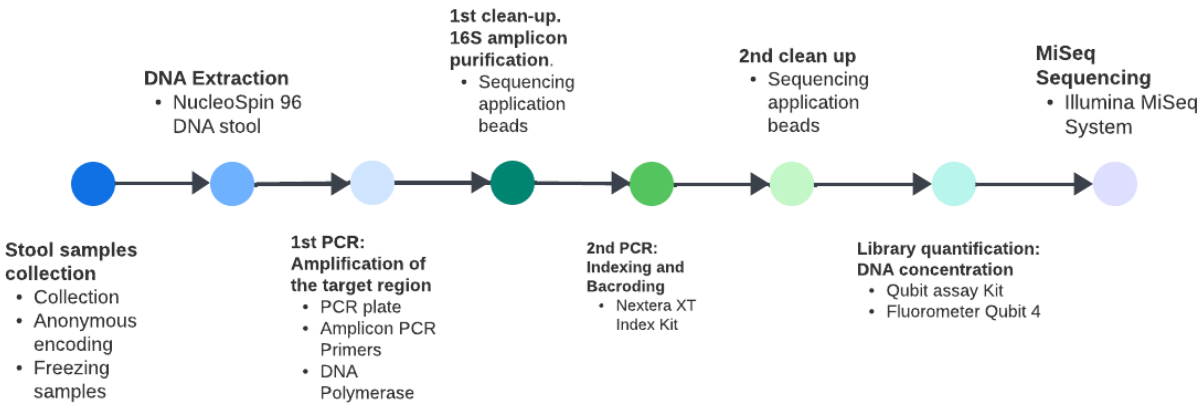

***Figure 8**. Procedure for collection, storage, DNA extraction and processing of faecal samples. Abbreviations: DNA, Deoxyribonucleic; PCR, Polymerase Chain Reaction.*

During the follow-up, faecal sample analysis will be conducted annually on those participants who, when called to undergo the Patient Health Questionnaire-9 (PHQ-9), currently exhibit MDD (confirmed by the psychiatrist) and on their respective matched participants. It will also be conducted on those who have previously been diagnosed with MDD at the established cut-off point to assess the microbiota of persistent and remitting depressions.

To assess secondary objectives such as bacterial α-diversity, the Shannon index will be employed. This index, based on a formula that evaluates species diversity in a sample, will be analyzed alongside the ratio and microbiological profile. A higher index indicates greater diversity in the sample. It will be classified as a continuous quantitative variable.

7.5.2. Dependent variable

- **Presence of Major Depression Disorder.**

Confirmation of the presence of MDD will be made by a psychiatrist considering the DSM-V TR criteria *(Annex 1).*

This variable will be presented as a dichotomous qualitative variable depending on the compliance or not of the stablished criteria:

Yes: if participant meets diagnostic criteria of MDD

No: if the participant doesn't meet the criteria.

At the beginning of the study, the PHQ-9 will be used to evaluate the presence of depressive symptoms *(Annex 4).* A positive result excludes participation in the study.

During the cohort follow-up, a researcher will make a phone once a year to each participant to evaluate the PHQ-9 scale. If a significant score is obtained, the participant will be referred to a psychiatrist to make a confirmatory diagnosis, since the PHQ-9 is only a screening tool. Given that it is a long-term follow-up study, and the calls will be annual, clinical records will be assessed at the end of the study to determine the number of participants who have experienced MDD during the unassessed time interval.

7.5.3. Covariables

There are included as covariables those that have been related to possible changes in the composition of the microbiota, those that have been associated to develop MDD and those that affect both variables. For this reason, they will be collected in order to avoid confusion and obtain interpretable results. They are the following:

- **Age**. The age measured in years will be expressed as a quantitative discrete variable.
- **Gender**. Presented as a qualitative dichotomous variable in categories: male or female.
- **Body mass index**. Measured in kilos/metre^2. The result will be presented considering the following groups: underweight (<18,50kg/m^2), normal weight (18,50-24,99), overweight (25-29,99) and obesity (≥30,00). It will be expressed as a qualitative ordinal variable.
- **Diet**. It will be evaluated through the Food Frequency Questionnaire (FFQ) (*Annex 7*). This variable will be expressed as a qualitative nominal variable with 5 predominant nutrient categories: balance diet, high fat diet, high fiber diet, high carbohydrate diet or high protein diet.
- **Family history of MDD**. It will be measured as a dichotomous qualitative variable (Yes or no) in participant data collection.
- **Physical activity.** Assessed using the International Physical Activity Questionnaire (IPAQ) (*Annex 6*). The results are classified into low, moderate, and high physical activity. It is an ordinal qualitative variable.
- **Smoking:** Expressed as a qualitative polytomous nominal variable and participants will be classified as non-smoker, ex-smoker, and active smoker. It will be measured in participant data collection.
- **Alcohol and substance consumption.** Expressed as a dichotomous qualitative variable (Yes or no). It will be measured in patient data collection.
- **Socioeconomic conditions.** This variable will be assessed using Deeleck's poverty line approach regarding economic difficulties in making ends meet (81). It will be treated as a dichotomous qualitative variable as it categorizes participants based on whether they experience economic difficulties or have no trouble making ends meet. It will be measured in patient data collection.
- **Adverse childhood experiences.** It will be evaluated through the Adverse Childhood Experience Questionnaire (ACEQ) *(Annex 10),* and it will be presented as a discrete quantitative variable considering the number of positive responses from the 10 formulated questions.

- **Adverse life events**. Stressful life events related to the last year will be evaluated using the Holmes and Rahe Stress Scale questionnaire *(Annex 11).* Participants will be categorized into high stress (>300), moderate stress (299-150), and low stress (<150) groups. It will be expressed as an ordinal qualitative variable.

7.6. Measuring instruments

7.6.1. Diagnostic and Statistical Manual of Mental Disorders 5th edition Text Revision (DSM-V-TR)

It is a manual published by the American Psychiatric Association that provides diagnostic criteria for the classification of mental disorders. It is a reference tool used by mental health professionals such as psychiatrists and psychologists. The DSM-5 has undergone several revisions over the years, including the current one (DSM-V-TR). It will be used to assess the dependent variable, based on the diagnostic criteria for MDD. It will be used annually by a psychiatrist in those patients whose PHQ-9 has tested positive, in order to confirm the diagnosis of MDD.

7.6.2. Patient Health Questionnaire-9 (PHQ-9)

It consists of nine questions that address the most common depressive symptoms based on the DSM-5 diagnostic criteria, including loss of interest or pleasure, feelings of sadness, sleep problems, fatigue, alterations in appetite, feelings of worthlessness or guilt, difficulty concentrating, and psychomotor agitation or retardation. Participants answer the questions based on their experiences over the past two weeks. The cutoff points used are: 0-4 (no or minimal depressive symptoms), 5-9 (Mild Depression), 10-14 (Moderate Depression), 15-19 (Moderately Severe Depression), 20 or more (Severe Depression). From a score of 10 or higher, participants will be referred to a psychiatrist to confirm the diagnosis. This assessment will be conducted at the beginning of the study, and those participants who test positive will be excluded from the study. Subsequently, an annual follow-up call will be made to assess the PHQ-9.

7.6.3. Food Frequency Questionnaire (FFQ)

It is a tool used to gather information about dietary patterns. It focuses on the frequency and quantity of consumption of various foods and beverages over a specific

period. The nutritional information obtained will be analyzed with a computer-based-nutritional assessment program (''Nutritionist Pro Diet Analysis'') in order to obtain the average of total kilocalories and grams of macronutrients and micronutrients consumed each day. The macronutrients of the same food group will be grouped into carbohydrates, fats, proteins, and fiber. In case of any macronutrient increase equal or greater than 10% of the expected proportion (as marked by the Sociedad Española de Nutrición), the participant will be considered to follow a predominant diet: balance diet, high fat diet, high fiber diet, high carbohydrate diet or high protein diet.

7.6.4. International Physical Activity Questionnaire (IPAQ)

The IPAQ collects information about physical activity undertaken in various domains of daily life, such as work, transportation, recreational activities, and free time. The questionnaire assesses both moderate and intense physical activity and includes questions about the duration and frequency of these activities. Consequently, the total amount of physical activity can be calculated, and participants can be categorized into different levels. The results are classified into low, moderate, and high physical activity.

7.6.5. Adverse Childhood Experience Questionnaire (ACEQ)

It is a questionnaire designed to measure adverse experiences during childhood and their potential impact on health. A total of 10 questions are asked, focusing specifically on three main categories: physical abuse, emotional abuse, and neglect. Each experienced adverse event counts as one point, with a maximum total score of 10 points.

7.6.6. Holmes and Rahe Stress Scale

The scale assigns scores to a list of 43 life events, each of which has a numerical value representing the perceived magnitude of associated stress. The participants must mark the events they have experienced in the last year. The higher the total score, the greater the risk of experiencing health problems due to stress. Life events were selected based on clinical observations and interviews conducted by Holmes and Rahe. The risk of suffering health problems is classified into high (>300), moderate (299-150), and low (<150) groups.

8. STATITICAL ANALYSIS

8.1. Descriptive analysis

The dependent variable (presence of MDD) will be presented as a dichotomous qualitative variable, and thus, it will be presented through percentages.

The independent variable (*Bacteroidetes/Firmicutes* ratio) is a continuous quantitative variable. However, when categorizing into exposed and non-exposed groups, it is expressed as a dichotomous qualitative variable (increased ratio vs not increased). In the qualitative form, it will be presented as percentages, and concerning its quantitative nature, the mean and standard deviation will be estimated if the distribution is normal, or the median will be provided if the distribution is non-uniform.

8.2. Bivariate statistical inference

For the main hypothesis, to assess differences in the incidence of MDD between both groups (exposed and non-exposed), we will use the chi-square test to calculate the magnitude of the association with relative risk, as we are comparing the association between two qualitative variables.

To determine the change in microbiota between healthy and MDD state, we will treat the independent variable as a continuous quantitative variable. Consequently, we will employ the t-test or U Mann-Whitney test. The latter method will be applied in case of a non-parametric distribution.

8.3. Multivariate analysis

Multivariate binomial logistic regression will be used to analyze the relationship between a dichotomous dependent variable (development of MDD) and a continuous independent variable (*Bacteroidetes/Firmicutes* ratio), considering potential confounding variables that may interact, in order to observe the true association between the two variables.

RStudio 4.3.0 with the Compare Groups package will be used for the descriptive and bivariate analyses. Stata 16.1 will be used to perform regression analyses. All p values will 2-sided, and p less than 0.05 will be considered statistically significant.

9. WORK PLAN AND CHRONOGRAM

9.2. Work plan

9.2.1. Participating centers

The centers proposed to carry out the study are the 4 basic health areas of Girona (*Annex 2*): Santa Clara, Montilivi/Vila-roja, Can Gibert del Plà, Taialà.

9.2.2. Research team members

The following professionals will be part of the research team:

- The **Main Investigator** (MI) is in charge of overseeing the study. Their responsibilities include developing the research plan, creating a procedure guide, and designing data collection forms. Additionally, they ensure the involvement of different basic health areas and coordinating meetings.
- **Clinicians** (Cls) are tasked with actively engaging in the study and disseminating information about the project to the community. They play a key role in recruiting patients by sending out informed letters, and they are also responsible for diagnosing participants who have been referred to the study.
    - **Psychiatrists**: they will diagnose MDD in case that PHQ-9 Questionnaire results are positive.
    - **Family physicians**: They will be essential in the process of randomly sending letters to individuals who meet the specified inclusion and exclusion criteria, informing patients about the project.
- The **Microbiology Researchers** (MR) will be the professionals responsible for the mass sequency analysis of the biological samples following the standardized protocol.
- The **Bioinformaticians** (Bis) will be in charge of the construction and analysis of the database created from the mass sequencing results. They will generate a database using MiSeq Reporter and QIIME software to represent the various taxonomic classifications for each sample.
- The **Statisticians** (STs) will enable statistical analysis based on the results obtained through the optimal methods.

- **Questionnaire Technicians** (QT) for administering the PHQ-9. They will be trained for a uniform administration of the questionnaires.

An **External Team (ET)**, consisting of the nursing staff from each healthcare center. They will provide the informed consent and questionnaires in the scheduled visit, as well as supply the containers for faecal samples for subsequent analysis. They will be responsible for sample collection and the subsequent pseudo-anonymization of the stool samples.

9.2.3. Phases of the study

The study will be carried out from July 2024 to July 2029 (five years). The research will be divided into the following stages:

PHASE 1: ELABORATION OF THE PROTOCOL AND STUDY DESIGN (3 months)

In the inaugural phase, the principal investigator shall assume responsibility for an exhaustive literature review, the formulation of the protocol, and the compilation of data collection sheets for participants. Furthermore, the researcher will establish contact with the designated primary health areas in Girona, elucidate the study details to them, and actively promote their participation.

PHASE 2: ETHICAL APROVAL (4 months)

The research protocol will be submitted to the Comité d'Ètica d'Investigació Clínica (CEIC) to further approval. The recommendations and suggestions from the committee will be duly considered.

PHASE 3: COORDINATION, ORGANIZATION AND TRAINING (3 months)

At the outset of this phase, the protocol will be presented to the entire research team to provide a clear overview of the study's objectives and phases. Subsequently, more targeted coordination meetings will be conducted to delineate the specific roles and responsibilities of each member of the research team.

The main researcher and the microbiologists will be responsible for preparing the laboratory materials required for the mass sequencing.

Training sessions will be conducted for professionals tasked with administering questionnaires and collecting data. The aim is to standardize the methods used so that they can be uniformly applied across the entire sample.

PHASE 4: PARTICIPANTS RECRUITMENT (4 months)

The recruitment of participants will be conducted by family physicians from each of the four basic health areas in Girona. They will be responsible for mailing informative sheets detailing the clinical, legal, and ethical aspects of the project (*Annexes 4 and 8*), encouraging individuals who meet the established inclusion and none of the exclusion criteria to participate in the study, using a probabilistic and random method, proportionate to the reference population of each center. Those who decide to participate will need to visit their respective basic health area, where they will receive further information about the study and sign the informed consent forms (*Annexes 3 and 9)*. Additionally, this visit will be utilized to administer the remaining questionnaires and provide the corresponding faecal sample container for microbiological analysis.

PHASE 5: DATA COLLECTION AND CONSERVATION (5 years)

Subsequently, stool samples (in accordance with the established protocol) will be collected in the respective basic health areas. Once collected, they will be pseudo-anonymized by an external team using a code corresponding to each participant. These samples will then be sent to the Biobank at the Biomedical Research Institute of Girona for proper preservation and subsequent microbiological analysis and bioinformatic processing.

From this point forward, an annual follow-up call will be made to each participant to assess the presence of depressive symptoms based on the PHQ-9 scale. If positive, individuals will be referred to a psychiatrist for the final diagnosis of MDD. Patients diagnosed will be asked to provide a stool sample for analysis, which will be compared with an equal number of samples from healthy participants selected through matching based on age and sex. At the following cut-off points, those previously diagnosed with MDD will also be analyzed to evaluate changes in microbiota in persistent and

remitting depression. Furthermore, at the end of the study, participants' clinical histories will be examined to evaluate potential cases of MDD not assessed at the cut-off points, and these will be considered along with those diagnosed during the study.

PHASE 6: DATA ANALYSIS AND FINAL EVALUATION (8 months)

The microbiologists will conduct mass sequencing of the samples obtained at IdibGi. The samples will undergo processing, and their phylogenetic profiles will be analyzed. Species diversity and the prevalence of specific species will be quantified. Subsequently, the *Bacteroidetes/Firmicutes* ratio will be calculated.

Bioinformaticians will establish a database using MiSeq Reporter and QIIME software. The obtained results will be analyzed by statisticians and ultimately assessed by the main researchers.

PHASE 7: PUBLICATION AND DISSEMINATION (5 months)

The main investigators will draft a final report and take responsibility for submitting it for publication in various scientific journals. Additionally, the study results will be presented at both national (National Psychiatry Congress) and international (CINP International Meeting) congresses.

9.3. Study chronogram

Table 4. Chronogram. *Abbreviations: MI, Main Investigator; BHA, Basic Health Areas; CEIC, Comitè Ètic d'Investigació Clínica; Cls, Clinicians; MR, Microbiology researchers; Sts, Statiscian; Pts, Patients; BI, Bioinformatician; ET, External Team: QT, Questionnaire technicians.*

PHASES AND ASSIGNMENTS	STAFF	YEARS AND MONTHS																																												
		2023		2024											2025				2026				2027				2028				2029				2030											2031		
		N	D	J	F	M	A	J	J	A	S	O	N	D	J	F	N	D	J	F	N	D	J	F	N	D	J	F	N	D	J	F	N	D	J	F	M	A	J	J	A	S	O	N	D	J	F	M
PHASE 1: ELABORATION OF THE PROTOCOL AND STUDY DESIGN																																																
Bibliographic research	MI																																															
Protocol elaboration	MI																																															
Elaboration of the data collection sheet	MI																																															
Contact with BHA	MI																																															
PHASE 2: ETHICAL APROVAL																																																
Ethical evaluation and approval	CEIC																																															
PHASE 3: COORDINATION, ORGANIZATION AND TRAINING																																																
Meetings of the research team	MI,																																															
Training sessions	MI, MR																																															
Preparation of laboratory staff	Cls, MR																																															
PHASE 4: PARTICIPANTS RECRUITMENT																																																
Participants recruitment and administration of informed consent and questionnaires	Cls																																															
PHASE 5: DATA COLLECTION AND CONSERVATION																																																
First stool sample collection	Pts, Ex																																															
MDD sample collection and matched healthy participants	Pts, ET																																															
Coding data of stool samples	ET																																															
Follow-up phone call for the PHQ-9 assessment	QT																																															
Sample processing	MR																																															
PHASE 6: DATA ANALYSIS AND FINAL EVALUATION																																																
Database development	MR, BIs																																															
Statistical analysis	Sts, MR																																															
Final report	Sts, MI																																															
PHASE 7: PUBLICATION AND DISSEMINATION																																																
Scientific publication	MI																																															
Congresses	MI																																															

10. ETHICAL AND LEGAL CONSIDERATIONS

This study will be conducted according to the four ethical principles of Beauchamp and Childress (no maleficence, beneficence, autonomy, and justice) and with the requirements expressed in the Helsinki Declaration of Ethical Principles for Medical Research in Human Beings signed by the World Medical Association (WMA) in 1964 and last revised in October 2013.

The research protocol will be submitted to the IdibGi's CEIC (Comité Ètic d'Investigació Clínica of Institut d'Investigació Biomèdica de Girona) for evaluation and approval prior to the initiation of the study. Moreover, the recommendation given by the committee will be considered to proceed with the study.

The confidentiality of personal and clinical information of the participants involved in the study will be guaranteed, according to the '*Ley Orgánica 3/2018 de 5 de diciembre de Protección de Datos de Protección de Datos Personales y Garantía de los Derechos Digitales*' and in consideration with the *Regulation (EU) 679/2016 of the European Parliament* and of the *Council of 27 April 2016.* All the information will be only used for the purpose of the research. Patients will always be allowed to modify or destruct any of their collected data. Pseudo-anonymization will be carried out by an external team to protect personal data.

Articles included in the *'Ley 41/2002, de 14 de noviembre, Básica reguladora de la autonomía del paciente y derechos y obligaciones en materia de información y documentación clínica'* will be considered. All participants interested on being part of the study will be asked to sign voluntarily the informed consent *(Annex 3)* and they will be informed of the clinical, ethical, and legal considerations (*Annex 4*).

As biological samples are collected during the investigation, '*Ley 14/2007 de 3 de julio de investigación biomédica'* and '*Royal Decree 1716/201*1' will be implemented. Participant will also be asked to authorize the inclusion of their stool sample into the Institut d'investigació biomedical de Girona Dr Josep Trueta (IdibGi) Biobank. A specific information sheet and informed consent will be given to them (*Annexes 8 and 9*).

No conflict of interest has been declared by the authors.

11. STUDY LIMITATIONS

Analyzing the study, the following limitations have been considered:

- The study design is an observational cohort with a follow-up for 5 years. This long period has been considered necessary to increase the probability of observing Major Depression Disorder but on the other hand it carries a risk of loss to follow-up due to abandonment. This limitation has been corrected in the sample size calculation considering 15% dropouts. In addition, the participant will be asked for the phone number of a close contact in case of inability to contact him.
- This is the first study that proposes the *Bacteroidetes/Firmicutes* ratio as a risk factor for the development of MDD. For this reason, the results should be assessed with caution and will serve as a basis for future research.
- The microbiota is a variable that can be influenced by numerous factors such as age, gender, diet, and physical exercise. Therefore, they will be taken into account as confounding variables and will be stratified in the multivariate statistical analysis.
- An inherent limitation of this study is the dynamic nature of the gut microbiota, which may undergo temporal changes. The composition of the microbiota is known to be influenced by various factors, and these fluctuations over time could impact the interpretation and introduce variability in our findings. To address this limitation, annual microbiological analyses will be conducted to observe variations in the microbiota during the follow-up period.
- The study will take place in Girona, so the sample may not be representative of the general population, impacting external validity due to factors such as the diet or social stratum. To improve it, a multicenter study covering the four basic health areas has been proposed.
- The inclusion and exclusion criteria could favor certain groups or conditions, limiting the generalizability of the results to a broader population. To control the selection bias, we have established clear inclusion and exclusion criteria,

and a proportional probabilistic random sampling has been conducted for each basic health area's population.

- PHQ-9's results rely on participants' self-evaluation, which may introduce biases associated with the subjective interpretation of symptoms. Furthermore, the validity of responses could be influenced by factors such as memory, emotional disposition, and participant comprehension. To mitigate this limitation various strategies will be implemented. Prior to questionnaire administration, participants will be provided with detailed information to ensure a comprehensive understanding of the questions and response scales. The questionnaire will be administered during real-time telephone calls, allowing the opportunity to address any questions or concerns. A supportive and private environment will be encouraged to promote honest responses. Additionally, participants exhibiting depressive symptoms on the PHQ-9 questionnaire will be referred to a psychiatrist who will make the final diagnosis of MDD based on the criteria of the DSM-V.
- Conducting a study of this magnitude, which encompasses various phases such as data collection, sample analysis, and review by specialized professionals, incurs significant costs. To address this limitation, resources will be maximized to reduce costs without compromising the effectiveness of the study. Possible collaborations with academic institutions, research organizations, or entities that can provide financial support will be explored. Additionally, seeking external funding through grants or sponsorships will be explored.

12. FEASIBILITY

The four basic health areas of Girona (Santa Clara, Montilivi/Vila-roja, Can Gibert del Plà, and Taialà) will be contacted to carry out the recruitment of participants and the collection of biological samples. Coordination meetings will be held to ensure a standardized approach.

Qualified personnel, such as microbiologists and statisticians, have been enlisted to the project with well-defined protocol and stages. The involvement of healthcare workers (family physicians, nurses, and psychiatrists) will be crucial for participant recruitment, sample collection, and diagnosis. Furthermore, the procedure for assessing faecal samples has been extensively outlined and standardized to ensure proper analysis.

The timeline for this study spans five years of follow-up, plus the additional time needed for result publication and dissemination. A realistic schedule has been established to organize the different phases.

This protocol adheres to ethical considerations and regulatory standards. Additionally, it will undergo approval from the CEIC, safeguarding the rights and well-being of participants throughout the study.

Regarding the financial feasibility, every effort has been made to adjust it as much as possible, taking into consideration that it is an extensive follow-up study and the expensive nature of laboratory materials required for microbiota research. A detailed budget has been developed including staff expenses, material costs, and meeting and divulgation expenses. Collaborations with researcher organizations and entities as well as external funding will be explored.

The limitations regarding participant dropout and natural fluctuations in the microbiota have been anticipated.

In conclusion, the feasibility analysis involves meticulous planning of the study to ensure its proper implementation. Available resources and centers, collaboration with qualified personnel, project schedule, ethical compliance and management of limitations have been considered.

13. BUDGET

***Table 5**. Budget. Costs related to staff, materials, meetings, and dissemination expenses.*

EXPENSES	UNIT COST	UNIT	SUBTOTAL
Staff expenses			**34,700 €**
Laboratory staff	20 €/ hour	300h	6,000 €
Statisticians	20€ / hour	150h	3,000 €
Questionnaire Technicians	15 €/ hour	1700h	25,500 €
Transport of frozen samples to IdibGi's	50€ /transport	4 transports	200 €
Material			**40,818 €**
Stool containers	263€/case of 1200	2000 samples	526 €
DNA extraction kit: NucleoSpin® 96 DNA Stool (Macherey-Nagel)	1266€/kit (384 samples)	2000 samples	5,064
Thermo Scientific™ PCR Plate, 96-well	150€/25 units	2000 samples	300 €
Primers (Thermo Fisher Scientific)	1246€/kit (250 samples)	2000 samples, 8 kits	9,968 €
Polymerase KAPA HiFi HotStart Ready Mix (Fisher Scientific)	652€/unit (250 samples)	2000 samples, 8 units	5,216 €
Agarose gels (Thermo Fisher Scientific)	800 €/unit (384 samples)	2000 samples, 6 units	4,800 €
Nextera® XT Index Kit (Illumina)	1,226€/unit (384 samples)	2000 samples, 5 units	6,130 €
Qubit® assay Kit	433€/unit (500 samples)	2000 samples, 4 units	1,732
Fluorometer Qubit® 4	3,750 €	1 device	3,750 €
Axygen™ AxyPrep MAG PCR Clean-Up Kit	204€/unit (5mL)	2000 samples, 8 units	1,632 €
Standard laboratory supplies: pipettes, storage, tubes, gloves, etc.			1,500 €
Printing costs			200 €
Meeting expenses			**4,000 €**
Research team meetings (travel, meals expenses)		3 meetings/ year	4,000 €
Insuracy			**0 €**
Divulgation expenses			**4,800** €
Publication in open access journal (3)			3,600 €
Congresses and scientific meetings			1,200 €
TOTAL			**84,318 €**

Psychiatrists, family physicians and external research team (nurses) will not receive financial compensation for their work in the research.

The budget does not include MiSeq Reporter Software, as are already available in the microbiology laboratories.

No funds are required for insurance as there will be no interventions in this study.

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15. ANNEXES

ANNEX 1. DIAGNOSTIC CRITERIA FOR MAJOR DEPRESSION DISORDER (DSM 5-TR).

Table 6. *Extended version DSM 5-TR criteria.*

A	Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. **Note:** Do not include symptoms that are clearly attributable to another medical condition. 1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). (Note: In children and adolescents, can be irritable mood.) 2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation). 3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month) or decrease or increase in appetite nearly every day. (Note: In children, consider failure to make expected weight gain.) 4. Insomnia or hypersomnia nearly every day. 5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down). 6. Fatigue or loss of energy nearly every day. 7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick). 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others). 9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.
B	The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C	The episode is not attributable to the physiological effects of a substance or another medical condition. **Note**: Criteria A–C represent a major depressive episode. **Note**: Responses to a significant loss (e.g., bereavement, financial ruin, losses from a natural disaster, a serious medical illness or disability) may include the feelings of intense sadness, rumination about the loss, insomnia, poor appetite, and weight loss noted in Criterion A, which may resemble a depressive episode. Although such symptoms may be understandable or considered appropriate to the loss, the presence of a major depressive episode in addition to the normal response to a significant loss should also be carefully considered. This decision inevitably requires the exercise of clinical judgment based on the individual's history and the cultural norms for the expression of distress in the context of loss.
D	At least one major depressive episode is not better explained by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.
E	There has never been a manic episode or a hypomanic episode. **Note**: This exclusion does not apply if all of the manic-like or hypomanic-like episodes are substance-induced or are attributable to the physiological effects of another medical condition.

ANNEX 2. BASIC HEALTH AREAS OF GIRONA CITY.

The city of Girona has about 104,000 inhabitants and has four basic health areas (BHA). The BHA is the elementary territorial unit through which primary health care services are organized. The reference population assigned to this BHA is made up of general physicians, pediatricians, dentists, nurses, nursing assistants, social workers, and non-health personnel. The BHA found in Girona are the following:

- **Santa Clara.** It has a reference population of 25,212 inhabitants, of which 11,910 are men and 13,302 women.
- **Montilivi/Vila-roja**. It has a reference population of 36,422 inhabitants, of which 17,490 are men and 18,032 women.
- **Can Gibert del Plà**. It has a reference population of 31,504, of which 17,490 are men and 18,932 women.
- **Taialà**. It has a reference population of 15,937, of which 7,821 are men and 8,116 women.

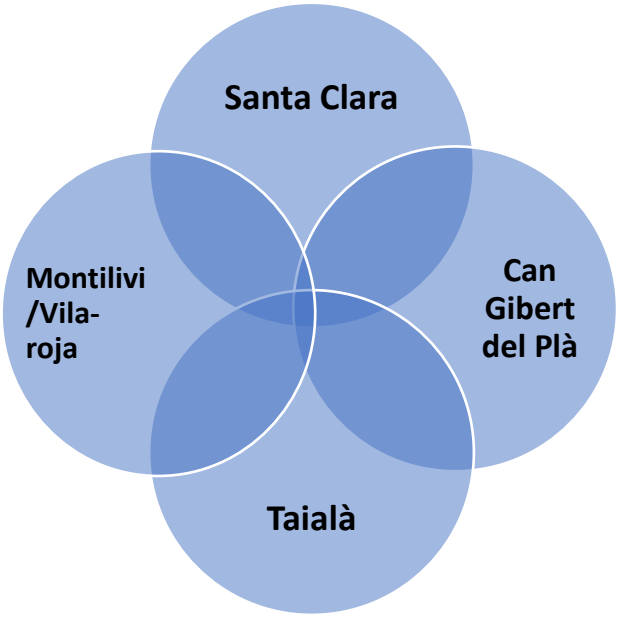

Figure 8. *Basic health areas in Girona.*

ANNEX 3. INFORMED CONSENT.

CONSENTIMENT INFORMAT

Títol de l'estudi: ''The Bacteroidetes/Firmicutes ratio: a relevant biomarker in Major Depression Disorder?''

Jo (Nom i cognoms) _____

Afirmo que:

- He llegit la fulla informativa sobre l'estudi que se m'ha entregat.
- He pogut fer totes les preguntes necessàries per resoldre els dubtes que tenia respecte l'estudi.
- He rebut tota la informació necessària sobre l'estudi.
- He entès la informació proporcionada sobre la meva participació a l'estudi.
- He estar informat per l'investigador _____ de las implicacions i finalitats del estudi.
- Entenc que la meva participació és voluntària.
- Entenc que les mostres obtingudes seran etiquetades amb un codi per tal de mantenir la confidencialitat de las meves dades.
- Entenc que puc revocar el meu consentiment de participació en l'estudi, sense haver de donar justificacions i sense afectar a la meva assistència sanitària.

Amb tot això present, ACCEPTO voluntàriament participar en el estudi.

I dono permís per tal que els investigadors del projecte contactin amb mi via telefònica per a concretar visita per a la recollida de mostres i, en cas de no ser possible, amb un telèfon de contacte que autoritzi.

Firma del participant Firma del investigador

_____, _____ de _____ de 20

ANNEX 4. INFORMATION DOCUMENT FOR THE STUDY.

FULL D'INFORMACIÓ PER AL PACIENT

Investigadors principals: Ariadna Iniesta Sánchez

Títol de l'estudi: ‘’The Bacteroidetes/Firmicutes ratio: a relevant biomarker in Major Depression Disorder?’’

Benvingut, benvinguda,

Agraïm el seu interès en l'estudi al qual el convidem a participar. La seva col·laboració és de gran ajuda per poder desenvolupar de forma correcta el projecte. A continuació, li exposem detalladament els motius del present estudi, així com tots els detalls que necessita conèixer. Si us plau, llegeixi atentament tota la informació i no dubti a consultar qualsevol aspecte amb la persona que li entrega el document quan ho consideri necessari.

Generalitats del projecte

L'estudi serà dut a terme per un grup de psiquiatres de la Xarxa de Salut Mental i Addiccions de Girona, en un període aproximadament de cinc anys. El projecte de recerca ha estat valorat i aprovat pel Comitè d'Ètica d'Investigació Clínica de l'IdibGi. Els participants en l'estudi col·laboraran en la recollida de mostres de les seves femtes i en la realització de qüestionaris sobre la salut mental i la dieta.

Objectius finals de l’estudi

Amb aquest estudi es pretén estudiar el perfil microbiològic en mostres de femtes de persones entre 18 i 30 anys, identificant la predominança i diversitat de les seves soques bacterianes i veure com això es relaciona amb el desenvolupament del trastorn de depressió major, així com establir les diferències de la microbiota entre pacients sans i pacients amb depressió.

Participació

La seva participació en l'estudi és totalment voluntària. El participant és lliure d'abandonar l'estudi si així ho desitja en qualsevol moment, sense necessitat de justificacions i sense que aquesta decisió afecti a la seva assistència sanitària. La participació en aquesta investigació és totalment gratuïta i no s'obtindrà cap compensació econòmica.

Confidencialitat y protecció de dades

S'aplicaran les mesures per garantir la confidencialitat de les seves dades segons el compliment de la Llei Orgànica 15/1999 i el nou reglament de la Unió Europea 2016/679. Per això, les dades seran recollides i gestionades de forma anònima i només s'utilitzaran amb finalitats de recerca. També es garantiran els articles descrits a la Llei 14/2007 d'investigació biomèdica.

Tasca del participant en la recollida de mostres

El participant haurà de recollir una mostra de femtes al principi de l'estudi, i, en alguns casos, se li indicarà més endavant que haurà de aportar una altra mostra. Les mostres hauran de ser

congelades immediatament i, al cap de quatre dies, hauran de ser portades al centre d'atenció primària al qual estigui vinculat per a la seva òptima conservació.

Resultats i beneficis de la investigació

El participant té dret a ser informat dels resultats obtinguts de la recerca, així com es respectarà la seva voluntat de no ser informat en cas que ho desitgi. Els resultats derivats de la investigació podran beneficiar les persones afectades per aquest trastorn i serviran de base per a futures investigacions.

ANNEX 5. PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9).

CUESTIONARIO SOBRE LA SALUD DEL PACIENTE-9 (PHQ-9)

Durante las <u>últimas 2 semanas</u>, ¿qué tan seguido ha tenido molestias debido a los siguientes problemas? *(Marque con un "□" para indicar su respuesta)*	**Ningún día**	**Varios días**	**Más de la mitad de los días**	**Casi todos los días**
1. Poco interés o placer en hacer cosas	0	1	2	3
2. Se ha sentido decaído(a), deprimido(a) o sin esperanzas	0	1	2	3
3. Ha tenido dificultad para quedarse o permanecer dormido(a), o ha dormido demasiado	0	1	2	3
4. Se ha sentido cansado(a) o con poca energía	0	1	2	3
5. Sin apetito o ha comido en exceso	0	1	2	3
6. Se ha sentido mal con usted mismo(a) – o que es un fracaso o que ha quedado mal con usted mismo(a) o con su familia	0	1	2	3
7. Ha tenido dificultad para concentrarse en ciertas actividades, tales como leer el periódico o ver la televisión	0	1	2	3
8. ¿Se ha movido o hablado tan lento que otras personas podrían haberlo notado? o lo contrario – muy inquieto(a) o agitado(a) que ha estado moviéndose mucho más de lo normal	0	1	2	3
9. Pensamientos de que estaría mejor muerto(a) o de lastimarse de alguna manera	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

Si marcó <u>cualquiera</u> de los problemas, ¿qué tanta <u>dificultad</u> le han dado estos problemas para hacer su trabajo, encargarse de las tareas del hogar, o llevarse bien con otras personas?

No ha sido difícil	**Un poco difícil**	**Muy difícil**	**Extremadamente difícil**
☐	☐	☐	☐

ANNEX 6: GLOBAL PHYSICAL ACTIVITY QUESTIONNAIRE (GPAQ).

Actividad física

A continuación, voy a preguntarle por el tiempo que pasa realizando diferentes tipos de actividad física. Le ruego que intente contestar a las preguntas aunque no se considere una persona activa.

Piense primero en el tiempo que pasa en el trabajo, que se trate de un empleo remunerado o no, de estudiar, de mantener su casa, de cosechar, de pescar, de cazar o de buscar trabajo *[inserte otros ejemplos si es necesario]*. En estas preguntas, las "actividades físicas intensas" se refieren a aquéllas que implican un esfuerzo físico importante y que causan una gran aceleración de la respiración o del ritmo cardíaco. Por otra parte, las "actividades físicas de intensidad moderada" son aquéllas que implican un esfuerzo físico moderado y causan una ligera aceleración de la respiración o del ritmo cardíaco.

	Pregunta	Respuesta	Código
En el trabajo			
49	¿Exige su trabajo una actividad física intensa que implica una aceleración importante de la respiración o del ritmo cardíaco, como [*levantar pesos, cavar o trabajos de construcción*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P 4*	P1
50	En una semana típica, ¿cuántos días realiza usted actividades físicas intensas en su trabajo?	Número de días ⌊__⌋	P2
51	En uno de esos días en los que realiza actividades físicas intensas, ¿cuánto tiempo suele dedicar a esas actividades?	Horas: minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ (hrs / mins)	P3 (a-b)
52	¿Exige su trabajo una actividad de intensidad moderada que implica una ligera aceleración de la respiración o del ritmo cardíaco, como caminar deprisa *[o transportar pesos ligeros]* durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P7*	P4
53	En una semana típica, ¿cuántos días realiza usted actividades de intensidad moderada en su trabajo?	Número de días ⌊__⌋	P5
54	En uno de esos días en los que realiza actividades físicas de intensidad moderada, ¿cuánto tiempo suele dedicar a esas actividades?	Horas: minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ (hrs / mins)	P6 (a-b)
Para desplazarse			
	En las siguientes preguntas, dejaremos de lado las actividades físicas en el trabajo, de las que ya hemos tratado. Ahora me gustaría saber cómo se desplaza de un sitio a otro. Por ejemplo, cómo va al trabajo, de compras, al mercado, al lugar de culto [*insertar otros ejemplos si es necesario*]		
55	¿Camina usted o usa usted una bicicleta al menos 10 minutos consecutivos en sus desplazamientos?	Sí 1 No 2 *Si No, Saltar a P 10*	P7
56	En una semana típica, ¿cuántos días camina o va en bicicleta al menos 10 minutos consecutivos en sus desplazamientos?	Número de días ⌊__⌋	P8
57	En un día típico, ¿cuánto tiempo pasa caminando o yendo en bicicleta para desplazarse?	Horas: minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ (hrs / mins)	P9 (a-b)
En el tiempo libre			
	Las preguntas que van a continuación excluyen la actividad física en el trabajo y para desplazarse, que ya hemos mencionado. Ahora me gustaría tratar de deportes, fitness u otras actividades físicas que practica en su tiempo libre [inserte otros ejemplos si llega el caso].		
58	¿En su tiempo libre, practica usted deportes/fitness intensos que implican una aceleración importante de la respiración o del ritmo cardíaco como [*correr, jugar al fútbol*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Si No, Saltar a P 13*	P10
59	En una semana típica, ¿cuántos días practica usted deportes/fitness intensos en su tiempo libre?	Número de días ⌊__⌋	P11
60	En uno de esos días en los que practica deportes/fitness intensos, ¿cuánto tiempo suele dedicar a esas actividades?	Horas: minutos ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ (hrs / mins)	P12 (a-b)

SECCIÓN PRINCIPAL: Actividad física (en el tiempo libre) sigue.			
Pregunta		**Respuesta**	**Código**
61	¿En su tiempo libre practica usted alguna actividad de intensidad moderada que implica una ligera aceleración de la respiración o del ritmo cardíaco, como caminar deprisa, [*ir en bicicleta, nadar, jugar al volleyball*] durante al menos 10 minutos consecutivos? *(INSERTAR EJEMPLOS Y UTILIZAR LAS CARTILLAS DE IMÁGENES)*	Sí 1 No 2 *Sí No, Saltar a P16*	P13
62	En una semana típica, ¿cuántos días practica usted actividades físicas de intensidad moderada en su tiempo libre?	Número de días ⌊__⌋	P14
63	En uno de esos días en los que practica actividades físicas de intensidad moderada, ¿cuánto tiempo suele dedicar a esas actividades?	Horas: minutos ⌊__⌋ : ⌊__⌋ hrs mins	P15 (a-b)
Comportamiento sedentario			
La siguiente pregunta se refiere al tiempo que suele pasar sentado o recostado en el trabajo, en casa, en los desplazamientos o con sus amigos. Se incluye el tiempo pasado [ante una mesa de trabajo, sentado con los amigos, viajando en autobús o en tren, jugando a las cartas o viendo la televisión], pero no se incluye el tiempo pasado durmiendo. *[INSERTAR EJEMPLOS] (UTILIZAR LAS CARTILLAS DE IMÁGENES)*			
64	¿Cuándo tiempo suele pasar sentado o recostado en un día típico?	Horas: minutos ⌊__⌋ : ⌊__⌋ hrs mins	P16 (a-b)

ANNEX 7: FOOD FREQUENCY QUESTIONNAIRE (FFQ).

Para cada alimento, señalar cuantas veces como media ha tomado la cantidad que se indica desde la última entrevista. Debe tener en cuenta las veces que toma el alimento solo y cuando lo añade a otro alimento o plato. Por ejemplo, en el caso del huevo, considere cuando lo toma solo (Ej. frito o cocido) y cuando lo toma añadido o mezclado con otros platos. Si en estos tres meses ha venido comiendo una tortilla de 2 huevos cada 2 días, deberá marcar "1 por día". No debe considerar el huevo que va con los productos de bollería o dulces.

I. LACTEOS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
1. Leche entera (1 vaso o taza, 200 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
2. Leche semi-desnatada (1 vaso, 200cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
3. Leche desnatada (1 vaso, 200cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
4. Leche condensada (1 cucharada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
5. Nata o crema de leche (1 cucharada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
6. Yogur entero (uno, 125 gramos)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
7. Yogur desnatado (uno, 125 gramos)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
8. Requesón, queso blanco o fresco (una porción o ración, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
9. Queso curado, semi-curado, o cremoso (un trozo, 50 gramos)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
10. Natillas, flan, puding (uno)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
11. Helados (1 cucurucho, vasito o bola)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
II. HUEVOS, CARNES, PESCADOS	**Nunca ó <1 mes**	**1-3 por mes**	**1 por sem**	**2-4 por sem**	**5-6 por sem**	**1 por día**	**2-3 por día**	**4-5 por día**	**6+ por día**
12. Huevos de gallina (uno)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
13. Pollo CON piel (1 plato mediano o pieza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
14. Pollo SIN piel (1 plato mediano o pieza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
15. Carne de ternera, cerdo, cordero como plato principal (1 plato mediano o pieza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
16. Carne de caza: conejo, codorniz, pato (1 plato)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
17. Hígado de ternera, cerdo, pollo (1 plato, ración o pieza mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
18. Vísceras: callos, sesos, mollejas (1 ración, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
19. Embutidos: jamón, salchichón, salami, mortadela, (1 ración de unos 50 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
20. Salchichas y similares (una mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
21. Patés, foie-gras (media ración, 50 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
22. Hamburguesa (una mediana, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
23. Tocino, beicon, panceta (2 tiras o lonchas, 50 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
24. Pescado frito variado (1 plato mediano o ración)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
25. Pescado hervido o plancha BLANCO: merluza, lenguado, dorada (1 plato o ración)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
26. Pescado hervido o plancha AZUL: atún, emperador, bonito, (plato o ración)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
27. Otros pescados azules: caballa, sardinas, boquerón/anchoas, salmón	①	②	③	④	⑤	⑥	⑦	⑧	⑨
28. Una lata pequeña de conserva de atún o bonito en aceite	①	②	③	④	⑤	⑥	⑦	⑧	⑨
29. Una lata pequeña de conserva de sardinas o caballa en aceite	①	②	③	④	⑤	⑥	⑦	⑧	⑨
30. Pescados en salazón y/o ahumados: anchoas, bacalao, salmón (media ración, 50g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
31. Almejas, mejillones, ostras (1 ración, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
32. Calamares, chipirones, sepia, choco, pulpo (1 ración o plato, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
33. Marisco: gambas, cangrejo, langostino, langosta (1 ración 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨

III. VERDURAS, LEGUMBRES.	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
34. Espinacas o acelgas cocinadas (1 plato mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
35. Col, coliflor, brócolis cocinadas (1 plato mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
36. Lechuga, endibias, escarola (1 plato mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
37. Tomate (uno mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
38. Cebolla (una mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
39. Zanahoria, calabaza (una o plato pequeño)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
40. Judías verdes cocinadas (1 plato)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
41. Berenjenas, calabacines, pepinos (uno)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
42. Pimientos (uno)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
43. Alcachofas (una ración o plato mediano, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
44. Espárragos (una ración o plato)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
45. Maíz hervido (plato o lata pequeña, 82 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
46. Legumbres: lentejas, garbanzos, judías pintas o blancas (1 plato mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
IV. FRUTAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
47. Naranjas, mandarinas (Una)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
48. Zumo de naranja natural (un vaso pequeño, 125 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
49. Plátano (uno)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
50. Manzana, pera (una mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
51. Melocotón, nectarina, albaricoque (uno mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
52. Sandía, melón (1 tajada o cala, mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
53. Uvas (un racimo mediano o plato de postre)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
54. Prunas, ciruelas frescas/secas (una, 37 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
55. Kiwi (una unidad)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
56. Aceitunas (un platito o tapa de unas 15 unidades pequeñas)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
57. Frutos secos: almendras, cacahuetes, piñones, avellanas (1 platito o bolsita, 30g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
V. PAN, CEREALES Y SIMILARES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
58. Pan blanco (Una pieza pequeña o 3 rodajas de pan de molde, 60 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
59. Pan integral (Pieza pequeña o 3 rodajas de pan de molde)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
60. Cereales desayuno (30 g en seco)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
61. Patatas fritas (1 ración o plato, 100 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
62. Patatas cocidas, asadas (1 patata mediana)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
63. Bolsa de patatas fritas (1 bolsa pequeña, 25-30 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
64. Arroz cocinado (1 plato mediano)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
65. Pastas: espaguetis, fideos, macarrones y similares (1 plato)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
66. Pizza (1 porción o ración, 200 g)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
VI. ACEITES, GRASAS Y DULCES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
67. Aceite de oliva añadido en la mesa a ensalada, pan y a platos (1 cucharada sopera)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
68. Otros aceites vegetales (ídem): girasol, maíz, soja (1 cucharada sopera)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
69. Margarina añadida al pan o la comida (1 cucharada o untada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
70. Mantequilla añadida al pan o la comida (1 cucharada o untada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
71. Galletas tipo María (1 galleta)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
72. Galletas con chocolate (1 galleta doble)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
73. Bollería: croissant, donut, magdalena, bizcocho, tarta o similar (uno o porción)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
74. Chocolate, bombones y similares (1 barrita o 2 bombones)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
75. Chocolate en polvo, cola-cao y similares (1 cucharada sopera)	①	②	③	④	⑤	⑥	⑦	⑧	⑨

VII. BEBIDAS Y MISCELANEAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
76. Vino tinto (1 vaso, 125 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
77. Vino blanco o rosado (1 vaso, 125 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
78. Jerez, vinos secos, vermú (copa, 50 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
79. Cerveza (una caña o botellín 1/5, 200 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
80. Cerveza sin alcohol (una caña o botellín 1/5, 200 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
81. Licores (20-25º): de frutas (manzana), de crema (Catalana, *Bayleys*) (1 copa, 50 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
82. Brandy, ginebra, ron, whisky, vodka, aguardientes 40º (1 copa, 50 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
83. Refrescos normales de cola, naranja, limón (ej. *coca-cola, fanta*) (Uno, 250 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
84. Refrescos sin azúcar cola, naranja, limón (ej. *coca-cola o pepsi light*) (Uno, 250 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
85. Agua del grifo (1 vaso, 250 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
86. Agua embotellada sin gas (1 vaso, 250 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
87. Agua embotellada con gas (1 vaso, 250 cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
88. Zumo de frutas envasado (1 vaso o envase de 200cc)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
89. Café (1 taza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
90. Café descafeinado (1 taza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
91. Sopa o puré de verduras (un plato)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
92. Croquetas de pollo, jamón (una)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
93. Croquetas, palitos o delicias de pescado fritos (una)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
94. Mayonesa (1 cucharada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
95. Salsa de tomate (media taza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
96. Ketchup ó catchup (1 cucharada sopera)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
97. Sal añadida a los platos en la mesa (1 pizca del salero o pellizco con dos dedos)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
98. Ajo (1 diente)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
99. Mermeladas, miel (1 cucharada)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
100. Azúcar (ej. en el café, postres, etc.) (1 cucharadita)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
101. Té o Infusiones (1 taza)	①	②	③	④	⑤	⑥	⑦	⑧	⑨
¿Consume algún otro alimento regularmente al menos una vez a la semana?									
_____	①	②	③	④	⑤	⑥	⑦	⑧	⑨
_____	①	②	③	④	⑤	⑥	⑦	⑧	⑨

No olvidar marcar todas las casillas

Consumo de suplementos vitamínicos o minerales. Referido a los meses previos, desde la última entrevista hasta ahora. ¿Ha tomado suplementos de vitaminas o minerales?...

	Marca y presentación	Dosis semanal (comp/sem)	Fecha inicio (mes/año)	¿Sigue tomándolo?	Si no, fecha de finalización
a. Sal yodada	_____	_____	___/_____	① Si ②No	___/_____
b. Leche con vit A+D	_____	_____	___/_____	① Si ②No	___/_____
c. Leche rica en Calcio	_____	_____	___/_____	① Si ②No	___/_____
d. Fibra/supl ricos en fibra	_____	_____	___/_____	① Si ②No	___/_____
e. Multivitaminas	_____	_____	___/_____	① Si ②No	___/_____
f. Acido fólico	_____	_____	___/_____	① Si ②No	___/_____
g. Complejo A + E	_____	_____	___/_____	① Si ②No	___/_____
h. Vitamina A	_____	_____	___/_____	① Si ②No	___/_____
i. Vitamina E	_____	_____	___/_____	① Si ②No	___/_____
j. Vitamina C	_____	_____	___/_____	① Si ②No	___/_____
i. Hierro	_____	_____	___/_____	① Si ②No	___/_____
j. Calcio	_____	_____	___/_____	① Si ②No	___/_____
l. Complejo B	_____	_____	___/_____	① Si ②No	___/_____
m. Zinc	_____	_____	___/_____	① Si ②No	___/_____
n. Otros Suplementos	_____	_____	___/_____	① Si ②No	___/_____

1. ¿Ha seguido usted algún tipo de dieta desde la última entrevista?

(Si responde NO pasar a pregunta 3)

① No ② Sí ⑨ No sabe/No contesta

2. ¿Podría indicar el motivo de seguir esta dieta? ***Puede marcar más de una respuesta***

① para controlar su peso
② porque tiene colesterol
③ porque tiene azúcar o diabetes
④ porque tiene problemas de estómago
⑤ porque tiene problemas de vesícula o hígado
⑥ porque tiene problemas de tensión alta o de corazón
⑦ porque tiene problemas de riñón
⑧ porque tiene alergia a algunos alimentos
⑨ porque tiene ácido úrico o gota
⑩ porque es vegetariana
①① por otro motivo, ¿cual? _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

3. Desde la última entrevista ¿cómo ha cambiado su ingesta para los siguientes grupos de alimentos, con respecto a la del año antes del embarazo?

	Eliminado	↓	Igual	↑	Ns/Nc
a. Lácteos y derivados	①	②	③	④	⑨
b. Huevos	①	②	③	④	⑨
c. Carne	①	②	③	④	⑨
d. Pescado	①	②	③	④	⑨
e. Verduras	①	②	③	④	⑨
f. Legumbres	①	②	③	④	⑨
g. Frutas	①	②	③	④	⑨
h. Pan	①	②	③	④	⑨
i. Aceite de oliva	①	②	③	④	⑨
j. Mantequilla/margarina	①	②	③	④	⑨
k. Azúcar/dulces	①	②	③	④	⑨
l. Bebidas alcohólicas	①	②	③	④	⑨

4. ¿Con qué frecuencia come comidas fritas?

① A diario.
② 5-6 veces por semana.
③ 2-4 veces por semana.
④ 1 vez por semana.
⑤ Menos de 1 vez por semana. ⑨ Ns/Nc

5. ¿Cuándo come carne, cómo de hecha le gusta comerla?

① No como carne (*pasar a pregunta 9*)
② Cruda
③ Poco hecha
④ Hecha
⑤ Muy hecha. ⑨ Ns/Nc

6. ¿Qué hace Vd. con la grasa visible, cuando come carne?

① La quita toda.
② Quita la mayoría.
③ Quita un poco.
④ No quita nada. ⑨ Ns/Nc

7. ¿Cómo suele comer la carne

	Nunca	Veces al Mes	Semana	Día	Ns/Nc
a. A la plancha	___	___	___	___	___
b. A la parrilla (grill)	___	___	___	___	___
c. Asada (horno)	___	___	___	___	___
d. Frita en aceite	___	___	___	___	___
e. Guisada	___	___	___	___	___

8. ¿Cómo de frecuente come lo tostado o quemado de la carne?

① Nunca o menos de una vez al mes
② Una vez al mes
③ 2-3 veces al mes
④ 1 vez a la semana
⑤ 2 o más veces a la semana ⑨ Ns/Nc

9. ¿Cómo de frecuente come la parte tostada del pescado?

① Nunca o menos de una vez al mes
② Una vez al mes
③ 2-3 veces al mes
④ 1 vez a la semana
⑤ 2 o más veces a la semana ⑨ Ns/Nc

10. ¿Cómo de frecuente come el tostado (*socarrat*) de la paella?,

① Nunca o menos de una vez al mes
② Una vez al mes
③ 2-3 veces al mes
④ 1 vez a la semana
⑤ 2 o más veces a la semana ⑨ Ns/Nc

11. ¿Qué clase de grasa o aceite usa para:

	Mantequilla	Margarina	Ac.Oliva	Ac.Ol virgen	Ac. Veg	Mezcla Ac.
ALIÑAR	___	___	___	___	___	___
COCINAR	___	___	___	___	___	___
FREIR	___	___	___	___	___	___

ANNEX 8. BIOBANK INFORMATION DOCUMENT.

FULL D'INFORMACIÓ AL PACIENT

dIBGi Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta	**Consentiment Informat** **Biobanc de l'Institut d'Investigació Biomèdica de Girona**	Bio Banc

A l'Hospital Universitari de Girona Dr Josep Trueta (HUDJT) i/o altres Centres Hospitalaris adscrits, igual que en la majoria d'hospitals, a més de l'assistència als pacients, es realitza investigació biomèdica. La finalitat d'aquesta investigació es progressar en el coneixement de les malalties y en la seva prevenció, diagnòstic i tractament. Aquesta investigació biomèdica requereix recollir dades clíniques y mostres biològiques de pacients i donants per analitzar-los i obtenir conclusions amb l'objectiu de conèixer millor las malalties i avançar cap el seu diagnòstic i/o tractament. Les mostres i dades clíniques obtingudes, una vegada utilitzades amb aquesta finalitat, resulten també útils i necessàries per la investigació. De fet, molts del avenços científics obtinguts en aquest últims anys en medicina son fruit d'aquest tipus d'estudis.

Sol·licitem la seva autorització per a l'obtenció d'una mostra biològica addicional que se li extraurà a l'GUGJT i/o altres Centres Hospitalaris adscrits, amb la finalitat de dipositar-la al Biobanc IDIBGI, així com la seva autorització per utilitzar la informació clínica associada a aquest material biològic per prosseguir amb la investigació biomèdica.

Seguint el que estableix la *Llei 14/2007*, d'investigació Biomèdica, la *Llei Orgànica 15/1999*, de Protecció de Dades Personals, i las seves normes de desenvolupament, li sol·licitem que llegeixi detingudament aquest document d'informació i el consentiment informat que se li adjunta al final per a la seva firma, si està d'acord en participar en aquesta proposta.

FINALIDAD DE LA INVESTIGACIÓ: Progressar en el coneixement de les malalties. La finalitat de la investigació és millorar el nostre coneixement de les malalties. Las mostres, les dades clíniques i analítiques i les proves d'imatge s'utilitzaran per a la recerca biomèdica.

MOSTRES BIOLÒGIQUES I INFORMACIÓ ASSOCIADA: Les mostres obtingudes es custodiaran i conservaran en el Biobanc IDIBGI fins a la seva extinció. Es guardarà i disposarà de la mostra biològica addicional de femta per a realitzar estudis d'investigació biomèdica, sense que aquest fet li causi molèsties addicionals. La donació d'aquestes mostres cedides al Biobanc IDIBGI no impedirà que vostè o la seva família puguin usar-les quan sigui necessari per motius de salut. Les mostres i la informació associada a questes es custodiaran i conservaran al Biobanc fins a la seva extinció.

Aquest Biobanc és un establiment sense ànim de lucre i inscrit en el Registro Nacional de Biobancos dependent de l'Institut de Salud Carlos III amb la referencia B.0000872, que acull col·leccions organitzades de mostres biològiques i informació associada en les condicions i garanties de seguretat que exigeix la legislació anteriorment referida i els codis de conducta aprovats per els Comitès d'Ètica. Les esmentades mostres i la seva informació associada queden disponibles però aquells investigadors que ho sol·liciten al Biobanc.

Qualsevol estudi d'investigació per al qual se sol·liciti la utilització d'aquestes dades o mostes haurà de disposar sempre de l'aprovació del Comitè d'Ètica Clínica (CEIC) competent, que

vetllarà per a què els investigadors desenvolupin els seus estudis seguint sempre les més estrictes normes ètiques i legals. A més, el comitè científic del Biobanc garantirà que els projectes siguin d'excel·lència científica. A partir de les mostres donades, en els casos en que la investigació ho requereixi, es realitzaran estudis genètics, i a partit d'ells es port obtenir informació sobre la seva salut i la dels seus familiars. Sempre s'actuarà vetllant per la protecció d'aquesta informació. En el cas de ser necessària alguna mostra addicional, la institució sanitària es podria posar en contacte amb vostè per a sol·licitar-li novament la seva col·laboració. En aquest cas se li informarà dels motius i se li sol·licitarà de nou el seu consentiment.

PROTECCIÓ DE DADES I CONFICENCIALITAT: Les mostres es conservaran codificades. Les dades personals que es recullin seran obtingudes, tractades i emmagatzemades complint en tot moment el deure del secret, d'acord amb la legislació vigent en matèria de protecció de dades de caràcter personal. La identificació de mostres biològiques del Biobanc serà sotmesa a un procés de codificació. A cada mostra se li assigna un codi d'identificació, que serà utilitzat per els investigadors. Nomes el personal autoritzat per el Biobanc podrà relacionar la seva identitat amb els citats codis. Mitjançat aquest procés els investigador que sol·licitin mostres al Biobanc no podran conèixer cap dada que reveli la seva identitat. De la mateixa manera, encara que els resultats obtinguts de la investigació realitzada amb les seves mostres es publiquen en revistes científiques, la seva identitat no serà facilitada. Les seves mostres i dades clíniques associades a les mateixes passaran a formar part del fitxer del Biobanc, inscrit en l'Agencia de Protecció de Dades sota al responsabilitat de l'Institut d'Investigació Biomèdica de Girona (IDIBGI). Vostè podrà exercir els seus drets d'accés, rectificació, cancel·lació i objecció, així com obtenir informació de l'ús de les seves mostres i dades associades, dirigint-se a:

DIRECCIÓ DEL BIOBANC IDIBGI	Avinguda de França s/n
Hospital Universitari de Girona Dr. Josep Trueta	17007 Girona
Biobanc@IDIBGI.org	Tlfn. 972 940 282

CARÀCTER ALTRUISTA DE LA DONACIO: **La cessió de mostres biològiques que vostè realitza al Biobanc IDIBGI és gratuïta**. La donació te per disposició legal caràcter altruista, per la qual cosa vostè no obtindrà ni ara ni en el futur cap benefici econòmic de la mateixa, ni tindrà drets sobre possibles beneficis comercials dels descobriments que es puguin aconseguir com a resultat de la investigació biomèdica.

PARTICIPACIÓ VOLUNTARIA: **La seva negativa NO repercutirà en la seva assistència medica, present o futura**. La seva participació és totalment voluntària. Si firma el consentiment informat, confirmarà que desitja participar. Port negar-se a participar o retirar el seu consentiment en qualsevol moment posterior a la firma sense haver d'explicar els motius i que això repercuteix en la seva assistència medica, present o futura.

COST I RISCOS ASSOCIATS: **La seva donació no li suposa CAP cost. L'extracció de la mostra no suposarà cap cost econòmic per vostè**. En el cas d'una mostra de femta, l'extracció es realitzarà en el context assistencial, de manera que no afegeix cap risc addicional per a vostè. Mai es realitzarà una intervenció exclusivament per l'obtenció de mostres per a investigació.

REVOCACIÓ DEL CONSENTIMENT: **Si vostè decideix firmar aquest consentiment podrà també cancel·lar-lo lliurament.** Això comportarà la destrucció de les seves mostres.

Si en un futur vostè volgués anul·lar el seu consentiment, les seves mostres biològiques serien destruïdes i les dades associades a les mateixes serien retirades del Biobank. També podria sol·licitar l'anonimització de les mostres, de manera que en aquest cas s'eliminaria la relació entre las seves dades personals i les seves mostres biològiques i dades clíniques associades. Els efectes d'aquesta cancel·lació o anonimització no es podrien estendre a la investigació que ja s'hagi realitzat. Si desitges cancel·lar el consentiment, ho hauria de sol·licitar per escrit a la Direcció del Biobanc IDIBGI, a l'adreça anteriorment mencionada.

INFORMACIÓ SOBRE ELS RESULTATS DE LA INVESTIGACIÓ: **Se li proporcionarà informació si vostè la desitja rebre.** En el cas que vostè ho demani expressament, el Biobanc podrà proporcionar informació sobre quines son les investigacions en que s'han utilitzat les seves mostes i dels resultats globals d'aquestes investigacions, excepte en el cas de cancel·lació o anonimització. Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la practica clínica, per el que no han de ser considerats amb valor clínic per a vostè. Malgrat això, en el cas que aquestes investigacions proporcionin dades que poguessin ser clínica o genèticament rellevants per a vostè i interessar a la seva salut o a la seva família, li seran comunicats si així ho estima oportú. Així mateix, podria donar-se el cas que s'obtingui informació rellevant per a la seva familiar. En aquest supòsit, li correspondrà a vostè decidir si vol o no que aquesta informació li sigui comunicada. En cas afirmatiu, ha de consignar-ho a la casella que apareix al final d'aquest document. Si vostè no desitja aquesta informació, tingui en compte que la llei estableix que, quan la informació obtinguda sigui necessària per a evitar un greu perjudici per a la salut dels seus familiar biològics, un Comitè d'experts estudiarà el cas i haurà de decidir si es convenient informar als afectats o als seus representants legals.

Si us plau, pregunti al personal sanitari que li ha comunicat aquesta informació sobre qualsevol dubte que pugui tenir, ara o en el futur, en relació a aquest consentiment. Així mateix, port comentar els seus dubtes al seu metge, que el posarà en contacte amb el personal sanitari autoritzat.

BIOBANC IDIBGI

ANNEX 9: BIOBANK CONSENT FORM.

CONSENTIMENT INFORMAT

dIBGi Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta	**Consentiment Informat** **Biobanc de l'Institut d'Investigació Biomèdica de Girona**	Bio Banc

Si ha comprès la informació que si li ha proporcionat en el document informatiu, resolt qualsevol dubte que pogués tenir i decideix col·laborar amb el Biobanc IDIBGI en els termes abans explicats, si us plau, llegeixi i firmi a continuació aquest full:

Qui signa el present document autoritza a l'HUGTJ i/o altres Centres Hospitalaris adscrits a obtenir la mostra biològica addicional de FEMTA per tal que puguin ser incorporada al Biobanc IDIBGI, el qual podrà emmagatzemar i utilitzar científicament tant la informació clínica i assistencial del seu historial mèdic com les proves d'imatge i les mostres biològiques obtingudes, amb la finalitat de desenvolupar projectes d'investigació biomèdica, sempre que aquests comptin amb l'obligada aprovació del Comitè d'Ètica d'Investigació competent.

Confirmo que:

1. Autoritzo que la mostra biològica cedida i la informació clínica associada s'utilitzi en investigacions:
   Nacionals: ☐ Sí ☐ No Internacionals: ☐ Sí ☐ No

2. Desitjo que se'm comuniqui la informació derivada de la investigació que realment sigui rellevant i aplicable per a la meva salut o la de la meva família:
   ☐ Sí ☐ No Telèfon o email de contacte.....

3. Autoritzo a ser contactat en el cas de necessitar més informació o mostres biològiques addicionals:
   ☐ Sí ☐ No Telèfon o email de contacte.....

4. He expressat el meu desig de que se'm respectin les següents excepcions respecte a l'objectiu i mètodes de les investigacions:
   .....
   .....

DONANT	PERSONA QUE INFORMA	TESTIMONI/TUTOR
Nom Cognoms DNI Edat Signatura	Nom Cognoms DNI Edat Signatura	Nom Cognoms DNI Edat Signatura

A ....., a .....de.....de.....

ANNEX 10: ADVERSE CHILDHOOD EXPERIENCE QUESTIONNAIRE (ACEQ)

1. Algun dels seus pares o algun altre adult a casa seva freqüentment o molt freqüentment... Li va cridar, el va insultar, el va fer sentir menys, o el va humiliar?

O

¿Es va comportar d'alguna manera que va fer que sentís por o que se sentís físicament ferit?

☐ Sí ☐ No

2. Algun dels seus pares o algun altre adult a casa seva freqüentment o molt freqüentment…Li va empènyer, li va agafar bruscament, li va donar una bufetada, o li va tirar amb alguna cosa?

O

El va colpejar tan fort que li deixo marques o ferides?

☐ Sí ☐ No

3. Algun adult o una altra persona almenys 5 anys més gran que vostè alguna vegada… Va tocar el cos o el va obligar a tocar el cos d'una manera sexual?

O

Va intentar o va tenir sexe oral, anal o vaginal amb vostè?

☐Sí ☐ No

4. Sent freqüentment o molt freqüentment…Que ningú a la seva família li ha volgut o ha pensat que era important o especial?

O

Que a la seva família no es protegien mútuament, ni eren propers els uns als altres, ni sigui recolzaven mútuament

☐Sí ☐No

5. Sent amb freqüència…Que no té prou menjar que ha d'usar roba bruta, o que no té qui el protegeixi?

O

Que els seus pares estaven molt borratxos o drogats per cuidar-lo o portar-lo al doctor si era necessari?

☐Sí ☐No

6. Va perdre algun dels seus pares biològics com a resultat de divorci, abandó, o alguna altra raó?

☐Sí ☐No

7. Alguna vegada la seva mare o madrastra: Freqüentment o molt freqüentment va ser empesa, agafada amb brusquedat, bufetejada, o li van llençar algun objecte?

O

De tant en tant, freqüentment, o molt freqüentment va ser petejada, mossegada, enganxada amb el puny, o enganxada amb algun objecte dur?

O

Alguna vegada va ser copejada repetidament per alguns minuts o amenaçada amb pistola o ganivet?

☐Sí ☐No

8. Heu viscut amb algú que té problemes amb l'alcohol, que és/va ser alcohòlic, o

que consumia drogues?

☐Sí ☐No

9. Algun membre de la seva llar patia de depressió o malaltia mental, o algun

membre de la seva llar intent suïcidar-se?

☐Sí ☐No

10. Algú a casa seva va estar a la presó?

☐Sí ☐No

ANNEX 11: HOLMES AND RAHE STRESS SCALE

Anoteu el valor que correspon amb cadascuna de les situacions enumerades a continuació si s'han presentat durant l'últim any i sumi el total obtingut.

1. Mort del cònjuge	100
2. Divorci	73
3. Separació	65
4. Privació de la llibertat	63
5. Mort d'un familiar proper	63
6. Malaltia o incapacitat, greus	53
7. Matrimoni	50
8. Perdre la feina	47
9. Reconciliació de la parella	45
10. Jubilació	45
11. Malaltia dun parent proper	44
12. Embaràs	40
13. Problemes sexuals	39
14. Arribada d'un nou membre a la família	39
15. Canvis importants a la feina	39
16. Canvis importants a nivell econòmic	38
17. Mort d´un amic íntim	37
18. Canviar feina	36
19. Discussions amb la parella (canvi significatiu)	35
20. Demanar una hipoteca d'alt valor	31
21. Fer efectiu un préstec	30
22. Canvi de responsabilitats a la feina	29
23. Un fill/a abandona la llar (matrimoni, universitat)	29
24. Problemes amb la llei	29
25. Assoliments personals excepcionals	28
26. La parella comença o deixa de treballar	26
27. S'inicia o s'acaba el cicle d'escolarització	26
28. Canvis importants en les condicions de vida	25
29. Canvi en els hàbits personals	24
30. Problemes amb el cap	23
31. Canvi en l'horari o les condicions de treball	20
32. Canvi de residència	20
33. Canvi a una escola nova	20
34. Canvi en la forma o freqüència de les diversions	19
35. Canvi en la freqüència de les activitats religioses	19
36. Canvi en les activitats socials	18
37. Demanar una hipoteca o préstec menor	17
38. Canvis en els hàbits del son	16
39. Canvis al nombre de reunions familiars	15
40. Canvi en els hàbits alimentaris	15
41. Vacances	15
42. Nadal	12
43. Infraccions menors de la llei	11

ANNEX 12: DATA COLLECTION SHEET.

FULL D' INFORMACIÓ MÈDICA

EIPP:_____ Codi del participant:_____ Data: __/__/___

Telèfons de contacte: Participant:_____ Altres:_____

DADES PERSONALS

- Gènere: ☐ Masculí ☐ Femení
- Edat:
- Ètnia: ☐ Caucàsica ☐ Africana ☐ Asiàtica ☐ Magrebí ☐ Altres
- Alçada (cm): _____ Pes (kg): _____

ANTECEDENTS FAMILIARS:

- El seu pare o la seva mare han estat diagnosticats d'un trastorn depressiu?

  Pare: ☐ Sí ☐ No

  Mare: ☐ Sí ☐ No

ANTECEDENTS PATOLÒGICS:

- Historial mèdic:

ANTECEDENTS TÓXICS:

- De las drogues que apareixen a continuació, quines ha consumit durant el darrer any?

  Alcohol: ☐ Sí ☐ No

  Cànnabis: ☐ Sí ☐ No

  Opiacis: ☐ Sí ☐ No

  Cocaïna: ☐ Sí ☐ No

  Amfetamines: ☐ Sí ☐ No

- En cas de resposta afirmativa en la pregunta anterior, quin ha estat el seu consum?

  Alcohol: ☐ Un cop/dia ☐ Un cop/setmana ☐ Un cop/mes ☐ Un cop/any

  Cànnabis: ☐ Un cop/dia ☐ Un cop/setmana ☐ Un cop/mes ☐ Un cop/any

  Opiacis: ☐ Un cop/dia ☐ Un cop/setmana ☐ Un cop/mes ☐ Un cop/any

Cocaïna: ☐ Un cop/dia ☐ Un cop/setmana ☐ Un cop/mes ☐ Un cop/any

Amfetamines: ☐ Un cop/dia ☐ Un cop/setmana ☐ Un cop/mes ☐ Un cop/any

- Consumeix tabac? ☐ Sí ☐ No
- En cas de resposta afirmativa indiqui el número de cigarrets consumides por dia:

TRACTAMENT FARMACOLÓGIC

- Ha estat en tractament amb antibiòtics durant el darrer any? ☐ Sí ☐ No
- Actualment està en tractament amb algun fàrmac? ☐ Sí ☐ No

En cas de resposta afirmativa indiqui el nom del fàrmac:

DADES SOCIOECONÒMIQUES

- Pensant en l'ingrés total mensual de la vostra llar, indiqui com diria que arriba econòmicament el seu llar a final de mes?

☐ Amb moltes o alguna dificultat
☐ Molt fàcilment o fàcilment