MOLECULAR STUDY OF ORAL DYSBACTERIOSIS IN PEOPLE WITH OBESITY AND PREDIABETES OR DIABETES (SMILE)

ENDOCANNABINOIDS IN ORAL DYSBACTERIOSIS

29 JUNE 2023

HOSPITAL REGIONAL UNIVERSITARIO DE MALAGA (MALAGA, SPAIN).
Unidad Endocrinología y Nutrición, Hospital Civil, Pabellón 7, planta 2

VERSION 3

1. SUMMARY

1.1. STUDY TITLE

MOLECULAR STUDY OF ORAL DYSBACTERIOSIS IN PEOPLE WITH OBESITY AND PREDIABETES OR DIABETES (*SMILE*)

1.2. PRINCIPAL INVESTIGATOR OF THE TRIAL

Dr. Rodolfo Matias Ortiz Flores
Dr. Francisco Javier Bermudez Silva

1.3. CENTER WHERE THE TEST IS PLANNED TO BE CARRIED OUT

Hospital Regional Universitario de Malaga (Malaga).

1.4. RESEARCH ETHICS COMMITTEE THAT EVALUATED THE TRIAL

Investigación Provincial de Málaga Ethics Committee

1.5. MEDICATION/SUBSTANCE WITH WHICH THE TEST IS GOING TO BE CARRIED OUT

No medication or substance will be tested on patients, it is a descriptive observational study.

1.6. MAIN OBJECTIVE OF THE TRIAL

Investigate the possible differences in endocannabinoid levels and their correlation with inflammatory cytokines and microbial load in the oral cavity depending on the presence or absence of oral cavity disease in people with obesity and prediabetes or diabetes.

1.7. TRIAL DESIGN

Descriptive observational study.

1.8. ILLNESS OR DISORDER UNDER STUDY

- Prediabetes
- Diabetes mellitus type 2
- Obesity
- Oral dysbiosis
- Oral cavity disease (OCD)

1.9. MAIN AND SECONDARY VARIABLES

As MAIN VARIABLE, endocannabinoids in saliva and plasma (2-AG, AEA, PEA, OEA, DHEA, 2-LG, 2-OG; pmol/ml) will be studied .

And as SECONDARY VARIABLES are going to be studied:

- The bacteriological profile of the oral cavity (the following bacterial strains: *Aggregatibacter actinomycetemcomitans , Porphyromonas gingivalis , Tannerella forsythia , Treponema denticola , Prevotella intermedia, Fusobacterium nucleatum, Parvimonas micra, Campylobacter rectus, Eikenella corroe, Veillonella kindergarten* and *Actinomyces naeslundii* for periodontal disease; and *streptococci mutans , S. sanguis, S. mitior, S. salivarius* and *S. milleri* for dental caries) ,
- The concentration of inflammatory cytokines in saliva and blood (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-17, leptin, adiponectin, vascular endothelial growth factor [VEGF], Interferon [IFN] -γ and Tumor Necrosis Factor [TNF]-α),
- Glucose management (fasting blood glucose (mg/ dL), insulin (mUI/mL), HbA1c (%), HOMA-IR, HOMA2-IR, QUICKY, HOMA2%S, HOMA2%B) ,
- Adiposity and atherogenic profile [BMI (kg/m²)], waist circumference (cm), waist/hip ratio, waist/height ratio, blood pressure (mmHg), triglycerides (mg/dL),

total cholesterol (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), total cholesterol/HDL, LDL/HDL and Triglycerides/HDL ratios) ,

- Salivary parameters through sialometry (salivary flow), salivary viscosity, salivary pH and the impact profile on oral health (OHIP-14sp; WHO questionnaire) .

1.10. STUDY POPULATION AND TOTAL NUMBER OF PATIENTS

A total of 60 adult patients are expected to be included: 15 diagnosed with obesity and prediabetes with OCD, 15 diagnosed with obesity and prediabetes without OCD, 15 diagnosed with obesity and diabetes with OCD, and 15 diagnosed with obesity and diabetes without OCD.

1.11. TEST DURATION

Twelve months.

1.12. SCHEDULE AND EXPECTED COMPLETION DATE

- Study authorization date: Second quarter of 2023.
- Date of inclusion of the first patient: Fourth quarter of 2023.
- Date of inclusion of the last patient: Second quarter of 2024.
- Study closing date: Fourth quarter of 2024.

2. GENERAL INFORMATION

2.1. TEST IDENTIFICATION

Title and acronym :

MOLECULAR STUDY OF ORAL DYSBACTERIOSIS IN PEOPLE WITH OBESITY AND PREDIABETES OR DIABETES - ***SMILE***

2.2. DESCRIPTION OF THE MEDICATION/SUBSTANCE UNDER STUDY

No medication or substance will be tested on patients, this is a descriptive observational study

2.3. DATA RELATING TO THE PROMOTER

Fundación Pública Andaluza para la Investigación de Málaga en Biomedicina y Salud (FIMABIS)

2.4. DATA RELATING TO THE PRINCIPAL INVESTIGATOR

- Dr. Rodolfo Matias Ortiz Flores
- Dr. Francisco Javier Bermudez Silva

UGC Endocrinología y Nutrición, Hospital Regional de Málaga - Instituto de Investigación Biomédica de Málaga (IBIMA)

Hospital Civil, Pabellón 2, sótano, Laboratorio de Investigación

2.5. CENTER WHERE THE TEST IS PLANNED TO BE CARRIED OUT

Hospital Regional Universitario de Málaga
Pabellón C (Hospital Civil)
Plaza del Hospital Civil, s/n
29009 Málaga.

2.6. TEST DURATION

Twelve months.

3. RATIONAL BASIS OF THE ESSAY

3.1. INTRODUCTION

According to the International Diabetes Federation, 537 million adults (20-79 years) are living with **diabetes,** 1 in 10 people worldwide. This number is projected to rise to 643 million

by 2030 and 783 million by 2045 [1]. Type 2 diabetes (T2D) is the most common type of diabetes, accounting for more than 90% of all diabetes worldwide [2,3]. It has been estimated that, in 2021, 541 million adults worldwide (10.6% of the total) had glucose intolerance and some 319 million adults (6.2% of the world's adult population), had altered basal glucose (ABG) [1], and it is estimated that by the year 2045, these figures will increase to 730 million adults (11.4%) and 441 million adults (6.9%), respectively [1].

Both glucose intolerance and ABG are conditions of elevated blood glucose levels above the normal range and below the diagnostic threshold for T2D, both situations being collectively called **prediabetes**. Prediabetes implies a greater risk of future development of T2D and the appearance of cardiovascular disease (CVD) [3]. It is estimated that the cumulative incidence of progression of T2D at five years after the diagnosis of glucose intolerance or GBA is 26% and 50%, respectively. T2D, in the absence of proper control, can cause damage to multiple organs and systems, leading to disabling and life-threatening health complications, such as CVD, nerve damage (neuropathy), kidney damage (nephropathy), amputation of lower limbs, vision loss and even blindness. Prediabetes and T2D are also important risk factors for **oral cavity diseases** (**OCD)**, with this risk being three times higher than among people without diabetes [4,5]. Oral health depends on the maintenance of stable microbial communities, appearing oral disease occurs when pathogenic species exceed the normal flora [6].

In the oral cavity, the presence of representative pathogenic bacteria is usually associated with two main diseases; *streptococcus mutans* and *Lactobacilli* with dental caries, and *Porphyromonas gingivalis* , *Fusobacterium nucleatum* and *Treponema denticola* with periodontal disease [7]. Gingivitis is a frequent form of inflammation of the gums, a step prior to periodontitis. ECHO are complications associated with T2D, being generally underestimated and underdiagnosed. Interestingly, there is evidence of a bidirectional relationship between the T2D condition and said oral conditions, with salivary levels of different pathogens correlated with blood glucose levels [8–10]. However, although inflammation seems to play a central role, the molecular mechanisms that link these pathological conditions are currently poorly understood.

The **endocannabinoid system (ECS)** is a lipid signaling machinery that regulates various biological processes within the body [11,12]. Originally, it was composed of the lipid signaling molecules anandamide (AEA) and 2-arachidonoylglycerol (2-AG), two receptors, CB1 and CB2, and five enzymes responsible for the biosynthesis and degradation of these ligands: N - acyl phosphatidylethanolamine phospholipase D (NAPE-PD), diacylglycerol lipase alpha and beta (DAGL) for the synthesis of endocannabinoids, and fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) for the catabolism of AEA and 2-AG, respectively [13]. This original conception of the SEC, however, has turned out to be too simplistic. Receptor heterodimers, non-canonical cannabinoid-sensitive receptors, and the ability of some ligands to act as receptor allosteric modulators have amplified this signaling system.[14,15] constituting what is now called **an endocannabinoidome** [16,17]. It is now well known that the endocannabinoidome plays a key role in energy homeostasis and related diseases such as obesity, prediabetes, and T2D by acting on different organs, including the gut microbiota [18,19], and modulating key processes such as inflammation and oxidative stress.

For all these reasons, the present study aims to unravel the possible role of endocannabinoids and related lipids as mediators of OCD, which could help to develop new therapies based on this signaling system aimed at preventing and treating OCD in the context of prediabetes/T2D.

The association between T2D and periodontitis is bidirectional. Results from mechanistic studies indicate that T2D leads to a hyperinflammatory response in the periodontal microbiota and also affects inflammation resolution and repair, leading to accelerated periodontal destruction. On the other hand, it has been reported that periodontitis negatively affects glycemic control in patients with diabetes mellitus and contributes to the development of complications [20]. Mechanistic links between periodontitis and T2D involve elevations in interleukin (IL)-1-β, tumor necrosis factor-α, IL-6, activator of nuclear factor receptor-kappa B ligand/ osteoprotegerin, oxidative stress, and expression Toll -Like Receptor (TLR)-2/4 [21]. Therefore, inflammation is a central feature of the pathogenesis of diabetes and periodontitis.

However, the relationship between the oral microbiota and T2D is less explored. Several studies indicate that there are probably subtle differences in the microbial composition of the subgingival biofilm between people with and without T2D [22], but the clinical relevance of this is not fully clear. Such differences may arise from the effect of T2D by altering the local environment within the periodontal pocket, in such a way that the growth of certain bacterial species is favored [23]. Unfortunately, the specific mechanisms that exacerbate inflammation, oral microbiota dysbiosis, and periodontal disease in people with prediabetes and T2D are still unknown.

3.2. JUSTIFICATION

Diseases of the oral cavity are more frequent in people with prediabetes and diabetes, implying a decrease in the quality of life of these patients, adding to the multiple complications that these people develop during the progression of the disease. Precisely, one of the possible mechanisms that mediate the relationship between inflammation and oral dysbiosis in T2D is the endocannabinoidome. The novelty of this proposal consists precisely in studying the possible correlation between endocannabinoids and related lipids, inflammatory markers and oral dysbiosis in people with prediabetes and T2D, determining the levels of these molecules in saliva and blood samples. To date, there are no studies that have addressed the analysis of these relationships, which may open the door to new ways to prevent and treat OCD in people with prediabetes and T2D.

3.3. HYPOTHESIS

For all of the above, our **hypothesis** is the following: **the endocannabinoidome has a similar role in the oral microbiota as in the intestinal one, so that its deregulation produces dysbiosis and an inflammatory state in the oral cavity, which may be linked to the OCD appearance.**

In fact, elevated levels of the endocannabinoids AEA and 2-AG have been found in the saliva of obese people [24]. Furthermore, ECS has been described in the oral cavity [25–27]and several studies have demonstrated the presence and physiological relevance of the endocannabinoidome in the salivary glands [25,28].

3.4. AIMS

Based on this hypothesis, we propose the following main objective: **to study the possible differences in the levels of endocannabinoids (2-AG, AEA, PEA, OEA, DHEA, 2-LG, 2-OG; pmol/ml), both in saliva and plasma, in a sample of patients with/without oral cavity disease, and with obesity and prediabetes, or with obesity and T2D.**

As secondary objectives will be studied:

- The bacteriological profile of the oral cavity (increase in the bacterial 16S rRNA amplicon , compared to the reference genes, for the following bacterial strains: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola , Prevotella intermedia, Fusobacterium nucleatum , Parvimonas micra, Campylobacter rectus , Eikenella corroe, Veillonella kindergarten* and *Actinomyces naeslundii* for periodontal disease; and *streptococci mutans , S. sanguis, S. mitior , S. salivarius* and *S. milleri* for dental caries).
- Inflammatory cytokines in saliva and plasma (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-17, leptin, adiponectin, vascular endothelial growth factor [VEGF], Interferon [IFN]-γ and Tumor Necrosis Factor [TNF]-α).
- Glucose management through fasting blood glucose levels (mg/dL), insulin (mUI/mL), HbA1c (%), HOMA-IR, HOMA2-IR, QUICKY, HOMA2%S, HOMA2%B.
- Adiposity and atherogenic profile, through BMI (kg/m²), waist circumference (cm), waist/hip ratio, waist/height ratio, blood pressure (mmHg), triglycerides (mg/dL), total cholesterol (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), total cholesterol/HDL, LDL/HDL, and Triglycerides /HDL ratios.
- Salivary parameters (sialometry (salivary flow), salivary viscosity, salivary pH and the impact profile on oral health (OHIP-14sp; WHO questionnaire)).

3.5. BIBLIOGRAPHIC REFERENCES

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4. STUDY DESIGN

4.1. TYPE OF STUDY

Descriptive observational study.

A study with four groups is proposed: a group of obese diabetic subjects with a healthy mouth, a group of obese diabetic subjects with a diseased mouth, a group of obese pre-diabetic subjects with a healthy mouth, and a group of obese pre-diabetic subjects with sick mouth. All patients will be recruited on a voluntary and unpaid basis. After a meeting with each participant, where a detailed explanation about the study will be given, written informed consent will be obtained, as well as the necessary information to complete the medical evaluation questionnaire, which will allow determining their state of health and the criteria of inclusion and exclusion. Once the patient's eligibility is confirmed, they will be scheduled to fast for a saliva and blood extraction and an oral health questionnaire and/or an oral clinical examination.

The samples of the patients included in the study will be processed and frozen at -80ºC until their subsequent analysis following standardized protocols. The samples will be stored in the biobank of the Hospital Regional de Málaga-IBIMA, which is part of the biobank of the public health system of Andalucía (BSSPA).

5. SUBJECT SELECTION

5.1. STUDY POPULATION

A population of patients of both sexes aged 40-65 years, with obesity and diagnosed with pre-diabetes, and with obesity and diabetes, and who meet the rest of the inclusion criteria, as well as who do not present any of the criteria, will be studied. of exclusion, and who agree to participate voluntarily in the study. For this, three studies led by our unit will be based on: The Pizarra study, an epidemiological study of the UGC of Endocrinología y Nutrición del Hospital Regional de Málaga that has provided detailed information on anthropometric, biochemical, eating habits and style parameters. of life in a cohort of about 1,700 subjects, the di@bet.es study, a national epidemiological study carried out on a sample of more than 5,000 people, coordinated by this Unit, and the April study, a nutritional intervention study on people with obesity and prediabetes. In addition, the databases of the obesity consultations of the UGC of Endocrinología y Nutrición del Hospital Regional de Málaga will be available. Based on these databases, we will proceed to contact the people who could meet the inclusion criteria, in order to arrange a recruitment visit for the study, following the guidelines of the Declaration of Helsinki (Fortaleza 2013) and the Standards of Good Clinical Practices. Personal data will be processed in accordance with REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of April 27, 2016 on the protection of natural persons with regard to the processing of personal data and the free movement of these dates; and Organic Law 3/2018, of December 5, Protection of Personal Data and guarantee of digital rights

5.2. SAMPLE SIZE

Based on previous publications that have reported the levels of endocannabinoids in saliva (20), at least 12 subjects are needed to detect as significant differences of 0.5pmol/ml of AEA and 25pmol/ml of 2-AG, which are the differences found between healthy people and obese people. It is intended to recruit 15 people with obesity, prediabetes and a healthy mouth, 15 people with obesity, prediabetes and a diseased mouth, 15 people with obesity, diabetes and a healthy mouth, and 15 people with obesity, diabetes and diseased mouth. To differentiate a healthy mouth from a diseased mouth, a complete oral evaluation will be carried out on all participants, which includes a review of the mucous membranes, an evaluation of the bacterial plaque and the presence of dental caries, and an evaluation of the health of the teeth. gums, including the depth of probing and the presence of gingival bleeding (see point 5.6).

5.3. INCLUSION CRITERIA

- Adults (40-65 years)
- With obesity and prediabetes: BMI 30-40 and HbA1c 5.7-6.4
- With obesity and diabetes: BMI 30-40 and previous diagnosis of diabetes

5.4. EXCLUSION CRITERIA

- Pregnant women
- Diagnosis of some type of neoplasia or treatment with radiotherapy and/or chemotherapy in the last year.
- Ongoing inflammatory diseases (Crohn's disease, ulcerative colitis, arthritis, etc.) and/or anti-inflammatory treatments
- Presence of systemic diseases of vital organs
- Participants in treatment with drugs that could alter salivary flow
- Smokers.
- Participants who have not followed the specifications prior to sampling
- Participants who did not sign the informed consent

5.5. ELIMINATION CRITERIA

- Inadequate amount of saliva to perform the tests

5.6. HEALTHY MOUTH vs DISEASE MOUTH CRITERIA

The differentiation between a healthy mouth and a diseased mouth will be made through a detailed observation and a clinical examination carried out by a dentist. This will be done in accordance with the guidelines of the World Health Organization (WHO; https://www.who.int/publications/m/item/draft-global-oral-health-action-plan-[2023-2030]).
The signs that indicate a healthy mouth are:

- Firm, pink gums that do not bleed during brushing or flossing.
- Clean teeth without stains, without cavities or fractures.
- Absence of pain or dental sensitivity.
- No presence of bad breath or halitosis.
- No signs of inflammation or infection in the gums or around the teeth.

On the other hand, the signs that indicate a sick mouth are:

- Red, swollen or bleeding gums.
- Teeth with stains, cavities or fractures.
- Tooth pain or sensitivity, especially when biting or chewing food.
- Bad breath or chronic halitosis.
- Presence of abscesses or infections in the teeth or gums.

In the present study, based on these criteria, the dentist will make the diagnosis of "without oral cavity disease" or "with oral cavity disease" for each subject.

5.7. SUBJECT IDENTIFICATION

Patients will be identified with a sequential numerical identification code, which will be assigned to each patient according to the correlative order of inclusion when they give their informed consent. In the event that a patient is included in the study and is considered unfit to continue in the study or the patient withdraws their consent, the code assigned to that patient cannot be reused for the next consecutively assigned patient.

The promoter will only be able to identify the subjects by the code that has been assigned to them, their date of birth, sex and date of signing the informed consent. The principal investigator will keep a record, protected by password, with the names of the patients and the assigned identification code.

6. DEVELOPMENT OF THE TRIAL AND EVALUATION OF THE RESPONSE

6.1. ASSESSMENT OF EFFICACY

Not applicable.

6.2. SAFETY ASSESSMENT

Not applicable.

6.3. DEVELOPMENT OF THE TEST

Selection of patients and survey. Participants will attend the recruitment appointment after fasting for at least 8 hours. After verifying that they meet the inclusion and exclusion criteria and sign the informed consent, they will be given a survey that collects data on affiliation and lifestyle habits related to health and daily oral hygiene, including smoking and alcohol consumption; medical history that includes the medication taken, last visit to the dentist and risk factors and symptoms of oral disease, through questions that are easy to answer when self-identifying general symptoms compatible with gingivitis, periodontitis and dental caries (eg localized tooth sensitivity and in gums, bleeding gums, loose teeth, hypersensitivity in teeth to cold and/or heat, persistent bad breath, elongated teeth with receding and/or separated gums, modified bite, dry mouth, change of color in teeth, etc.).

Collection of salivary and blood samples. After completing the medical history, all patients will have their anthropometric data such as height, weight, waist circumference, hip circumference, systolic blood pressure, and diastolic blood pressure recorded. The research team dentist will perform a complete oral health diagnosis on the study subjects, using the World Health Organization (WHO) guidelines for diagnosing oral cavity disease [29 ,30]. Likewise, the oral health questionnaire will be passed. After the oral examination, **saliva** samples will be collected under resting conditions using the passive drooling method, in 15 mL tubes , every 1 min, for 5 min in the same tube. Together with the saliva samples, trained personnel will collect 9 ml of **blood samples** , under resting conditions, in EDTA tubes. The tubes will be centrifuged and the supernatant will be collected and stored at -80ºC in the Biobank.

Analysis and detection of eCBs levels and profile of inflammatory biomarkers. In saliva and plasma, the analysis and detection of endocannabinoids and related lipids (2-arachidonoyl-glycerol [2-AG], N- arachidonoylethanolamine [AEA], N- palmitoylethanolamine [PEA], N- oleoylethanolamine [OEA], N- docosahexanoylethanolamine [DHEA], 2-linoleoylglycerol, [2-LG] and 2-oleoylglycerol [2-OG]). The analysis and detection of these factors will be carried out by HPLC chromatography. Inflammatory markers will also be quantified in saliva and plasma (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-17, leptin, adiponectin, vascular endothelial growth factor [VEGF], Interferon [IFN]-γ and Tumor Necrosis Factor [TNF]-α), using Procarta multiplex assay (TermoFisher Scientific) following the manufacturer's instructions. In addition, biochemical parameters such as blood glucose, insulin, HbA1c, triglycerides , total cholesterol, HDL cholesterol, LDL cholesterol will be analyzed in the hospital laboratory in the blood samples .

Analysis of oral bacteriological profiles. The bacteriological profiles in the saliva samples will be analyzed using massive sequencing techniques and amplification of the bacterial 16S rRNA amplicon of the following microbial strains: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum, Parvimonas micra, Campylobacter rectus , Eikenella corroe, Veillonella parvula and Actinomyces naeslundii* for periodontal diseases ; and *streptococci mutans , S. sanguis, S. mitior, S. salivarius* and *S. milleri* for tooth decay. In addition, saliva will be analyzed for sialometry (salivary flow), salivary viscosity, salivary pH and the impact profile on oral health (OHIP-14sp; WHO questionnaire).

7. STATISTIC ANALYSIS

7.1. COLLECTED VARIABLES

The following groups of variables are included in this essay:

- Anthropometric data: weight, height, body mass index, waist circumference, hip circumference, blood pressure.
- Clinical data: Previous or ongoing illnesses, current medication.
- Blood test data: Standard biochemistry (plasma glucose, insulin, plasma HbA1c, lipid profile, renal function, liver function, lymphocytes, hs-CRP, etc.), inflammatory markers (panel of inflammatory cytokines, including IL-6 , IL- 1beta, and TNF-alpha), endocannabinoids (2-AG, AEA, PEA, OEA, DHEA, 2-LG, 2-OG).
- Saliva data: Salivary flow (to calculate unstimulated oral saliva flow), salivary viscosity, salivary pH, salivary protein concentration. Inflammatory markers (panel of inflammatory cytokines , including IL-6, IL-1beta and TNF-alpha), endocannabinoids (2-AG, AEA, PEA, OEA, DHEA, 2-LG, 2-OG), Bacteriological profiles. In addition, the oral health impact profile (OHIP-14sp) will be evaluated, both through the dental examination and through questions.

7.2. STATISTICAL ANALYSIS

For the statistical study, the statistical software package SPSS 25.0 will be used. A database equivalent to the data collection notebook will be created with all the variables. First, the sample will be studied (descriptive statistics) using absolute and relative frequencies and then inferential statistics will be performed. The parametricity of the variables will be studied using a normality test. The parametric analysis will be carried out using ANOVA, followed by the corresponding post-test analysis. If the distribution of the variable is not normal, it will be converted to log or a non-parametric test will be used to contrast the differences. All hypothesis contrasts will be made with a significance level of 5%.

8. ADVERSE EVENTS

Given the nature of this trial, no type of adverse event or adverse reaction is anticipated.

9. ETHICAL ASPECTS

The collection and processing of human saliva and peripheral blood samples and personal and anthropometric data, as well as the data obtained in the aforementioned tests will be carried out in accordance with current legal regulations on the use of human samples, preserving and codifying adequately the identity of the subjects involved in the study. A copy of the informed consent to be signed by each subject included in the study is attached. This study will not begin until it is informed and approved by the Provincial CEI of Malaga. Once the trial is finished, the rest of the human samples obtained in this study will be stored in the Biobank of the Hospital Regional Universitario de Málaga - IBIMA (which is part of the Biobank of the SSPA), so that they can be used by any researcher who considers them useful.

CONSENTIMIENTO INFORMADO - INFORMACIÓN AL PACIENTE

Antes de proceder a la firma de este consentimiento informado, lea atentamente la información que a continuación se le facilita y realice las preguntas que considere oportunas.

Nombre del Estudio:

ESTUDIO MOLECULAR DE LA DISBACTERIOSIS ORAL EN PERSONAS CON PREDIABETES Y DIABETES - *SMILE*

Naturaleza:
Las enfermedades de la cavidad oral son más frecuentes en personas con prediabetes y diabetes, suponiendo una disminución de la calidad de vida de estos pacientes, uniéndose a las múltiples complicaciones que desarrollan estas personas durante la progresión de la enfermedad. Por tanto, el Hospital Regional de Málaga está realizando un estudio que permitan aclarar esta relación y descubrir nuevas dianas moleculares que permitan prevenir y/o tratar estas afecciones. Para ello, los participantes acudirán a la cita de reclutamiento tras un ayuno de al menos 10 horas y se les entregará una encuesta a rellenar, que recoge datos de filiación y hábitos de estilo de vida relacionados con la salud y la higiene oral diaria. Luego se procede a la recogida de una muestra de **sangre venosa**, y de repetidas muestras (una por cada minuto) de **saliva total** (en reposo) utilizando el método de babeo pasivo, durante 5 min.

Importancia:
Toda la información recogida servirá para determinar el vínculo causal entre la disglicemia y las enfermedades de la cavidad oral. El sistema endocannabinoide podría ser el mediador de estas alteraciones, siendo asi diana terapéutica potencial. Este estudio persigue dilucidar si el sistema de señalización intercelular por endocannabinoides está alterado en la cavidad oral de personas con prediabetes y diabetes que tienen enfermedades de la cavidad oral.

Implicaciones para el donante/paciente:
La donación/participación es totalmente voluntaria.
El donante/paciente puede retirarse del estudio cuando así lo manifieste, sin dar explicaciones y sin que esto repercuta en sus cuidados médicos.
• Todos los datos carácter personal, obtenidos en este estudio son confidenciales y se tratarán conforme a la Ley Orgánica de Protección de Datos de Carácter Personal 15/99.
• La donación/información obtenida se utilizará exclusivamente para los fines específicos de este estudio.

Riesgos de la investigación para el donante/paciente:
La donación de sangre apenas tiene efectos secundarios; lo más frecuente es la aparición de pequeños hematomas en la zona de punción que desaparecen transcurridos 1 ó 2 días.
Si requiere información adicional se puede poner en contacto con nuestro personal de Investigación en el teléfono: 951 29 02 26 o en el correo electrónico: smile@ibima.eu.

CONSENTIMIENTO INFORMADO - INFORMACIÓN AL PACIENTE

Estudio molecular de la disbacteriosis oral en personas con prediabetes y diabetes - *SMILE*

Yo (Nombre y Apellidos):_____

- He leído el documento informativo que acompaña a este consentimiento (Información al Paciente).
- He podido hacer preguntas sobre el **Estudio molecular de la disbacteriosis oral en personas con prediabetes y diabetes - *SMILE*.**
- He recibido suficiente información sobre el **Estudio molecular de la disbacteriosis oral en personas con prediabetes y diabetes - *SMILE*.**
- He hablado con el profesional sanitario informador:_____.
- Comprendo que mi participación es voluntaria y soy libre de participar o no en el estudio.
- Se me ha informado que todos los datos obtenidos en este estudio serán confidenciales y se tratarán conforme establece la Ley Orgánica de Protección de Datos de Carácter Personal 15/99.
- Se me ha informado de que la donación/información obtenida sólo se utilizará para los fines específicos del estudio.
- **Deseo** ser informado/a de los datos que se obtengan en el curso de la investigación, incluidos los descubrimientos inesperados que se puedan producir, siempre que esta información sea necesaria para evitar un grave perjuicio para mi salud o la de mis familiares biológicos.

☐ Si ☐ No

Comprendo que puedo retirarme del estudio:

- Cuando quiera
- Sin tener que dar explicaciones
- Sin que esto repercuta en mis cuidados médicos

Presto libremente mi conformidad para participar en el *proyecto titulado*
Estudio molecular de la disbacteriosis oral en personas con prediabetes y diabetes - *SMILE*.

Firma del paciente (o representante legal en su caso) Nombre y apellidos:_____ Fecha: _____	Firma del profesional sanitario informador Nombre y apellidos:_____ Fecha: _____