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END OF TERM PROJECT

METABOLIC SYNDROME, INFLAMMATION AND MICROBIOTA

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ABSTRACT

Background and justification: The criteria for metabolic syndrome include obesity, dyslipidemia, elevated blood pressure and elevated fasting glucose. Altered gut microbial have been associated with metabolic disorders in animals and humans, including MeTS . Different microbiota have been observed depending on the individual's BMI (normal/obese) or the country where people live, among others.
There is paucity of information regarding which microbiota are beneficial/detrimental, microbiota's composition among Spanish population and its composition depending on the micronutrients consumed.

Objectives: The main aim is to evaluate the difference of the gut microbiota between males and females patients (pre-/post-menopause) with metabolic syndrome vs. without metabolic syndrome. Other objectives are: a) to determine the composition of microbiota in individuals with and without obesity depending on the presence of metabolic syndrome;
b) to evaluate the composition of microbiota in individuals with subclinical inflammation depending on the presence of metabolic syndrome; and c) to determine which factors (age, sex, obesity, inflammation, metabolic syndrome and diet) are independently associated with the gut microbiota.

Methods: This is a cross-sectional study in 131 patients recruited between January 2016 and October 2017 (age range 27-67 years). Nonparametric Spearman correlations were used to determine the associations between quantitative variables. Finally, two kinds of stepwise multivariate linear regression were performed to assess the association of clinical, anthropometric, laboratory, dietetic parameters and metabolic syndrome with microbiota.

Results: Significant differences in the microbiota were confirmed between patients depending on the metabolic syndrome.
After Bonferroni correction, there was a trend ($p<0.01$) towards differences in the relative abundance of *Candidatus Atribacteria, Chrysiogenetes, Lentisphaerae, Planctomycetes and Tenericutes* according to the presence of metabolic syndrome. The RA of *Candidatus Moranbacteria, Tenericutes, Thermotogae, Candidatus Omnitrophica, Spirochaetes and Ascomycota* was associated with decreased BMI and decreased chronic inflammation in subjects without metabolic syndrome.

Conclusions: In summary, our work shows a connection between metabolic syndrome, inflammation and gut microbiota. Human intestinal microbiota may be linked with the prevention of developing metabolic syndrome by affecting the parameters of BMI, SBP, DBP and HDL. There is a special connection between microbiota from non-obese individuals and low CRP, without MetS.

Keywords: •metabolic syndrome •inflammation •microbiota •cardiovascular diseases •type 2 diabetes mellitus •obesity

ABBREVIATIONS

HDL-C	high-density lipoprotein
MetS	metabolic syndrome
MS	metabolic syndrome
BMI	body mass index
SCFAs	short-chain fatty acids
FMT	fecal microbiota transplantations
DBP	diastolic blood pressure
SBP	systolic blood pressure
us-CRP	ultra-sensitive C-Reactive Protein
IDF	International Diabetes Federation
NCEP ATP III	National Cholesterol Education Adult Treatment Panel
FPG	fasting plasma glucose
IFG	impaired fasting glucose
WHO	world health organization
T2DM	type 2 diabetes mellitus
OGTT	oral glucose tolerance test
CVD	cardiovascular disease
HIV	human immunodeficiency virus
TBC	tuberculosis
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CRF	Case Report Form
SD	Standard deviation
IQ	Interquartile range
RA	relative abundance

FIGURES

TABLES

1. INTRODUCTION

Metabolic syndrome is a cluster of conditions that is associated with the development of diabetes mellitus type 2 and cardiovascular disease.(1) It has become a global epidemic and the total cost, including the cost of health care and loss of potential economic activity, is in trillions. (2) MetS has been related with persistent low-grade inflammatory response and microbiota (3) This complex intestinal "superorganism" seems to affect host metabolic balance modulating energy absorption, gut motility, appetite, glucose and lipid metabolism, as well as hepatic fatty storage. (4) Inflammation leads to impaired insulin action, which promotes the development of metabolic abnormalities. (5) (6)

1.1. Metabolic syndrome

The first formalized definition of the metabolic syndrome was proposed in 1998 by a consultation group on the definition of diabetes for the World Health Organization (WHO). This group emphasized insulin resistance as the major underlying risk factor and required evidence of insulin resistance for diagnosis. According to WHO, the criteria was on the basis of several markers of insulin resistance plus 2 additional risk factors, including: obesity, hypertension, high triglyceride level, reduced high-density lipoprotein cholesterol level or microalbuminuria. Patients with T2DM were not excluded from diagnosis.

Nevertheless, ATP II (National Cholesterol Education Program Adult Treatment Panel III) in 2001 did not require demonstration of insulin resistance per se. Moreover, no single factor was required for diagnosis, but instead, ATP III made the presence of 3 of the following 5 factors the basis for establishing the diagnosis: Abdominal obesity (which is highly correlated with insulin resistance), elevated triglyceride, reduced high-density lipoprotein cholesterol, elevated blood pressure, and elevated fasting glucose (impaired fasting glucose or type 2 diabetes mellitus).

The IDF dropped the WHO requirement for insulin resistance but made abdominal obesity necessary as 1 of 5 factors required in the diagnosis, the other criteria reminded the same. (7)

ATP III and IDF are the most used definition on the publications (8), that is the reason why in this study will be used these two definitions.

1.1 IDF (International Diabetes Federation)

Table 1: International Diabetes Federation (IDF) criteria for diagnosing Metabolic Syndrome (9)

International Diabetes Federation (IDF)	
For a person to be defined as having the metabolic syndrome they must have: • **Central obesity** (defined as waist circumference* with ethnicity specific values) • **Plus** any two of the following four factors:	
Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) **or specific treatment for this lipid abnormality**
Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females **or specific treatment for this lipid abnormality**
Raised blood pressure	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg **or treatment of previously diagnosed hypertension**
Raised fasting plasma glucose	(FPG) ≥ 100 mg/dL (5.6 mmol/L), **or previously diagnosed type 2 diabetes** If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome

*European data. If BMI is >30 kg/m^2, central obesity can be assumed and waist circumference does not need to be measured.

Table 2: Ethnic specific values for waist circumference (9)

Country/Ethnic group		Waist circumference
Europids* In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purpose	Male	≥ 94 cm
	Female	≥ 80 cm
South Asians Based on a Chinese, Malay and Asian-Indian population	Male	≥ 90 cm
	Female	≥ 80 cm
Chinese	Male	≥ 90 cm
	Female	≥ 80 cm
Japanese**	Male	≥ 90 cm
	Female	≥ 80 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africans	Use European data until more specific data are available	
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available	

*In future epidemiological studies of populations of Europid origin, prevalence should be given using both European and North American cut-points to allow better comparisons.
** Originally different values were proposed for Japanese but new data support the use of the values shown above.

1.2 NCEP ATP III (The National Cholesterol Education Program. Adult Treatment Panel III)

According to this definition, a subject has the MS if he or she has 3 or more of the following criteria:

Table 3: NCEP ATP III definition for diagnosing Metabolic Syndrome (10)

NCEP ATP III	
According to this definition, a subject has the MS if he or she has 3 or more of the following criteria:	
1. Abdominal obesity	WC ≥ 102 cm in men and ≥ 88 cm in women
2. Hypertriglyceridemia	≥ 150 mg/dL (1.7 mmol/L)
3. Low HDL-C	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females
4. High blood pressure	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg
5. High fasting plasma glucose	(FPG) ≥ 100 mg/dL (5.6 mmol/L),

The metabolic syndrome -otherwise called syndrome X, insulin resistance syndrome, Reaven syndrome, and the "dealdy quartet"- is the name given to the aggregate of clinical conditions comprising central and abdominal obesity, systemic hypertension, insulin resistance (or type 2 diabetes mellitus), and atherogenic dyslipidemia. In addition to inflammatory dermatoses, metabolic syndrome is also associated with accelerated atherosclerotic cardiovascular disease, hyperuricemia/gout, chronic kidney disease, obstructive sleep apnea (11,12), nonalcoholic fatty liver disease (12) and premature mortality (13)

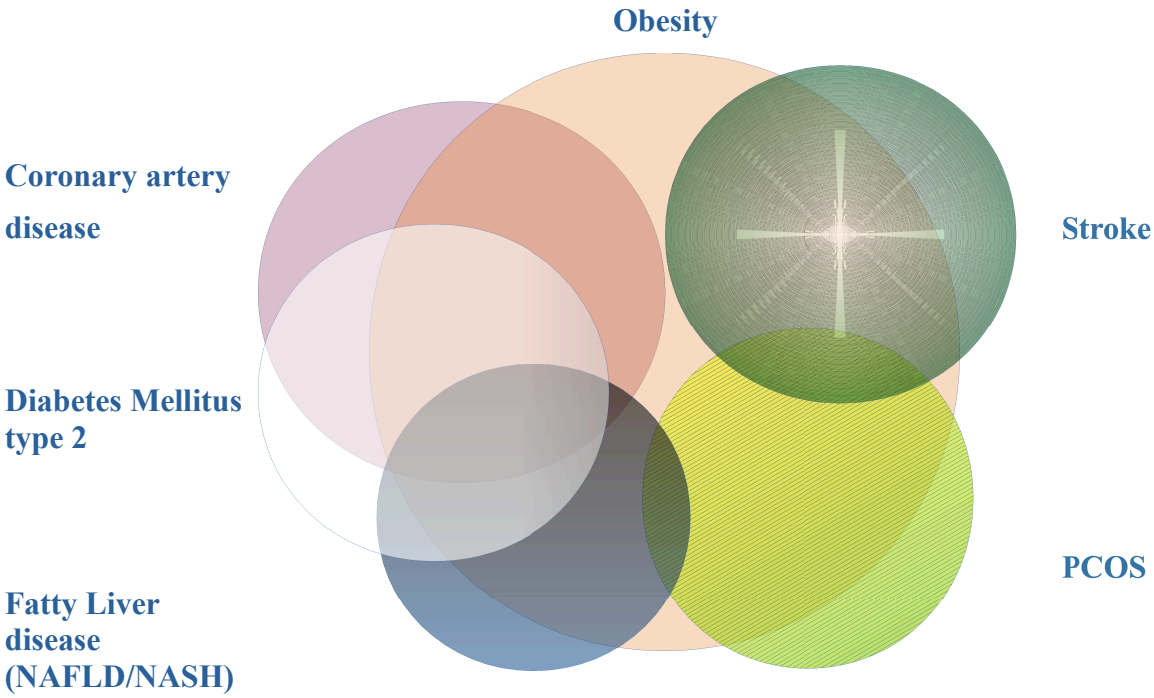

Figure 1: Metabolic Syndrome and Associated Diseases. Adapted from (11)

The metabolic syndrome is a prothrombotic and proinflammatory state characterized by increased inflammatory cytokine activity which will increase the risk for type 2 diabetes and atherosclerotic cardiovascular disease (11)

1.2. Inflammation

Chronic inflammation in people with obesity has been linked to help to the clinical progression of the metabolic syndrome and obesity-related pathologies such as type 2 diabetes and non-alcoholic fatty liver disease. (5) Inflammation leads to impaired insulin action, which promotes the development of metabolic abnormalities. (5,6)

Increased caloric intake, high fat accumulation and lipotoxicity activate the production of effector molecules (cytokines) (8–10) and cells that are primarily involved in innate immunity. This production provokes a chronic, low-grade inflammatory status, induces the recruitment and activation of many mature immune cells (including mast cells, macrophages and dendritic cells) in metabolic tissues and particularly in adipose tissues, and also induces recruitment and activation of

other cells, such as adipocytes , that modify the tissue milieu and reinforce the inflammatory process. (5)

One of the most interesting concepts about the association between obesity and cardiovascular complications include the low-grade systemic inflammatory reaction associated with obesity and characterized by the secretion of cytokines by the adipose tissue to the circulation (**Fig.2**).

Cytokines are low molecular weight proteins produced by the immunologic system in the inflammatory process. In the last years, obesity and metabolic syndrome has been associated with low-grade chronic inflammatory markers or subclinical. Because of the increase of CRP and other inflammatory factors, insulin resistance is related with the obesity, endothelial dysfunction and atherosclerosis. (5)

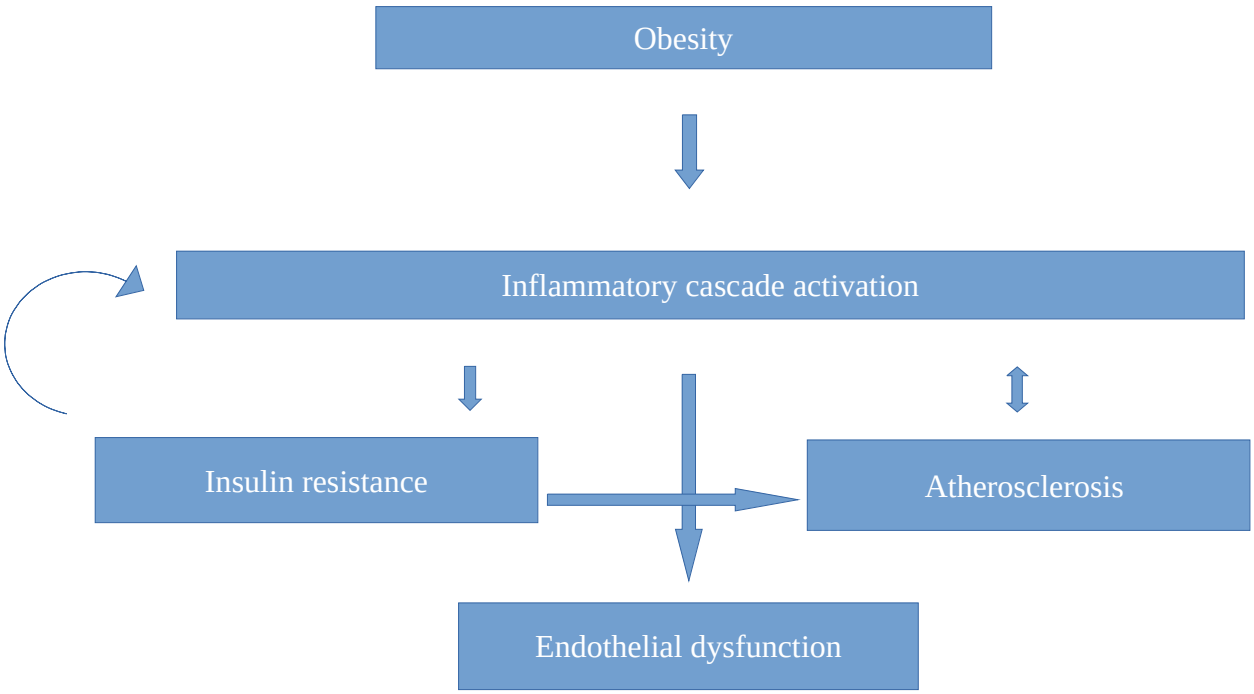

Figure 2: Relation of obesity - inflammation – insulin resistance – atherosclerosis. Adapted from (5)

1.3 Gut microbiota

The gut microbiota is composed by trillions of microbes which live in our gut. Even though some studies suggest that the development of the microbiota starts prenatally, through microbial transmission from mother to fetus, (16) others are still sceptical about the colonization with bacteria in *utero*. (17) Following birth, multiple factors contribute to the colonization by an array of microbes including gestational age, mode of delivery (whether natural or by Caesarean section), diet (breastfeeding or infant formula), hygiene and antibiotic therapy (18,19). By 2-5 years of age the human gut microbiota is similar to the adult when it comes to composition and diversity. Consequently, the diet in the first 3 years of life is fundamental for the establishment of the microbiota (20).

The recent scientific upsurge in the field of gut microbiota has firmly established its role in contemporary clinical medicine (**Fig. 3**) However, the history of fecal infusions in medicine is much longer. The first records of fecal transplantation date back to 4th century China, where "yellow soup" was applied in cases of severe food poisoning and diarrhea. Currently, FMT has been associated with extra-intestinal diseases, like metabolic syndrome. (21)

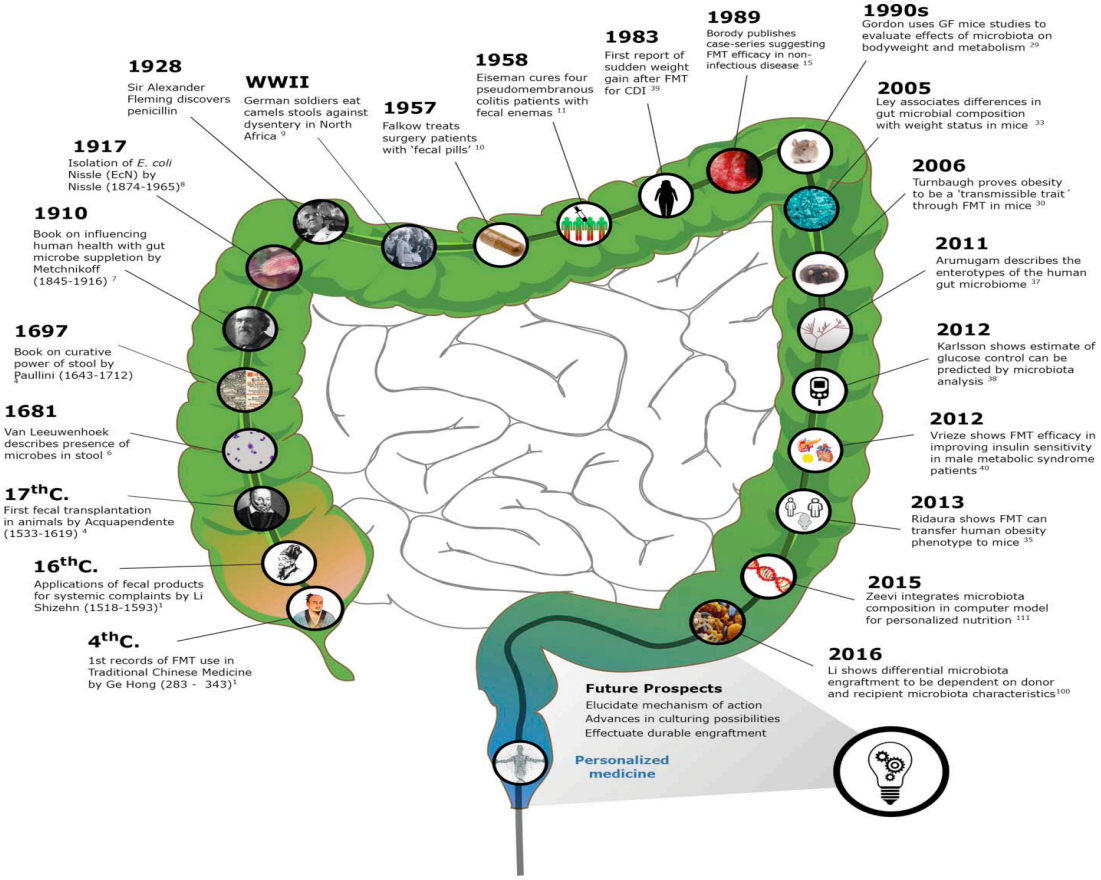

Figure 3: Timeline: Key contributions to FMT development and research.(21)

Table 4: Phyla and their genera in gut: putative relationships with metabolic and gut functions (22)

Phyla	Genera	Functions in Gut
Firmicutes **(Gram-positive)**	*Anaerostipes* *Bacillus* *Clostridium* *Coprococcus* *Enterococcus* *Eubacterium* *Faecalibacterium* *Lactobacillus* *Lactococcus* *Megasphaera* *Mycoplasma* *Peptostreptococcus* *Phascolarctobacterium* *Pseudobutyrivibrio* *Roseburia* *Ruminococcus* *Staphylococcus* *Streptococcus* *Veillonella*	They make up the largest portion of the human gut microbiome and have been shown to be involved in energy extraction, and potentially related to the development of diabetes and obesity.
Bacteroidetes **(Gram-negative)**	*Bacteroides* *Prevotella* *Corynebacterium*	They have implications for the normal development of the gut, including the interactions with the immune system. Gut *Bacteroidetes* generally produce butyrate, an end-product of colonic fermentation, that is thought to have antineoplastic properties and play a role in maintaining a healthy gut, with implications in the development of obesity.
Actinobacteria **(Gram-positive)**	*Eggerthella* *Olsenella*	Found in the human colon and feces, they cause ulcerative colitis, liver and anal abscesses, and systemic bacteremia.
Cyanobacteria **(Gram-negative)**	*Spirulina*	Spirulina (Arthrospira platensis) has hypolipidemic, hypoglycemic, and antihypertensive properties.
Proteobacteria **(Gram-negative)**	*Citrobacter* *Escherichia* *Helicobacter* *Klebsiella* *Salmonella* *Shigella* *Sutterella*	The phylum Proteobacteria is the most unstable in the course of the host life among the four main represented phyla of the gut microbiota and its imbalance is suggested as a potential diagnostic criterion for gut-related diseases
Spirochaetes **(Gram-negative)**	*Brachyspira*	The best-known species is Brachyspira hyodysenteriae, the agent of swine dysentery, which induces an extensive and severe mucohaemorrhagic colitis in growing pigs
Verrucomicrobia **(Gram-negative)**	*Akkermansia*	A. muciniphila is a common inhabitant of the human intestinal tract, comprising up to 1% of the total bacteria in the intestine. It grows optimally at 37 °C and it is capable of fermenting glucose, N-acetylglucosamine and N-acetylgalactosamine
Fusobacteria **(Gram-negative)**	*Fusobacterium (5 species in the gastrointestinal tract)*	Gut bacteria including Fusobacteria may have an influence on the development of CRC through interaction with the innate immune system or host factors

Since the genetic and species variability of the microbiota provides different enzymatic, biochemical, and metabolic pathways relating to the host's (**Table 4**), the result is the extraction of energy and absorbable substrates for the host, as well as the supply of energy and nutrients for the proliferation of the same resident bacterial species. For these reasons, the microbiota is considered a metabolic active organ. Commensal intestinal bacteria, mainly *Bacteroidetes (Bacteroides and Prevotella), Firmicutes and Actinobacteria* get energy from the fermentation and transformation of undigested food substrates. (22)

The microbiota has important metabolic functions (**Fig. 4**). Mammals have the capacity for absorbing simple sugars in the small intestine but their ability is limited when it comes to digest polysaccharides, so that's why all undigested food like the fiber are the principal substrate for the establishment and development of the intestinal flora, since the microbiota can extract the energy from it. (22) For example, the gut bacteria produces short-chain fatty acids (SCFAs) by fermenting the fibers. SCFAs influences insulin sensitivity in adipocytes and peripheral organs(18). SCFAs have some important biological action such as acting on glucose homeostasis (23)

Another study is more sceptical about the benefits of SCFAs and states that SCFAs provide extra energy to the individual, affect the synthesis of cholesterol and lipogenesis promoting metabolic diseases. (24)

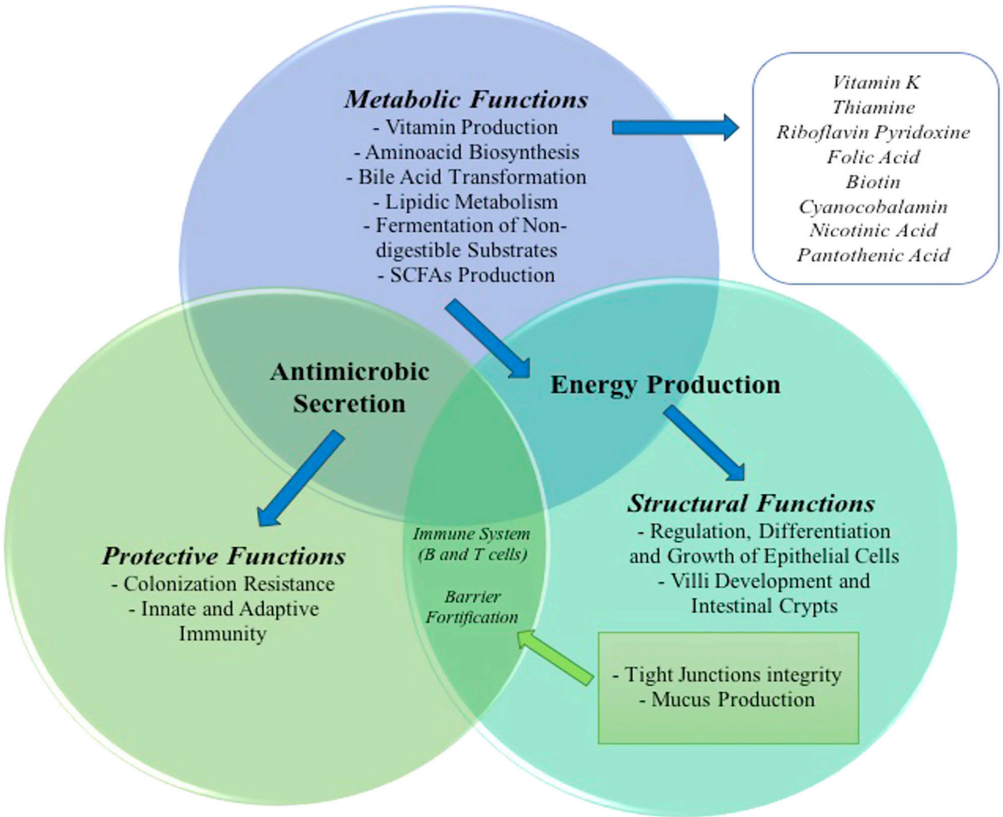

Figure 4: Gut microbiota functions. SCFAs short-chain fatty acids (22)

Microbiota is needed for the synthesis of different vitamins and enzymatic cofactors like folic acid, vitamin B1, B2, B6, B12, PP, K, H, pantothenic acid, etc) and for the absorption of calcium, magnesium and iron. As well there is some proof that microbes form part of the process of hepatic transformation of cholesterol into bile acids. (22)

In metagenomic studies in humans have identified various specific gut microbiota changes in individuals with metabolic syndrome including malevolent microbes that contribute to insulin resistance, such as *Prevotella copri* and *Bacteroides vulgatus*, but also beneficial species such as *Akkermansia municiphila,* and *Faecalibacterium prausnitzii* which are associated with increased insulin sensivity. Furthermore, it was discovered that metformin improved glucose homeostasis by increasing the population of *Akkermansia species*.(21)

A healthy microbiota comprises a balanced representation of symbionts (bacteria with health-promoting functions) and pathobionts (bacteria that potentially induce pathology) (**Fig. 5**). A shift toward dysbiosis results from a decrease in symbionts and/or an increase in pathobionts and is

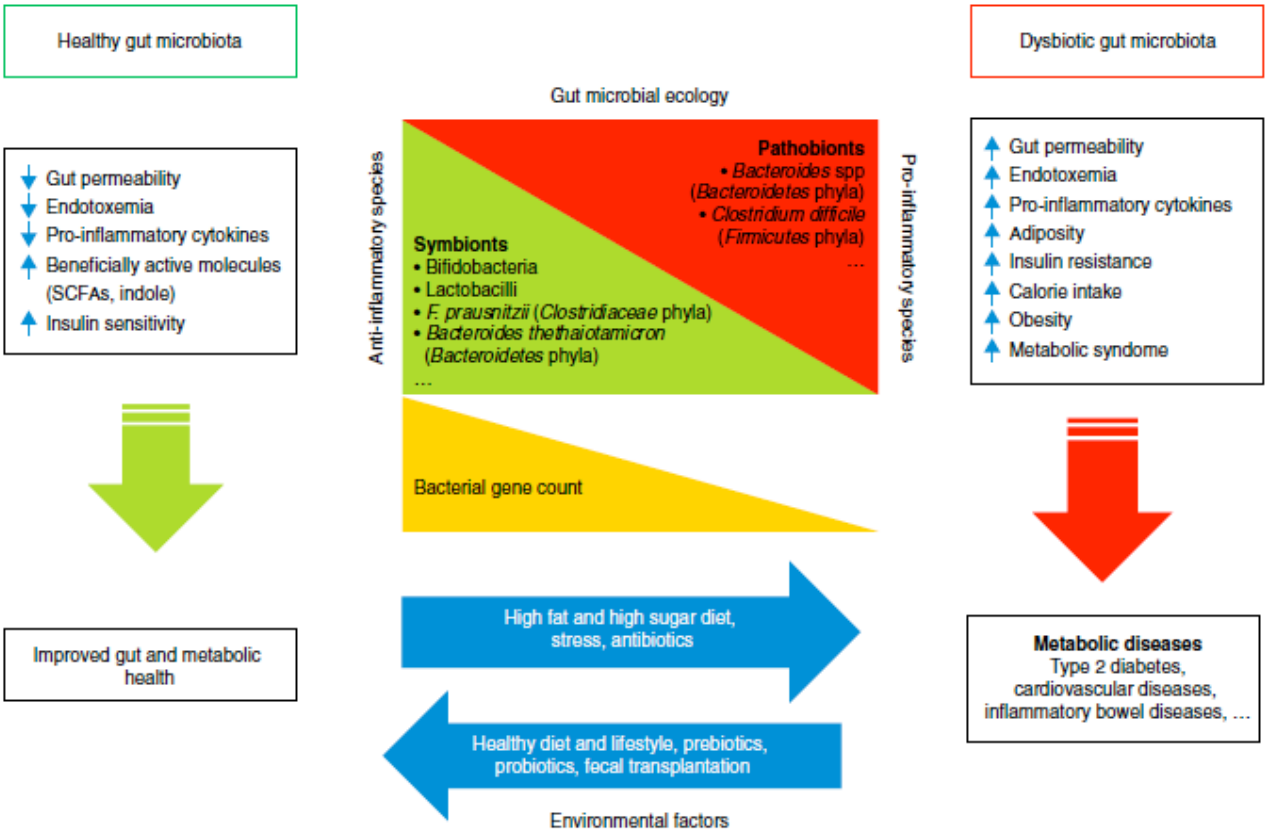

Figure 5: Effects of a healthy gut microbiota and dysbiosis on the gut and metabolic health of the host. (18)

likely to be triggered by environmental factors (such as diet, stress, antibiotics, and infections). Low bacterial gene counts have also been associated with altered gut microbial functions and dysbiosis and have been linked to increased fat accumulation, lipopolysaccharide-induced inflammation, insulin resistance, obesity, and the metabolic syndrome. Individuals with these characteristics are more likely to develop metabolic diseases (such as diabetes, cardiovascular diseases, and inflammatory bowel diseases). (18)

The gut microbiota diversity is likely altered at multiple stages by the diet. Unhealthy diets may promote an inflammatory state that, in turn, is strictly interconnected with the gut microbial configuration. The combination of these three factors (unhealthy diets, inflammation and a dysbiotic, low-diverse and pro-inflammatory microbial layout) may favor the onset of obesity. High physical activity may protect the human host from obesity, even when diet and microbiota are in a low-diversity and pro-inflammatory configuration. However, human genetics can lead the host to develop obesity, regardless of the microbiome configuration. (**Fig. 6**) (2)

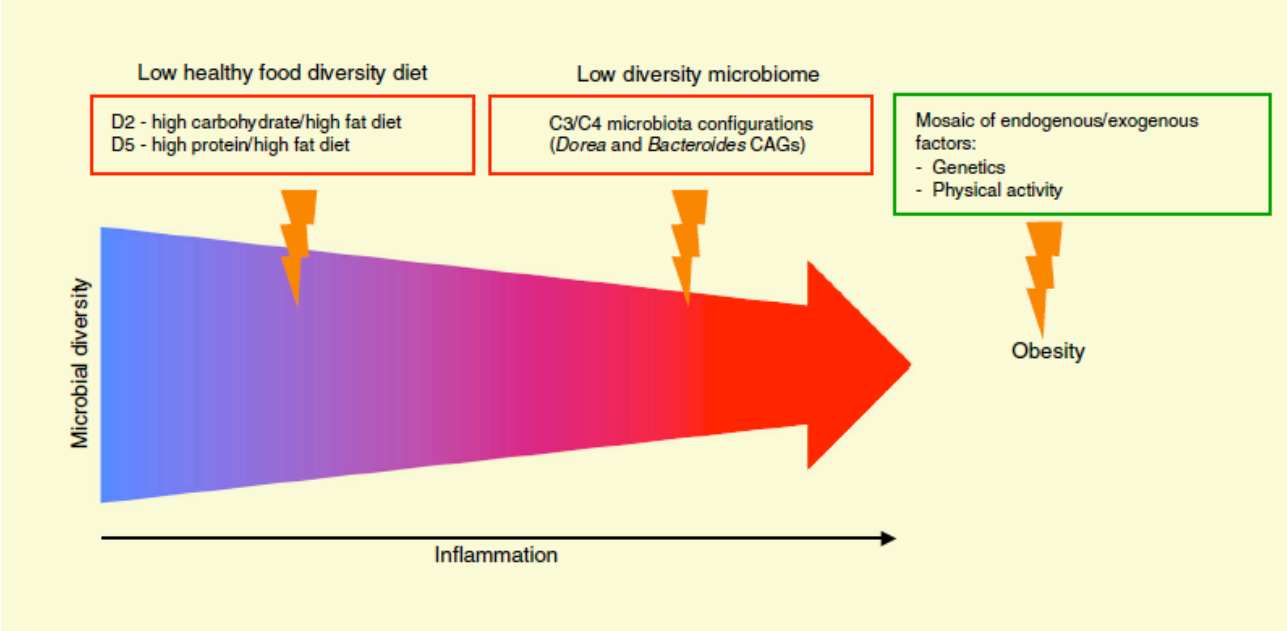

Figure 6: The mosaic etiology of obesity. (2)

There is little literature about Mediterranean diet and its association with the microbiota.

A study compared participants who had high adherence to the Mediterranean diet with patients who had it to fast food. This study observed that those who had a Mediterranean diet had lower Escherichia coli, higher bifidobacteria:E. Coli ratio, increased levels and prevalence of Candida albicans. It would be interesting to study further for getting to know which nutrients are beneficial for our intestinal microbiota in symbiosis. (25)

1.4. Relation between metabolic syndrome, inflammation and microbiota

Obesity-related inflammation and impaired insulin action are tightly connected; inflammation leads to impaired insulin action, which in turn promotes the development of metabolic abnormalities. The gut microbial dysbiosis is believed to contribute to metabolic diseases via stimulation of low-grade inflammation. (18)

There is more and more proof that the alteration of the microbiota (known as dysbiosis) can increase the risk to important diseases, including metabolic diseases, like obesity and diabetes, and cardiovascular diseases. (**Fig.7**) (22)

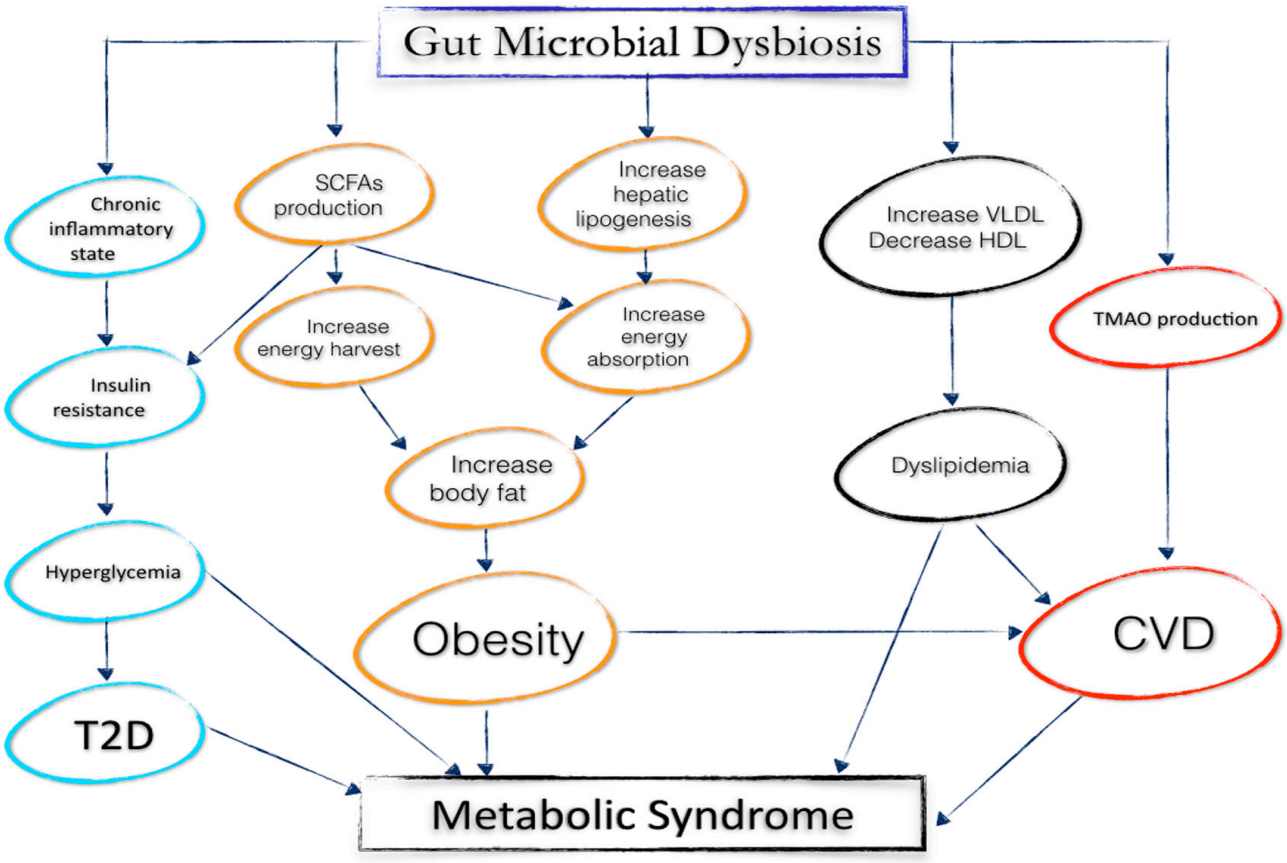

Figure 7: Overview of the interactions between the microbiota and the host, which may lead to metabolic changes and disease (22)

In the last decades, a solid base of evidence has linked the inflammatory state in metabolic syndrome to impaired gut barrier function and leakage of bacteria and/or bacterial components into the system.

Resulting low grade endotoxemia (and possibly bacteremia) chronically activates inflammatory pathways. Bacterial components may also migrate to target organs, leading to an influx of macrophages that contribute to local as well as systemic low grade inflammation and insulin resistance. (**Fig. 8**) (21)

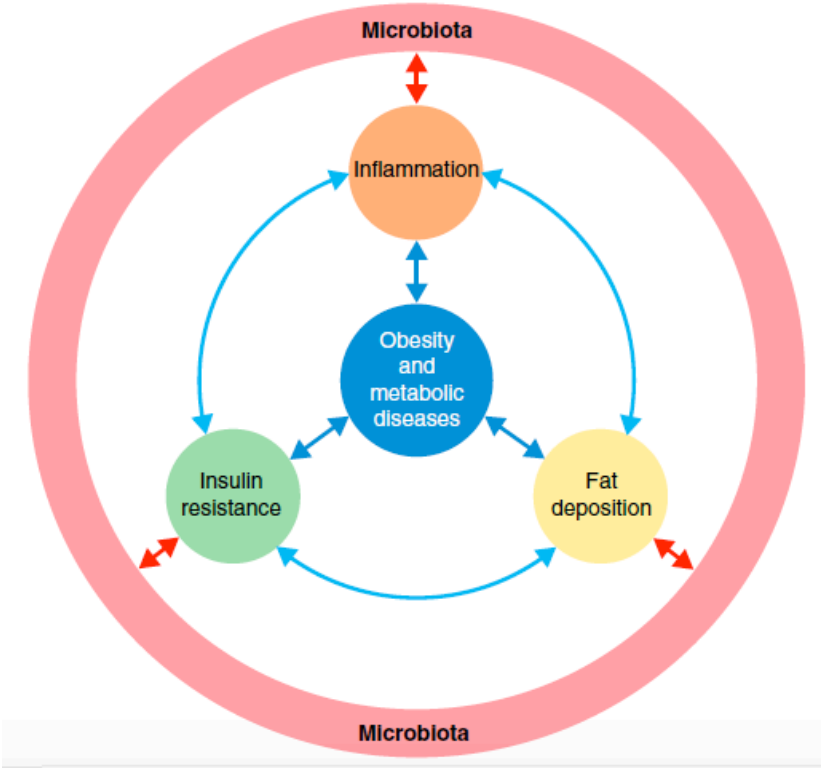

Figure 8: Crosstalk between the gut microbiota and the mammalian host in inflammation and metabolism. The gut microbiota can contribute to host insulin resistance, low grade inflammation, and fat deposition through a range of molecular interactions with the host and therefore can indirectly participate in the onset of obesity and metabolic diseases.(18)

2. JUSTIFICATION

Metabolic syndrome has become a global epidemic and the total cost of the malady including the cost of health care and loss of potential economic activity is in trillions. (2)

In the last decades an astounding amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease. (26) It has been studied that healthy microbiome may be related with preventing many cardiovascular diseases and also MeTS/diabetes (2). It has been shown that the gut microbiota forms part of basic human biological processes, including modulating the metabolic phenotype, influencing innate immunity and regulating epithelial development. (26)

The evidence for a strong contribution of the gut microbiota to the onset of obesity and metabolic diseases is growing. The modifications in the gut microbial ecology by dietary factors, antibiotics, probiotics or prebiotics that were observed in rodents and humans have further highlighted the key modulatory roles of the gut microbiota and its contribution to host obesity and metabolic diseases.(18)

However, how external factors (such as diet or age) affect the composition and the effectiveness of microbial functions in rodents and humans is still unclear.(18) Previous work has established that diet and geographical environment are two principal determinants of microbiome structure and function (27)

Conflicting data exist, with discordances about the relation of obesity, MeTS, pre/post menopause or CRP with microbiome and there is scarce literature of the relation of diet and microbiome in Girona.

Since we didn't possess enough data because we needed a bigger N, we decided to do a pilot study. In the future, a full-scale study could be considered.

This research will provide information about the relation between different factors (like sex, age obesity, hypertension, HDL-C or us-CRP) which will facilitate future clinical trials, including for fecal microbiota transplantations, being able to prevent and reverse the syndrome.

3. HYPOTHESIS

Microbiota determines the presence of inflammation and metabolic syndrome. Different composition of microbiota is associated with metabolic syndrome, grade of inflammation and obesity.

4. OBJECTIVES

- The main aim is to determine the difference of the gut microbiome between individuals with and without metabolic syndrome.

Secondaries:

- Evaluate the difference of the gut microbiota between males and females (pre-/post-menopause) with metabolic syndrome vs. without metabolic syndrome
- Assess the composition of microbiota in individuals with and without metabolic syndrome depending on the presence of obesity.
- Evaluate the composition of microbiota in individuals with and without metabolic syndrome depending on the grade of inflammation (CRP).
- Determine which factors: the presence of metabolic syndrome or their components (BMI, SBP, DBP, FPG, triglycerides, HDL-c levels), grade of inflammation or diet are independently associated with the gut microbiota.

5. METHODOLOGY

5.1 Study design

This is a cross-sectional study and it has been designed in order to observe the association between metabolic syndrome, inflammation and microbiota. The data have been extracted from a database compiled between January 2016 and October 2017.

5.2 Participants

The population studied are Caucasian from Girona who were recruited according to inclusion and exclusion criteria during the dates mentioned before.

A target population of 131 (89 are female and 42 are males) aged between 27,22 and 66,60 years old.

5.2.1 Inclusion criteria

To be enrolled in this study the subjects had to accomplish the following criteria:

- Aged 25 to 70 years
- Ability to understand study procedure
- Written informed consent obtained from the patient

5.2.2 Exclusion criteria

- Serious systemic diseases not related with obesity, such as cancer, severe kidney disease, or liver disease, type 1 or type 2 diabetes
- Systemic diseases with inflammatory activity, such as rheumatoid arthritis, Crohn's disease, asthma, chronic infection (HIV, TBC)
- Pregnancy and lactation
- Severe disorder of eating behavior
- Clinical symptoms and signs of infection in the previous month

- Antibiotic, antifungal and antiviral treatment for the last 3 months
- Anti-inflammatory chronic treatment(with steroidal and/or non-steroidal anti-inflammatory drugs) or insulin treatment
- Major psychiatric antecedents
- Serum liver enzymes (ALT and AST) activity over twice the upper limit of normal
- Excessive alcohol intake (40g a day (women) or 80gr/day (men) or drug abuse)

5.3 Sampling

5.3.1 Sample stratification

Sample recruitment took place in the period from January 2016 and October 2017 and non-probabilistic consecutive sampling method was used. Patients attending the Endocrinology outpatient clinic of the *Hospital Universitari de Girona Doctor Josep Trueta* were informed about the study and, if inclusion and exclusion criteria were met, the candidates received an explanation of the purpose and nature of all procedures (ANNEX 1) and they were invited to participate voluntarily by signing the informed consent (ANNEX 2). Eligible participants were individuals with obesity (BMI $\geq$ 30 kg/m^2) and their respective paired controls (BMI < 30 Kg/m^2) men, pre and postmenopausal women, range 27,22-66,60 years.

5.3.2 Sample size

Since we did not have sufficient data because we needed a bigger N, we decided to do a pilot study. In a two-side contrast with a level of significance α=5% , assuming a moderate difference and 131 participants, the statistical power was 82,89%.

The computations were carried out with the Prof. Marc Saez' software based on the library 'pwr' of the free statistical environment R (version 3.5.1).

5.4 Data collection

- **Clinical variables.** Age, sex and completed medical history was collected from all participants. The data was reported to the study database according to the Case Report Form (CRF) **(ANNEX 3)**, in which all variables are included.

- **Anthropometric variables.** Weight, height and waist circumference. Subjects were weigthed barefoot with a calibrated weighing scale. Standing height was measured with height rod, also barefoot, and it was assessed three times and the average was taken as the correct value. Waist circumference was measured locating the upper hip bone and the top of the right iliac crest, placing a measuring tape in a horizontal plane around the abdomen at the level of the iliac crest. The measurement is made at the end of a normal expiration.

- **Systolic and Diastolic Blood Pressure (SBP, DBP).** SBP and DBP were measured using an electronic sphygmomanometer. Every patient rested for 30 minutes before starting the procedure and the patient's arm was resting on a surface that was level with their arm. It was assessed three times and the average of two similar measurements was taken as the correct value.

- **Blood test.** Blood samples were obtained in the morning after an overnight fast (12 hours). Fasting plasma glucose (FPG) and lipid profiles (triglycerides and HDL) were measured by standard laboratory methods using an analyzer (Cobas® 8000 c702, Roche Diagnostics, Basel, Switzerland). Ultrasensitive C-reactive protein (usCRP) levels were determined by immunoturbidimetric method (Cobas® 8000 c702, Roche Diagnostics, Basel, Switzerland).

- **Microbiota.** Each sample collection was brought to the lab within 4 hours of collecting it and it was frozen to -80 ºC. Extraction of fecal genomic DNA and whole-genome shotgun sequencing was done. It was performed whole-genome shotgun sequencing of 131 fecal samples. On average, we obtained 38 million paired-end reads for each sample (ranging from 15 million to 116 million). Fecal genomic DNA was extracted from 100mg of frozen stools using the QIAamp DNA mini stool kit (Qiagen, Coutaboeuf, France) following repeated bead-beating ((6,500 r.p.m., 3 × 30 s). The DNA was extracted from 131 fecal samples, obtained from the participants at three different time points during the study. DNA fragments of approximately 300 bp were

sequenced on an Illumina NextSeq 500 instrument (150 bp; paired-end) at Genomics Core Facility at the Sahlgrenska Academy, University of Gothenburg.)

- **Diet.** Validated food frequency questionnaire was given to all participants (28) **(ANNEX 4).**

5.5 Variables

5.5.1 Primary variable

- **Metabolic syndrome.** Dichotomus qualitative variable. ATP III and IDF were used criteria on this study for diagnosing the metabolic syndrome patients. The patients diagnosed with MetS were exactly the same with both criteria, even though for IDF having abdominal obesity was a must while on ATP III wasn't a mandatory factor. (29) (10) Patients who didn't meet criteria for having metabolic syndrome were classified as 0 and patients who met them were classified as 1.

- **Microbiota.** Quantitative variable based on relative abundance of the different *Phyla*. *Phylum* which was detected in more than 20% of the subjects was analysed. The gut microbial dysbiosis is believed to contribute to metabolic diseases via stimulation of low-grade inflammation (18)

5.5.2 Secondary variable:

- **Age.** Continuous quantitative variable, expressed in years. Age is an important factor because of the physiological variations seen in the metabolism (30) and microbiota **(31)** .

- **Sex.** Dichotomus qualitative variable, expressed as male or female. It has been described as a percentage and absolute value. Male patients were classified as 1 and female as 2.

- **Menopause in women.** Dichotomus qualitative variable. Many chronic diseases can emerge after estrogen levels decline, understanding the role of the microbiota in women's health at the menopausal phase could help to improve strategies for microbiota modulation and prevent dysfunction. (29) Patients were classified as 0 for pre-menopausal women and 1 for menopausal women.

- **Body mass index (BMI).** Continuous quantitative variable, expressed in kg/m^2. It is calculated through weight (kg) and height (m^2). Regarding metabolic syndrome classification if BMI > 30 kg/m^2 central obesity can be assumed and it is a fundamental criteria according to IDF (9) definition and one of the possible criterias for ATP III. (10) Obesity has been defined as BMI ≥ $30kg/m^{2.}$

- **Systolic blood pressure (SBP).** Continuous quantitative variable, expressed in millimetres of mercury (mmHg). Systolic blood pressure is force exerted on the artery wall when the heart is pumpling (contracting) (32) Normal values are <140 mmHg. (33)

- **Diastolic blood pressure (DBP)**. Continuous quantitative variable, expressed in millimetres of mercury (mmHg). The force exerted when the hearts relaxes is known as diastolic blood pressure(32) Normal value are <85 mmHg. (33)

- **Lipid profile.** Lipid profile is defined for total cholesterol, high-density lipoprotein cholesterol (HDL-c) and triglycerides.
  Serum triglycerides and serum HDL-c are part of the criteria for the definition of the metabolic syndrome (9)

  - Triglycerides. Continuous quantitative variable, expressed in (mg/dL).

  - HDL. Continuous quantitative variable, expressed in (mg/dL).

- **IFG.** Continuous quantitative variable, expressed in (mg/dL). Impaired fasting glucose plasma is one of the criteria for diagnosing metabolic syndrome (fasting glucose 110-125 mg/dl). (34)

- **Ultrasensitive C-Reactive Protein (us-CRP).** Dichotomic qualitative variable based on median of our sample. Is an acute-phase reactant protein. The results are reported in mg/dL. (35) CRP is a key factor for the progression to insulin resistance in DM2. (5)The subjects who had a us-CRP under the median were classificated as 0 and the subjects who had it above the median were classificated as 1.

- **Diet.** A Validated Food Frequency Questionnaire was administrated. Macronutrients (carbohydrates, fiber, proteins, lipids), micronutrients (vitamin A, B1, B2, B3, B6, B12, C, D and E) and total kcal were analyzed. Macronutrients were measured per

g/day and vitamins were measured as mg/day or μm/day. The diet is essential since plays a significant role in shaping the microbiome(36)

- **Tobacco.** Categorized in smokers/non-smokers. Recent studies have indicated microbiome alterations in smokers. (37)

- **Alcohol.** Grams of alcohol consumed per day were measured. There is a strong negative influence of alcohol dependence on gut microbiota. (38)

6. STATISTICAL ANALYSES

- **Descriptive analyses.** Stratifying by patients with and without metabolic syndrome, firstly, normal distribution and homogeneity of variances were tested. Results are expressed as number and frequencies for categorical variables, mean and standard deviation (SD) for normal distributed continuous variables and median and interquartile range [IQ] for non-normal distributed continuous variables.

- **Bivariate inference.** To determine differences between study groups (participants with mand without metabolic syndrome), we used unpaired Student's t-test in normal quantitative and Mann-Whitney U test for non-normal quantitative variables and Chi-squared test for categorical variables. Nonparametric Spearman correlations were used to determine the associations between quantitative variables.

- **Multivariate analyses.** Two kinds of stepwise multivariate linear regression were performed to assess the association of clinical, anthropometric, laboratory, dietetic parameters and metabolic syndrome with microbiome. Phylum of microbiote which had significant differences on bivariate analyses or Mann-Whitney U test were used as the dependent variables. As the independent variables:
  - In the first models: age, sex, presence of metabolic syndrome, BMI, usCRP, kcal total and a parameters of the diet.
  - In the second sort of models: age, sex, BMI, SBP, DBP, FPG, Triglycerides, HDL-cholesterol, usCRP, tobacco, alcohol, total kcal and a parameters of the diet.

- Statistical significance was set at p value < 0.01. All statistical analyses were performed with SPSS, version 19 (SPSS, Inc, Chicago, IL).

7. ETHICAL CONSIDERATIONS

This study respects all ethical considerations and human rights reflected in the Declaration of Helsinki for medical research involving human subjects, developed by the World Medical Association (WMA).

All personal and clinical information will remain confidential and only used for the purpose of the research according to the "Ley Organica 3/2018, del 5 de diciembre, de Protección de Datos de Carácter Personal" published on BOE 294, 6th December 2018.

The "Ley 14/2007, del 3 de Julio, de investigación biomedica" concerning medical investigations has also been strictly followed.

Informed consent has been obtained from each subject (**ANNEX 2**) after full explanation of the purpose and nature of all procedures used (**ANNEX 1**).

In order to carry out this study it was necessary to present the research protocol to the Clinical research Ethics Committee (CEIC) of Hospital Universitari Josep Trueta.

Participants have the right to access, modify, oppose or remove their personal data.
The investigators of this project declare that there are no conflicts of interest, and that they didn´t receive any economic compensation to collaborate in the study.

8. RESULTS

8.1 Descriptive analyses

A target population of 131 patients was studied, 67.9% females. Metabolic syndrome (MS) was presented in 65 participants while 66 did not meet the criteria for MS. Both groups were similar in age, whereas subjects with MS had higher levels of fasting plasma glucose (FPG), triglycerides and us-CRP and lower levels of HDL-cholesterol. The mean values of BMI and waist circumference in the MS group were in range of obesity and central obesity respectively (**Table 5A**). We also analysed the diet and showed that vitamin B12 and B3 were slightly more elevated in patients with metabolic syndrome than without metabolic syndrome. Significant differences between patients with MetS and without were not found on the rest of micronutrients and macronutrients examined (**Table 5B**).

No significant differences were found with alcohol and metabolic syndrome and neither with tobacco and MetS.

Table 5A. Descriptive analyses of the study population.

n=131	Without metabolic syndrome (n=66)	With metabolic syndrome (n=65)	*p*
Female n (%)	47 (71.20)	42 (64.60)	
Age (years)	47.3 (35.0-58.0)	51.6 (41.8-58.0)	0.102
Alcohol (g/day)	1.25 (0-5.5)	1.1 (0-5.5)	0.74
Tobacco (smokers)	5 7.6	11 16.9	0.117
BMI (kg/m²)	25.7 (23.7-40.0)	41.8 (34.2-47.4)	**<0.001**
Waist circumference (cm)	93 (84.0-117.0)	124 (110.0-130.0)	**<0.001**
Systolic blood pressure (mmHg)	124.1 ± 17.3	141.6 ± 17.6	**<0.001**
Diastolic blood pressure (mmHg)	69.9 ± 10.8	80.5 ± 9.5	**<0.001**
Fasting plasma glucose (mg/dl)	94 (88.0-98.0)	99 (93-106)	**0.001**
Triglycerides (mg/dL)	74.5 (57.8-94.3)	126 (85.5-163.5)	**<0.001**
HDL Cholesterol (mg/dl)	51 (59.5-73.5)	47 (38.0-60.0)	**<0.001**
Ultrasensitive CRP (mg/dL)	1.1 (0.5-3.5)	3.9 (1.7-9.6)	**<0.001**

BMI, body mass index; HDL, high density lipoprotein; CRP, C-reactive protein

Table 5B. Descriptive analyses of the study population.

n=131	Without metabolic syndrome (n=66)	With metabolic syndrome (n=65)	*p*
Dietary components			
Total energy (Kcal/day)	1817 (1647.8-2382.3)	1974.4 (1595.8-2320.9)	0.642
Protein (g/day)	88.9 (74.7-103.9)	101.9 74.6-119.8	0.075
Carbohydrate (g/day)	181.7 (151.7-241.4)	192 (152.8-233.7)	0.0922
Lipids (g/day)	84.1 (73.8-113.2)	89.2 (70.3-112.4)	0.935
Fiber (g/day)	19.5 (17.0-25.5)	21.8 (15.7-25.4)	0.937
Vitamin A (µg/day)	1582 (1015-2144)	1754.5 (1247.8-2776.8)	0.088
Vitamin B1 (mg/day)	1.57 (1.34-2.12)	1.88 (1.48-2.19)	0.119
Vitamin B2 (mg/day)	1.81 (1.51-2.27)	1.94 (1.52-2.58)	0.38
Vitamin B3 (mg/day)	23.8 (19.7-27.3)	25.7 (21.2-32.4)	**0.047**
Vitamin B6 (mg/day)	1.9 (1.6- 2.2)	2 (1.6-2.4)	0.299
Vitamin B12 (µg/day)	7.4 (5.4-10.0)	9.3 (6.9-12.3)	**0.011**
Vitamin C (mg/day)	124.2 (99.5-176.2)	137.7 (96.1-191.8)	0.346
Vitamin D (µg/day)	4.1 (3.2-5.4)	5 (3.5-6.4)	0.073
Vitamin E (mg/day)	12.3 (9.7-15.0)	12 (9.5-14.7)	0.615
Vitamin K (µg/day)	206.4 (150.4-244.3)	216.7 (166.7-295.4)	0.09

The difference in microbiota composition in patients depending on the metabolic syndrome was analyzed (**Table 6**).

Subjects without MS had higher RA of *Candidatus Atribacteria,Chrysiogenetes, Lentisphaerae, Planctomycetes* and *Tenericutes* than patients who met criteria for MS, all of them with a $p<0.01$.

Table 6. Differences in microbiota composition in patients with and without metabolic syndrome.

Phylum	without MS (n=66)	with MS (n=65)	P value
Acidobacteria	$5.43x10^{-6}$ ($3.20x10^{-6}$, $7.83x10^{-6}$)	$3.80x10^{-6}$ ($2.97x10^{-6}$, $6.04x10^{-6}$)	0.019
Aquificae	$1.41x10^{-6}$ ($5.77x10^{-7}$, $2.88x10^{-6}$)	$7.77x10^{-7}$ ($3.94x10^{-7}$, $2.24x10^{-6}$)	0.037
Candidatus Aminecenantes	$4.31x10^{-7}$ (0, $9.93x10^{-7}$)	0 (0, $5.04x10^{-7}$)	0.041
Candidatus Atribacteria	$7.09x10^{-7}$ ($2.61x10^{-7}$, $1.34x10^{-6}$)	$3.89x10^{-7}$ (0, $7.69x10^{-7}$)	**0.005**
Candidatus Desantibacteria	$4.14x10^{-7}$ (0, $8.99x10^{-7}$)	0 (0, $5.15x10^{-7}$)	0.03
Candidatus Gottesmanbacteria	$4.27x10^{-7}$ (0, $6.77x10^{-7}$)	0 (0, $4.82x10^{-7}$)	0.015
Candidatus Magasanikbacteria	$5.68x10^{-7}$ (0, $1.40x10^{-6}$)	$4.02x10^{-7}$ (0, $8.46x10^{-7}$)	0.019
Candidatus Omnitrophica	$2.78x10^{-6}$ ($1.56x10^{-6}$, $5.86x10^{-6}$)	$1.92x10^{-6}$ ($1.37x10^{-6}$, $3.55x10^{-6}$)	0.033
Candidatus Woesebacteria	$3.96x10^{-7}$ (0, $8.42x10^{-7}$)	0 (0, $4.81x10^{-7}$)	0.028
Chrysiogenetes	$4.14x10^{-7}$ (0, $1.12x10^{-6}$)	0 (0, $4.74x10^{-7}$)	**0.006**
Fusobacteria	$5.88x10^{-5}$ ($4.46x10^{-5}$, $1.10x10^{-4}$)	$5.08x10^{-5}$ ($3.53x10^{-5}$, $6.87x10^{-5}$)	0.042
Lentisphaerae	$9.16x10^{-6}$ ($2.44x10^{-6}$, $3.76x10^{-7}$)	$3.83x10^{-6}$ ($1.36x10^{-6}$, $9.06x10^{-6}$)	**0.004**
Nitrospirae	$4.21x10^{-6}$ ($2.06x10^{-6}$, $6.66x10^{-6}$)	$3.02x10^{-6}$ ($1.49x10^{-6}$, $5.10x10^{-6}$)	0.045
Planctomycetes	$7.34x10^{-6}$ ($4.66x10^{-6}$, $1.19x10^{-5}$)	$5.05x10^{-6}$ ($2.76x10^{-6}$, $8.99x10^{-6}$)	**0.006**
Spirochaetes	$1.14x10^{-4}$ ($8.02x10^{-5}$, $1.83x10^{-4}$)	$8.76x10^{-5}$ ($5.86x10^{-5}$, $1.33x10^{-4}$)	0.014
Tenericutes	$3.42x10^{-5}$ ($1.92x10^{-5}$, $1.91x10^{-4}$)	$2.02x10^{-5}$ ($1.22x10^{-5}$, $4.17x10^{-5}$)	**0.008**
Candidate division Zixibacteria	$7.91x10^{-7}$ (0, $1.57x10^{-6}$)	$4.54x10^{-7}$ (0, $7.27x10^{-7}$)	0.032
Chlorophyta	$1.67x10^{-6}$ ($7.13x10^{-7}$, $2.49x10^{-6}$)	$9.75x10^{-7}$ ($4.39x10^{-7}$, $2.32x10^{-6}$)	0.029
Eukaryota na	$5.87x10^{-6}$ ($3.36x10^{-6}$, $2.71x10^{-4}$)	$3.92x10^{-6}$ ($2.21x10^{-6}$, $8.79x10^{-6}$)	0.013
Neocallimastigomycota	$1.17x10^{-6}$ ($5.10x10^{-7}$, $2.20x10^{-6}$)	$6.89x10^{-7}$ ($3.54x10^{-7}$, $1.33x10^{-6}$)	0.01

*On ANNEX 5 full table available.

Microbiota was analyzed depending on sex and metabolic syndrome (see table 7). Males without MS had lower relative abundance of *Acidobacteria, Candidatus Atribacteria, Candidatus Omnitrophica, Lentisphaerae, Planctomycetes, Tenericutes and Eukaryota na phylum* than males with MS.

Regarding females, no significant differences were observed in the RA of the Phylum studied. Moreover, females were classified depending on their menopausal status **(ANNEX 5)** but no significant differences were found neither.

Table 7. Differences in Phylum microbiota composition in males and females with and without metabolic syndrome.

	Male			Female		
Phylum	**without MS (n=19)**	**with MS (n=23)**	**P value**	**without MS (n=47)**	**with MS (n=42)**	**P value**
Acidobacteria	$6.17x10^{-6}$ ($4.25x10^{-6}$, $7.70x10^{-6}$)	$3.72x10^{-6}$ ($2.83x10^{-6}$, $4.51x10^{-6}$)	**0.002**	$5.28x10^{-6}$ ($2.68x10^{-6}$, $8.19x10^{-6}$)	$4.30x10^{-6}$ ($3.06x10^{-6}$, $6.91x10^{-6}$)	0.373
Candidatus Atribacteria	$9.46x10^{-7}$ ($3.62x10^{-7}$, $1.35x10^{-6}$)	0 (0, $5.53x10^{-7}$)	**0.008**	$6.40x10^{-7}$ (0, $1.28x10^{-6}$)	$4x10^{-7}$ (0, $9.57x10^{-7}$)	0.116
Candidatus Omnitrophica	$3.35x10^{-6}$ ($2.18x10^{-6}$, $5.72x10^{-6}$)	$1.89x10^{-6}$ ($1.46x10^{-6}$, $2.74x10^{-6}$)	**0.004**	$2.39x10^{-6}$ ($1.48x10^{-6}$, $5.90x10^{-6}$)	$1.93x10^{-6}$ ($1.36x10^{-6}$, $3.96x10^{-6}$)	0.406
Lentisphaerae	$1.04x10^{-5}$ ($4.50x10^{-6}$, $6.36x10^{-5}$)	$2.77x10^{-6}$ ($1.41x10^{-6}$, $4.85x10^{-6}$)	**0.001**	$7.94x10^{-6}$ ($2.07x10^{-6}$, $3.43x10^{-5}$)	$6.10x10^{-6}$ ($1.30x10^{-6}$, $1.32x10^{-5}$)	0.167
Planctomycetes	$8.69x10^{-6}$ ($6.31x10^{-6}$, $1.18x10^{-5}$)	$4.14x10^{-6}$ ($2.69x10^{-6}$, $5.93x10^{-6}$)	**<0.001**	$6.99x10^{-6}$ ($4.14x10^{-6}$, $1.33x10^{-5}$)	$6.34x10^{-6}$ ($2.91x10^{-6}$, $1.08x10^{-5}$)	0.312
Tenericutes	$4.57x10^{-5}$ ($2.63x10^{-5}$, $1.30x10^{-4}$)	$1.96x10^{-5}$ ($1.50x10^{-5}$, $2.74x10^{-5}$)	**0.001**	$2.98x10^{-5}$ ($1.63x10^{-5}$, $5.24x10^{-4}$)	$2.16x10^{-5}$ ($1.19x10^{-5}$, $7.91x10^{-5}$)	0.221
Eukaryota na	$1.21x10^{-5}$ ($4.37x10^{-6}$, $4.33x10^{-4}$)	$3.48x10^{-6}$ ($2.36x10^{-6}$, $1.33x10^{-5}$)	**0.002**	$5.63x10^{-6}$ ($2.06x10^{-6}$, $1.72x10^{-4}$)	$4.18x10^{-6}$ ($1.80x10^{-6}$, $8.53x10^{-6}$)	0.269

*On ANNEX 5 full table available

As can be seen in **Table 8**, the subjects were divided depending on metabolic syndrome and with or without obesity. In the group of absence of MS, subjects with obesity had lower RA of *Aquificae*, *Candidatus Moranbacteria*, *Candidatus Omnitrophica, Tenericutes*, *Spirochaetes, Thermotogae*, and *Ascomycota* than their counterparts without obesity.

In individuals with MS, those who did not meet obesity criteria (BMI ≥ 30 kg/m^2) presented higher RA of *Candidatus Perigrinibacteria*, *Chlorobi*, *Deinococcus Thermus*, *Chlorophyta*, and *Eukaryota na* and *uc* than subjects with MS and obesity.

Table 8. Differences in Phylum microbiota composition in subjects with and without metabolic syndrome depending on the presence of obesity.

	without MS			with MS		
Phylum	**without obesity (n=43)**	**with obesity (n=23)**	**P value**	**without obesity (n=11)**	**with obesity (n=54)**	**P value**
Aquificae	$1.75x10^{-6}$ ($1.02x10^{-6}$, $3.15x10^{-6}$)	$6.79x10^{-7}$ ($3.18x10^{-7}$, $2.04x10^{-6}$)	**0.008**	$2.33x10^{-6}$ ($5.01x10^{-7}$, $2.97x10^{-6}$)	$6.68x10^{-7}$ ($3.77x10^{-7}$, $1.76x10^{-6}$)	0.079
Candidatus Moranbacteria	$8.89x10^{-7}$ ($5.15x10^{-7}$, $1.81x10^{-6}$)	$3.48x10^{-7}$ (0, $5.50x10^{-7}$)	**0.001**	$4.66x10^{-7}$ (0, $1.08x10^{-6}$)	$6.40x10^{-7}$ (0, $1.45x10^{-6}$)	0.456
Candidatus Omnitrophica	$3.42x10^{-6}$ ($1.78x10^{-6}$, $6.78x10^{-6}$)	$2.09x10^{-6}$ ($9.49x10^{-7}$, $3.35x10^{-6}$)	**0.007**	$2.33x10^{-6}$ ($1.57x10^{-6}$, $5.01x10^{-6}$)	$1.88x10^{-6}$ ($1.31x10^{-6}$, $3.15x10^{-6}$)	0.142
Candidatus Peregrinibacteria	$9.51x10^{-7}$ (0, $2.24x10^{-6}$)	$3.68x10^{-7}$ (0, $5.95x10^{-7}$)	0.013	$1.89x10^{-6}$ ($5.24x10^{-7}$, $2.64x10^{-6}$)	$5.42x10^{-7}$ ($2.64x10^{-7}$, $1.02x10^{-6}$)	**0.003**
Chlorobi	$2.66x10^{-6}$ ($1.71x10^{-6}$, $4.67x10^{-6}$)	$2.38x10^{-6}$ ($9.55x10^{-7}$, $3.70x10^{-6}$)	0.172	$3.77x10^{-6}$ ($2.65x10^{-6}$, $5.76x10^{-6}$)	$1.92x10^{-6}$ ($1.12x10^{-6}$, $3.25x10^{-6}$)	**0.009**
Deinococcus Thermus	$2.53x10^{-6}$ ($1.77x10^{-6}$, $4.75x10^{-6}$)	$1.67x10^{-6}$ ($9.49x10^{-7}$, $2.86x10^{-6}$)	0.046	$4.71x10^{-6}$ ($2.50x10^{-6}$, $5.29x10^{-6}$)	$1.97x10^{-6}$ ($9.61x10^{-7}$, $3.48x10^{-6}$)	**0.002**
Spirochaetes	$1.27x10^{-4}$ ($9.06x10^{-5}$, $2.41x10^{-4}$)	$8.29x10^{-5}$ ($6.22x10^{-5}$, $1.28x10^{-4}$)	**0.001**	$1.28x10^{-4}$ ($7.92x10^{-5}$, $2.84x10^{-4}$)	$8.54x10^{-5}$ ($5.38x10^{-5}$, $1.19x10^{-4}$)	0.023
Tenericutes	$1.10x10^{-4}$ ($2.27x10^{-5}$, $1.09x10^{-3}$)	$2.25x10^{-5}$ ($1.63x10^{-5}$, $4.57x10^{-5}$)	**0.003**	$4.20x10^{-5}$ ($1.71x10^{-5}$, $1.34x10^{-4}$)	$1.96x10^{-5}$ ($1.19x10^{-5}$, $3.28x10^{-5}$)	0.025
Thermotogae	$5.14x10^{-6}$ ($3.79x10^{-6}$, $8.25x10^{-6}$)	$3.52x10^{-6}$ ($1.90x10^{-6}$, $5.36x10^{-6}$)	**0.006**	$5.52x10^{-6}$ ($3.77x10^{-6}$, $6.60x10^{-6}$)	$3.82x10^{-6}$ ($2.65x10^{-6}$, $4.99x10^{-6}$)	0.066
Ascomycota	$4.75x10^{-6}$ ($2.90x10^{-6}$, $1.37x10^{-5}$)	$3.13x10^{-6}$ ($2.04x10^{-6}$, $4.54x10^{-6}$)	**0.006**	$3.71x10^{-6}$ ($1.76x10^{-6}$, $7.40x10^{-6}$)	$3.50x10^{-6}$ ($1.93x10^{-6}$, $8.18x10^{-6}$)	0.834
Chlorophyta	$2.07x10^{-6}$ ($9.92x10^{-7}$, $2.86x10^{-6}$)	$1.09x10^{-6}$ (0, $1.78x10^{-6}$)	0.013	$2.50x10^{-6}$ ($1.40x10^{-6}$, $3.67x10^{-6}$)	$8.59x10^{-7}$ ($4x10^{-7}$, $1.28x10^{-6}$)	**0.001**
Eukaryota na	$1.11x10^{-5}$ ($4.59x10^{-6}$, $3.49x10^{-4}$)	$3.77x10^{-6}$ (0, $1.78x10^{-6}$)	0.012	$7.49x10^{-6}$ ($4.02x10^{-6}$, $9.69x10^{-5}$)	$3.18x10^{-6}$ ($1.80x10^{-6}$, $6.43x10^{-6}$)	**0.007**
Eukaryota uc	$4.61x10^{-7}$ (0, $1.83x10^{-6}$)	0 (0, $5.84x10^{-7}$)	0.188	$5.02x10^{-7}$ (0, $1.65x10^{-6}$)	0 (0, $4.68x10^{-7}$)	**0.004**

*On ANNEX 5 full table available.

Differences in microbiote composition in participants with and without metabolic syndrome depending on the grade of inflammation was analized in **Table 9**.

Subjects with MS and higher levels of us-CRP had lower RA of *Fibrobacteres phylum*. Regarding subjects without MS and low grade of inflammation, significant higher RA of *Armatinomadetes*, *Calditrichaeota*, *Candidatus Marinimicrobia*, *Candidatus Moranbacteria*, *Candidatus Omnitrophica*, *Candidatus Peregrinibacteria*, *Cyanobacteria*, *Deinococus Thermus*, *Elusimicrobia*, *Planctomycetes*, *Spirochaetes*, *Synergistetes*, *Tenericutes*, *Thermotogae*, *Ascomycota*, *Chlorophyta*, *Neocallimastigomycota* were observed.

Table 9. Differences in microbiote composition in subjects with and without metabolic syndrome regarding the grade of inflammation.

	without MS			with MS		
Phylum	**low grade of inflamation (n=46)**	**with inflammation (n=20)**	**P value**	**without inflammation (n=22)**	**with inflammation (n=43)**	**P value**
Armatimonadetes	$2.38x10^{-6}$ ($9.75x10^{-7}$, $3.40x10^{-6}$)	$6.83x10^{-7}$ (0, $2.42x10^{-6}$)	**0.009**	$1.31x10^{-6}$ ($8.47x10^{-7}$, $3.52x10^{-6}$)	$1.49x10^{-6}$ ($5.71x10^{-7}$, $2.71x10^{-6}$)	0.921
Calditrichaeota	$4.09x10^{-7}$ (0, $8.60x10^{-7}$)	0 (0, 0)	**0.007**	0 (0, $7.68x10^{-7}$)	0 (0, $4.02x10^{-7}$)	0.208
Candidatus Marinimicrobia	$5.14x10^{-7}$ (0, $1.13x10^{-6}$)	0 (0, $2.61x10^{-7}$)	**0.007**	$4.37x10^{-7}$ (0, $1.05x10^{-6}$)	0 (0, $4.73x10^{-7}$)	0.08
Candidatus Moranbacteria	$8.89x10^{-7}$ ($4.76x10^{-7}$, $1.72x10^{-6}$)	$3.76x10^{-7}$ (0, $6.38x10^{-7}$)	**0.002**	$4.71x10^{-7}$ (0, $1.32x10^{-6}$)	$4.91x10^{-7}$ (0, $1.66x10^{-6}$)	0.664
Candidatus Omnitrophica	$3x10^{-6}$ ($1.81x10^{-6}$, $6.90x10^{-6}$)	$1.72x10^{-6}$ ($6.49x10^{-7}$, $3.41x10^{-6}$)	**0.003**	$1.93x10^{-6}$ ($1.54x10^{-6}$, $4.11x10^{-6}$)	$1.92x10^{-6}$ ($1.15x10^{-6}$, $3.11x10^{-6}$)	0.332
Candidtus Peregrinibacteria	$9.51x10^{-7}$ ($4.41x10^{-7}$, $1.88x10^{-6}$)	0 (0, $4.08x10^{-7}$)	**0.001**	$1.08x10^{-6}$ ($4.05x10^{-7}$, $2.12x10^{-6}$)	$6.51x10^{-7}$ ($3.80x10^{-7}$, $1.09x10^{-6}$)	0.154
Cyanobacteria	$1.25x10^{-5}$ ($9.24x10^{-6}$, $1.68x10^{-5}$)	$7.88x10^{-6}$ ($3.60x10^{-6}$, $1.14x10^{-5}$)	**0.001**	$9x10^{-6}$ ($6.93x10^{-6}$, $1.36x10^{-5}$)	$9.39x10^{-6}$ ($6.09x10^{-6}$, $1.22x10^{-5}$)	0.663
Deinococcus Thermus	$2.55x10^{-6}$ ($1.86x10^{-6}$, $4.57x10^{-6}$)	$1.42x10^{-6}$ ($6.82x10^{-7}$, $2.74x10^{-6}$)	**0.001**	$2.96x10^{-6}$ ($1.50x10^{-6}$, $4.94x10^{-6}$)	$1.96x10^{-6}$ ($9.63x10^{-7}$, $3.25x10^{-6}$)	0.027
Elusimicrobia	$4.69x10^{-6}$ ($2.35x10^{-6}$, $6.75x10^{-6}$)	$1.62x10^{-6}$ ($8.43x10^{-7}$, $3.75x10^{-6}$)	**0.002**	$3.81x10^{-6}$ ($2.02x10^{-6}$, $5.43x10^{-6}$)	$2.19x10^{-6}$ ($9.79x10^{-7}$, $4.82x10^{-6}$)	0.028
Fibrobacteres	$3.54x10^{-5}$ ($2.30x10^{-5}$, $5.41x10^{-5}$)	$3.29x10^{-5}$ ($2.74x10^{-5}$, $5.26x10^{-5}$)	0.932	$4.13x10^{-5}$ ($2.91x10^{-5}$, $7.31x10^{-5}$)	$3.12x10^{-5}$ ($1.91x10^{-5}$, $4x10^{-5}$)	**0.005**
Planctomycetes	$8.69x10^{-6}$ ($6.58x10^{-6}$, $1.62x10^{-5}$)	$5.07x10^{-6}$ ($3.59x10^{-6}$, $7.51x10^{-6}$)	**0.003**	$5.93x10^{-6}$ ($2.83x10^{-6}$, $1.10x10^{-5}$)	$4.90x10^{-6}$ ($2.23x10^{-6}$, $8.06x10^{-6}$)	0.289
Spirochaetes	$1.28x10^{-4}$ ($8.99x10^{-5}$, $2.41x10^{-4}$)	$7.58x10^{-5}$ ($5.70x10^{-5}$, $1.16x10^{-4}$)	**0**	$8.76x10^{-5}$ ($7.11x10^{-5}$, $1.73x10^{-4}$)	$8.34x10^{-5}$ ($5.42x10^{-5}$, $1.23x10^{-4}$)	0.137
Synergistetes	$4.81x10^{-5}$ ($3.49x10^{-5}$, $1.36x10^{-4}$)	$2.81x10^{-5}$ ($1.44x10^{-5}$, $5.12x10^{-5}$)	**0.008**	$3.97x10^{-5}$ ($2.44x10^{-5}$, $8.15x10^{-5}$)	$3.44x10^{-5}$ ($2.31x10^{-5}$, $7.60x10^{-5}$)	0.675
Tenericutes	$5.40x10^{-5}$ ($2.52x10^{-5}$, $7.50x10^{-4}$)	$2.09x10^{-5}$ ($1.04x10^{-5}$, $4.36x10^{-5}$)	**0.003**	$2.20x10^{-5}$ ($1.58x10^{-5}$, $6.56x10^{-5}$)	$1.88x10^{-5}$ ($1.19x10^{-5}$, $3.24x10^{-5}$)	0.089
Thermotogae	$5.36x10^{-6}$ ($3.94x10^{-6}$, $7.56x10^{-6}$)	$2.85x10^{-6}$ ($1.32x10^{-6}$, $4.60x10^{-6}$)	**<0.001**	$4.11x10^{-6}$ ($3.28x10^{-6}$, $6.51x10^{-6}$)	$3.75x10^{-6}$ ($2.54x10^{-6}$, $4.96x10^{-6}$)	0.243
Ascomycota	$5.28x10^{-6}$ ($2.91x10^{-6}$, $1.25x10^{-5}$)	$3.17x10^{-6}$ ($1.79x10^{-6}$, $4.44x10^{-6}$)	**0.009**	$3.01x10^{-6}$ ($1.69x10^{-6}$, $1.90x10^{-5}$)	$3.55x10^{-6}$ ($2.06x10^{-6}$, $5.79x10^{-6}$)	0.957
Chlorophyta	$2.07x10^{-6}$ ($1.16x10^{-6}$, $2.86x10^{-6}$)	$7.84x10^{-7}$ (0, $1.69x10^{-6}$)	**0.004**	$1.36x10^{-6}$ ($6.59x10^{-7}$, $2.65x10^{-6}$)	$8.75x10^{-7}$ ($3.52x10^{-7}$, $1.40x10^{-6}$)	0.023
Neocallimastigomycota	$1.43x10^{-6}$ ($8.33x10^{-7}$, $2.58x10^{-6}$)	$4.96x10^{-7}$ ($3.26x10^{-7}$, $1.20x10^{-6}$)	**0.001**	$8.47x10^{-7}$ ($4.37x10^{-7}$, $1.89x10^{-6}$)	$6.89x10^{-7}$ (0, $1.25x10^{-6}$)	0.241

*On ANNEX 5 full table available.

Non-parametric correlations can be found in **Table 10**. A negative correlation between the BMI and quantification of *Acidobacteria, Aquificae, Candidatus Atribacteria, Candidatus Omnitrophica, Candidatus Peregrinibacteria, Chlorobi, Chlorophyta, Cyanobacteria, Deinococcus Thermus, Elusimicrobia, Eukaryota na, Lentisphaerae, Planctomycetes, Spirochaetes, Synergistetes, Tenericutes* and *Thermotogae* was verified.

A negative correlation between *Acidobacteria, Aquificae, Candidatus Atribacteria, Candidatus Omnitrophica, Chlorobi, Chlorophyta, Cyanobacteria, Deinococcus Thermus, Elusimicrobia, Eukaryota na, Lentisphaerae, Neocallimastigomycota, Planctomycetes, Spirochaetes, Synergistetes, Tenericutes* and *Thermotogae* and waist was found.

A negative correlation was seen also between SBP and *Chlorophyta.*

Between triglycerides and *Candidatus Atribacteria* a negative correlation was verified.

Moreover, a negative correlation was found between *Chlorophyta, Elusimicrobia, Eukaryota na Eukaryota uc, Lentisphaerae, Neocallimastigomycota,Planctomycetes, Spirochaetes, Tenericutes* and *Thermotogae* and usCRP.

In DBP, FPG and HDL-C no significant correlation between evaluated microorganisms was observed.

Table 10. Non-parametric correlations.

Non-parametric correlations	BMI (kg/m²)		Waist (cm)		SBP (mmHg)		DBP (mmHg)		FPG (mg/dL)		Triglycerides (mg/dL)		HDL-C (mg/dL)		us-CRP (mg/dL)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Acidobacteria (RA)	**-.360**	**.000**	**-.418**	**.000**	-.158	.073	-.150	.088	-.200	.022	-.228	.009	.225	.010	-.256	.004
Aquificae (RA)	**-.343**	**.000**	**-.391**	**.000**	-.024	.790	-.103	.242	-.129	.142	-.247	.004	.158	.071	-.224	.011
Armatimonadetes (RA)	-.266	.002	-.292	.001	-.133	.133	-.186	.034	-.152	.083	-.052	.554	.112	.201	-.241	.006
Ascomycota (RA)	-.213	.015	-.201	.023	.037	.674	.007	.933	.013	.880	-.106	.229	.007	.936	-.163	.068
Calditrichaeota (RA)	-.204	.020	-.217	.014	-.162	.066	-.147	.096	-.086	.327	-.047	.595	.008	.932	-.236	.008
Candidatus Atribacteria (RA)	**-.308**	**.000**	**-.280**	**.001**	-.072	.418	-.123	.163	-.088	.316	**-.325**	**.000**	.212	.015	-.215	.015
Candidatus Marinimicrobia (RA)	-.171	.051	-.146	.101	-.038	.671	-.038	.667	.048	.584	.000	1.000	.102	.245	-.188	.034
Candidatus Moranbacteria (RA)	-.234	.007	-.225	.011	-.067	.448	-.044	.623	-.124	.158	-.056	.524	.082	.350	-.202	.022
Candidatus Omnitrophica (RA)	**-.326**	**.000**	**-.364**	**.000**	-.183	.037	-.252	.004	-.099	.259	-.170	.052	.121	.169	-.271	.002
Candidatus Peregrinibacteria (RA)	**-.285**	**.001**	-.231	.009	-.049	.579	-.061	.489	.011	.897	-.084	.338	.092	.294	-.230	.009
Chlorobi (RA)	**-.307**	**.000**	**-.330**	**.000**	-.086	.329	-.027	.762	-.102	.248	-.116	.188	.174	.047	-.232	.009
Chlorophyta (RA)	**-.338**	**.000**	**-.353**	**.000**	**-.309**	**.000**	-.254	.004	-.102	.244	-.200	.022	.228	.009	**-.309**	**.000**
Chrysiogenetes (RA)	-.255	.003	-.232	.008	.013	.886	-.054	.545	-.125	.155	-.099	.259	.066	.456	-.186	.036
Cyanobacteria (RA)	**-.349**	**.000**	**-.333**	**.000**	-.167	.058	-.192	.029	-.134	.128	-.144	.101	.193	.027	-.271	.002
Deinococcus Thermus (RA)	**-.303**	**.000**	**-.325**	**.000**	-.056	.524	-.064	.472	-.127	.148	-.091	.300	.080	.364	-.303	.001
Elusimicrobia (RA)	**-.311**	**.000**	**-.311**	**.000**	-.142	.106	-.133	.132	-.092	.299	-.189	.030	.178	.042	**-.327**	**.000**
Eukaryota na (RA)	**-.308**	**.000**	**-.306**	**.000**	-.233	.008	-.248	.004	-.222	.011	-.185	.034	.139	.114	**-.302**	**.001**
Eukaryota uc(RA)	-.194	.026	-.203	.022	-.217	.013	-.231	.008	-.147	.094	-.188	.031	.072	.414	**-.364**	**.000**
Fibrobacteres (RA)	-.101	.249	-.041	.643	-.056	.530	.047	.596	-.045	.609	-.065	.463	-.012	.888	-.256	.004
Lentisphaerae (RA)	**-.380**	**.000**	**-.394**	**.000**	-.168	.056	-.232	.008	-.128	.145	-.239	.006	.145	.098	**-.297**	**.001**
Neocallimastigomycota (RA)	-.290	.001	**-.285**	**.001**	-.083	.350	-.052	.556	-.006	.944	-.172	.049	.105	.232	**-.343**	**.000**
Planctomycetes (RA)	**-.353**	**.000**	**-.367**	**.000**	-.215	.014	-.269	.002	-.158	.071	-.179	.041	.207	.018	**-.292**	**.001**
Spirochaetes (RA)	**-.399**	**.000**	**-.421**	**.000**	-.181	.040	-.234	.007	-.106	.228	-.230	.008	.219	.012	**-.383**	**.000**
Synergistetes(RA)	**-.325**	**.000**	**-.302**	**.001**	-.078	.380	-.139	.115	-.036	.681	-.113	.199	.173	.048	-.207	.020
Tenericutes (RA)	**-.375**	**.000**	**-.366**	**.000**	-.149	.091	-.194	.027	-.225	.010	-.248	.004	.158	.071	**-.345**	**.000**
Thermotogae (RA)	**-.316**	**.000**	**-.317**	**.000**	-.129	.145	-.053	.552	-.053	.548	-.102	.247	.203	.020	**-.372**	**.000**

RA (relative abundance), BMI (basal mass index), SBP (systolic blood pressure), DBP (diastolic blood pressure), FPG (fasting plasma glucose), HDL-C (high-density lipoprotein) and us-CRP (ultra-sensitive C-Reactive Protein).

B, p and R^2 adjusted values can be seen in **Table 11** as a result of a multiple regression analyses of components of metabolic syndrome, that were independently associated with the relative abundance of some Phyla.

Obesity had an independent and negative association with *Acidobacteria, Aquificae, Armatimonadetes, Candidatus Marinimicrobia, Candidatus Moranbacteria, Candidatus Omnitrophica, Candidatus Peregrinibacteria, Chlorobi, Chlorophyta, Chrysiogenetes, Cyanobacteria Neocallimastigomycota, Planctomycetes and Thermotogae.* Especially obesity was negatively associated with *Acidobacteria, Candidatus Omnitrophica* and *Thermotogae.* HDL-C was a predictor for *Synergistetes* and a major one for *Candidatus Atribacteria*.

DBP was associated independently and having a negative association for *Chlorophyta*; whereas us-CRP had an independent and negative association with *Fibrobacteres*.

Finally, IGT, as an independent variable, had a negative association with *Cyanobacteria*.

Independent associations between age, sex, SBP,triglycerides, tobacco, alcohol, total kcal and fiber (as independent variables) and the microbiota Phyla (as a dependent) were not found (data not shown).

Table 11. Linear regression model.

	Linear regression model	β	p	R^2 adjusted
Obesity	*Acidobacteria (RA)*	-0.366	<0.001	0.126
	Aquificae (RA)	-0.303	0.001	0.083
	Armatimonadetes (RA)	-0.288	0.002	0.075
	Candidatus Marinimicrobia (RA)	-0.196	0.039	0.030
	Candidatus Moranbacteria (RA)	-0.233	0.014	0.046
	Candidatus Omnitrophica (RA)	-0.358	<0.001	0.120
	Candidatus Peregrinibacteria (RA)	-0.318	0.001	0.093
	Chlorobi (RA)	-0.282	0.003	0.071
	*Chlorophyta (RA)**	-0.191	0.048	0.025
	Chrysiogenetes (RA)	-0.231	0.015	0.045
	*Cyanobacteria (RA)***	-0.319	0.001	0.117
	Neocallimastigomycota (RA)	-0.316	0.001	0.092
	Planctomycetes (RA)	-0.262	0.005	0.082
	Thermotogae (RA)	-0.329	<0.001	0.100
HDL-C	*Candidatus Atribacteria (RA)*	0.251	0.008	0.055
	Synergistetes (RA)	0.193	0.043	0.028
DBP	*Elusimicrobia (RA)*	-0.238	0.012	0.030
	Chlorophyta (RA)	-0.257	0.008	0.096
	Planctomycetes (RA)	-0.190	0.042	0.027
us-CRP	*Fibrobacteres (RA)*	-0.205	0.031	0.033
IGT	*Cyanobacteria (RA)*	-0.208	0.021	0.035

Dependent variable: Phylum. **Independent variables:** BMI, DBP, IGT, Triglycerides, HDL-C, usCRP.

Some associations between independent variables (BMI, HDL, DBP and us-CRP) with dependent variable (Phylum) are graphically depicted in **Figures 9A-9F**.

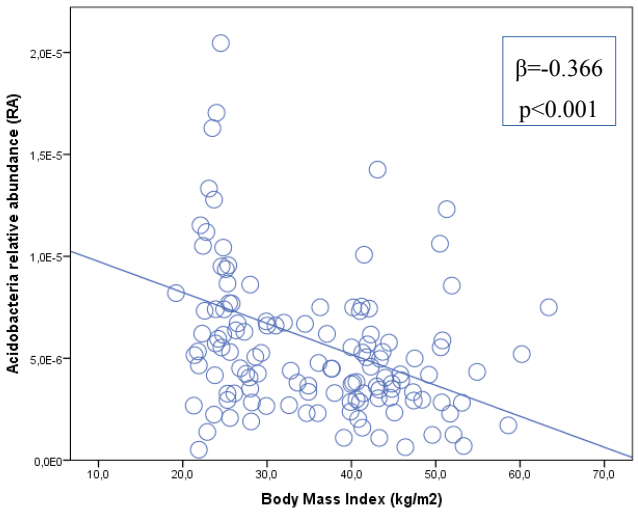

Figure 9A. Dispersion graph. β and p of the independent association variable BMI with *Acidobacteria*.

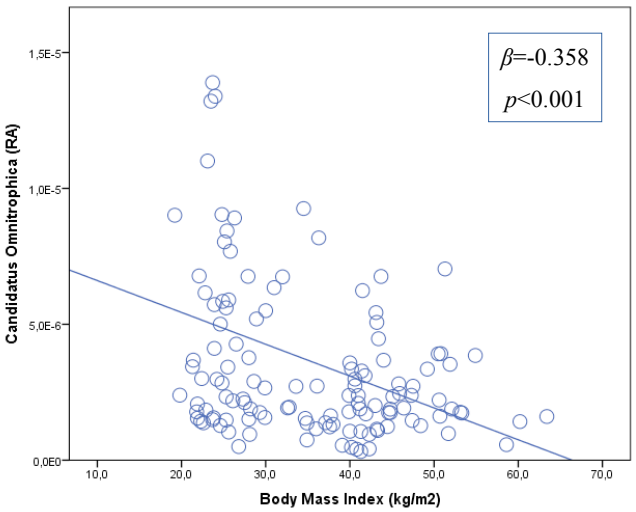

Figure 9B. Dispersion graph. β and p of the independent association variable BMI with *Candidatus Omnitrophica*.

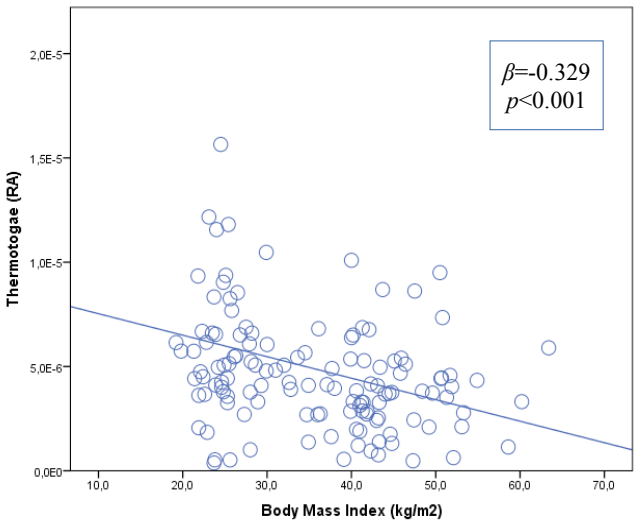

Figure 9C. Dispersion graph. β and p of the independent association variables BMI with *Thermotogae*.

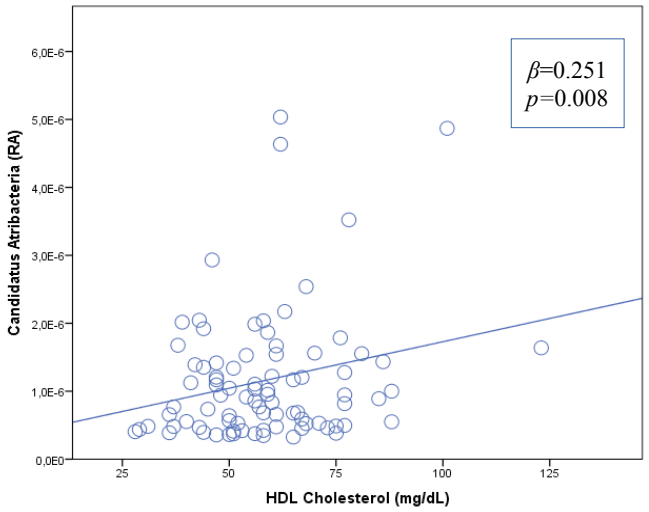

Figure 9D. Dispersion graph. β and p of the independent association variable HDL with *Candidatus Atribacteria*.

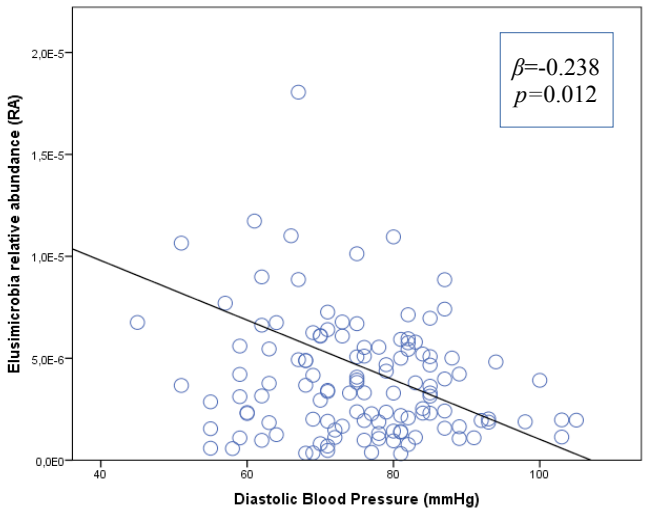

Figure 9E. Dispersion graph. β and p of the independent association variables DBP with *Elusimicrobia*.

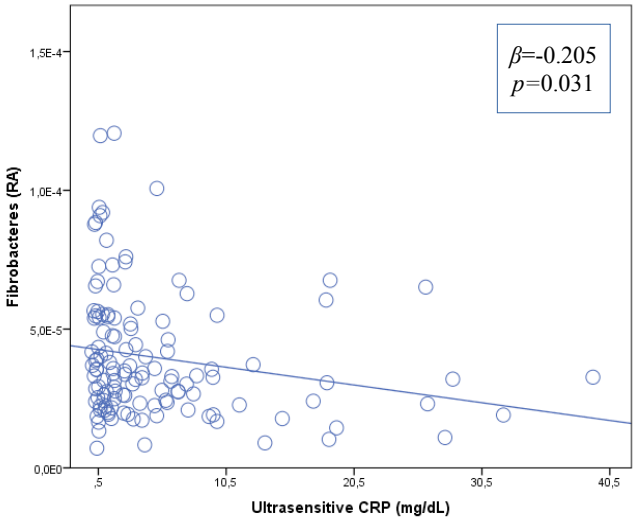

Figure 9F. Dispersion graph. β and p of the independent association variables us-CRP) with *Fibrobacteres*.

9. DISCUSSION

The evidence for a strong contribution of the gut microbiota to the onset of obesity and metabolic diseases is growing. (18) The results of this cross-sectional study indicated that microbiome of patients without metabolic syndrome had an increased presence of *Candidatus Atribacteria, Chrysiogenetes, Lentisphaerae, Planctomycetes* and *Tenericutes* compared with subjects with metabolic syndrome. Suggesting that these last microbiota mentioned might be potentially beneficial for preventing developing metabolic syndrome. Evidence about this association is scarce. Earlier studies mentioned the positively association of *Firmicutes* and *Proteobacteria* with MetS. (39)

The results suggested that gut microbiota may differ betwen men and women. Earlier study has shown such difference. (40) Microbiota differences in men depending on metabolic syndrome was observed as well, however, regarding women, no significant differences were found in the RA of *Phylum* studied and it wasn't found any significant difference neither on females depending on their menopausal status.

Interestingly, males without metabolic syndrome had lower relative abundance of *Acidobacteria*, *Candidatus Atribacteria*, *Candidatus Omnitrophica, Lentisphaerae, Planctomycetes, Tenericutes* and *Eukaryota na* than males with metabolic syndrome. This finding is interesting because it suggests that there may be some confounding variables and probably some interferences with the sex, since *Candidatus Atribacteria, Lentisphaerae, Planctomycetes* and *Tenericutes* had higher relative abundance in gut microbiome between general individuals depending on the metabolic syndrome. There is scarce evidence about such association and about differences of microbiota between sex. A study did not observe differences at Phyla level between men and women, just some differences were found with *Firmicutes/Bacteriodetes* when men and women were stratified according to their BMI. (41)

The gut microbiota in individuals without metabolic syndrome who were non-obese had higher relative abundance of *Aquificae, Candidatus Moranbacteria, Candidatus Omnitrophica, Tenericutes, Thermotogae* and *Ascomycota* than their counterparts with obesity, being associated as potential beneficial microbiota against obesity.

There is some previous evidence about an association between *Ascomycota* and

obese participants compared to non-obese individuals. (42) Literature was not found about the rest of the associations mentioned.

Candidatus Perigrinibacteria, Chlorobi, Deinococcus Thermus, Chlorophyta and Eukaryota na and *uc* have been succesfully indentified more abundant in patients who were non-obese with metabolic syndrome than those who were obese, being associated as potential beneficial microbiota for preventing obesity, as well. Previously reported data associated *Chlorobi* and *Deinococcus-Thermus* markedly lower in the high-fat diet mice group, (42) but data about their relation with BMI was not found.

This research showed that the gut microbiota of subjects with MetS and high levels of CRP had more abundance of *Fibrobacteres* than those who had low levels of CRP, suggesting that *Fibrobacteres* could be beneficial. No literature was found about association between *Fibrobacteres* and C-reactive protein, just a significantly higher populous of it on obese and diabetic leptin-resistant mice compared to the lean cohort, which wouldn't support the relation between inflammation and obesity before mentioned on the introduction. (44)
Patients without metabolic syndrome and low CRP had high abundance of *Armatinomadetes*, *Calditrichaeota*, *Candidatus Marinimicrobia*, *Candidatus Moranbacteria*, *Candidatus Omnitrophica*, *Candidatus Peregrinibacteria*, *Cyanobacteria*, *Deinococus Thermus*, *Elusimicrobia*, *Planctomycetes*, *Spirochaetes*, *Synergistetes*, *Tenericutes*, *Thermotogae*, *Ascomycota*, *Chlorophyta*, *Neocallimastigomycota,* suggesting having a potential beneficial for preventing subclinical inflammation. There is scarce evidence about these microbiota and inflammation, Wang et al. recently showed that *Cyanobacteria* have enhanced limonenene production (45) which is known for its anti-inflammatory effect. (46)
As mentioned before, one of the hallmarks of obesity and obesity-related pathologies is the occurrence of chronic low-grade inflammation, linking the gut. (18)
This statement has a relation with our findings since we observed that *Candidatus Moranbacteria, Tenericutes, Candidatus Omnitrophica, Spirochaetes, Thermotogae* and *Ascomycota* were significantly more abundant in patients without metabolic syndrome, who were not obese and decreased markers of chronic inflammation.

It was observed that BMI, DBP, FPG, HDL and us-CRP were independently associated with the gut microbiota. Surprisingly, independent associations between age, sex, SBP, triglycerides, tobacco,

alcohol, total kcal and fiber (as independent variables) and the microbiota Phyla (as a dependent variable) were not found.
In our study has been reported the association between obesity and *Acidobacteria, Candidatus Omnitrophica* and *Thermotogae,* among others. Obesity was independently and negatively associated with the relative abundance of these last Phyla mentioned. Studies have analyzed the relation between microbiota and BMI, but focusing mainly on *Firmicutes*/*Bacteroidetes*.(41,47)

There was an association as well betwen HDL-C and *Candidatus* Atribacteria and *Synergistetes*. HDL-C might be a factor which influences with higher relative abundance of these Phyla. A systematic review suggested significantly beneficial effects on HDL-C by consuming synbiotic food containing *Lactobacillus sporogenes,*(48) but it was not found literature about *Candidatus Atribacteria* and its association with HDL-C.

Chlorophyta, Elusimicrobia and *Planctomycetes* were associated with the DBP, meaning that participants with lower DBP had higher relaive abundance of them.
It really seems that *Fibrobacteres* had a remarkable link with *u*s-CRP, since in our results has been linked with it in different analyses, including in the linear regression where a negative association could be observed between both of them. IGT was associated independently and negatively with *Cyanobacteria.* There literature was scarce about these last microbiota mentioned and the association with the components of metabolic syndrome in general with microbiota. More research is needed.

In summary, there is an association between metabolic syndrome, inflammation and microbiota. Differences between gut microbiota in men and women were observed. BMI, DBP, FPG, HDL and us-CRP were independently associated with the gut microbiota. Moreover, interestingly, *Candidatus Moranbacteria, Candidatus Omnitrophica, Tenericutes, Spirochaetes, Thermotogae* and *Ascomycota* suggested to be potentially beneficial for preventing metabolic syndrome, obesity and chronic inflammation. Further studies are warranted to confirm our results and contribute to the development of new strategies for preventing and reversing metabolic syndrome. Gut microbiota will have to be analysed in different regions before making the fecal microbiota transplant a reality, more research about microbiota in every region is needed. It would be interesting in the future to collect data regarding environmental factors that may affect microbiota like stress.

10. CONCLUSIONS

In summary, our work shows that there is an association between metabolic syndrome, inflammation and gut microbiota. Gut microbiota was different between men and women. There were no differences found in the RA of *Phylum* studied among women, and neither depending on their menopausal status. Differences were observed in the gut microbiota when all participants were divided depending on metabolic syndrome and when we examined the data of the microbiota just in men depending on the metabolic syndrome. This finding is interesting because it suggests that there may be some confounding variables and probably some interferences with the sex.

There is a special connection between some microbiota in patients without metabolic syndrome, non-obese and low us-CRP, which need further investigation. Further studies are warranted to confirm our results and contribute to the development of new strategies for preventing and reversing metabolic syndrome by doing fecal microbiota transplants.

11. STRENGTH AND LIMITATIONS

We consider our data of interest because we found new posible microbiota associated with preventing obesity, inflammation, hypertension and dyslipidemia, which form part of the criteria for metabolic syndrome. The hypotheses and results assesed can be used for future investigations in a field with scarce evidence, providing more knowledge about the metabolic role of microbiota in our body as a factor for preventing and causing metabolic syndrome, among others diseases.

Moreover, the analysis was made with readily available data, being less time consuming and costly. For all these reasons mentioned, it has been highly feasible.

Several potential limitations that warrant consideration. Since it is an observational study, we can't determine cause and effect. Only a longitudinal approach and clinical trials could determine the real effects of microbiota on our metabolism. It is possible to have confounding variables and possible interactions, which we may not be aware of for scarce evidence about this innovative field.

12. PROJECT IMPACT ON THE NATIONAL HEALTH SERVICE

Metabolic syndrome has become a global epidemic and the total cost, including the cost of health care and loss of potential economic activity, is in trillions.(2)

Finding out which microbiota are associated with protection from this metabolic disorder and understanding its mechanism will be the key for future fecal microbiota transplantations. In this way, metabolic syndrome could be prevented or even reversed. With further investigation, we will avoid many morbidities of metabolic syndrome, like the risk for cardiovascular diseases and mortality risk (49) and extend life of our population, moreover, it could also reduce the national health services resources used to treat their morbidities, saving a lot of money to the hospitals.

This study showed the association not just between microbiota and metabolic syndrome, but also with inflammation which has been related with several metabolic alterations in our body (18) and even cancer. (50)

Understanding better the intestinal microbiome will help to develope novel therapeutic interventions and having an individualized health care.

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15. ANNEXES

ANNEX 1

FULL D'INFORMACIÓ AL PACIENT I CONSENTIMENT INFORMAT

DADES DE L'ESTUDI

Títol: Estudi del contingut de ferro en el teixit adipós, múscul i cervell en subjectes obesos. Interacció amb la composició de la microbiota i efectes de la pèrdua de pes.
Investigador Principal: Dr. José Manuel Fernández-Real, Secció Endocrinologia i Nutrició, Hospital Universitari Josep Trueta de Girona. Tel. 972.940.200. ext. 2325
Referència: IRONMET
Centre: Hospital Universitari Josep Trueta de Girona

Ens dirigim a vostè per informar-lo sobre un projecte d'investigació en el qual se'l convida a participar. Aquest projecte ha estat aprovat pel Comitè Ètic d'Investigació Clínica del nostre Centre.

La nostra intenció és que rebi la informació correcta i suficient perquè pugui avaluar i jutjar si vol o no participar en aquest projecte. Per a això, llegiu aquest full informatiu amb atenció, i nosaltres li aclarirem els dubtes que li puguin sorgir després de l'explicació. Pot consultar amb les persones que consideri oportú, a més de l'Investigador Principal del projecte, Dr. José Manuel Fernández-Real.

1.- ANTECEDENTS

La societat actual, està immersa en una forma de vida que afavoreix l'aparició de determinades malalties, producte entre altres coses del canvi d'hàbit alimentari i el sedentarisme. Així, tenim una elevada prevalença de patologies com l'obesitat o la diabetis mellitus, que en el moment actual afecten gairebé al 25% de la població del nostre país. Aquestes malalties comporten una menor esperança de vida i una pitjor qualitat de vida entre els que les pateixen. Entre les causes que s'estudien per explicar l'increment tan espectacular d'aquestes malalties, es considera el paper que el teixit adipós (greix) de l'organisme pot jugar en el seu desenvolupament i manteniment a llarg termini. El teixit adipós és un òrgan molt actiu a diferència del que es pensava fa uns anys, capaç de produir una sèrie de substàncies que poden afavorir un ambient propici per a l'aparició d'obesitat i diabetis.

Cada dia existeix més evidencia del paper del dipòsit excessiu de ferro associat a obesitat i diabetis. Es coneix com el pacient obès i diabètic tipus 2 tendeix a acumular ferro a nivell de diferents òrgans i teixits com són el fetge, el múscul, el cervell i el teixit adipós. Hi ha una evidència cada dia més gran de les relacions bidireccionals entre el metabolisme del ferro i la el metabolisme de la glucosa en el pacient obès. Diverses vies del metabolisme del ferro es modifiquen en presència d'alteracions en la tolerància oral a la glucosa, mentre que la resistència a la insulina es veu influenciada pels canvis en l'abundància relativa de ferro. Aquestes interrelacions bidireccionals s'exerceixen a múltiples nivells.

Els nexes entre el dipòsit de ferro a nivell del cervell o el teixit adipós humà i la seva relació amb la obesitat i la diabetis tipus 2 no han estat encara estudiats en profunditat.

El nostre grup està investigant quins són aquests nexes d'unió entre el dipòsit de ferro a nivell del cervell o el teixit adipós humà i la seva relació amb la obesitat i la diabetis tipus 2.

Alhora, sabem que a l'intestí de totes les persones s'alberguen multitud de bacteris que formen part de la flora normal de l'intestí, i que s'han relacionat amb el desenvolupament de diverses malalties, entre elles la obesitat i la DM2. L'anàlisi d'aquests bacteris, associat a l'acumulació de ferro en els diferents òrgans i teixits, ens pot ajudar a entendre molt millor aquests processos.

En el seu cas, ja sigui perquè pateix d'obesitat o perquè compleix criteris com a grup control, és convidat a participar en aquest estudi

2.- CONSENTIMIENT

L'estudi al que es convida a participar, està constituït per dos grups de pacients. En funció de quin sigui el seu índex de masa corporal (IMC), vostè serà inclòs en un o altre grup. Així,

- Si el seu IMC és inferior a 30, serà inclòs en el grup control i estudiat només en un únic punt de tall.

- Si el seu IMC és superior a 30, serà inclòs en el segon grup i se li demanarà de repetir les mateixes proves en 2 visites, separades un any entre sí.

La participació a l'estudi implica els següents procediments:

1. L'obtenció d'una mostra de sang (aproximadament 30 ml) a fi de realitzar un estudi analític bàsic (15mL) i uns altres 15mL que es procesaran i guardaran en forma de plasma, suero, linfocits i ADN per tal de fer altres determinacions analítiques vinculades a l'estudi.

2. L'obtenció d'una mostra de femta (recollirà vostè la mostra el mateix dia de l'estudi) per a l'estudi de la flora intestinal. En el cas que vostè pertanyi al grup amb IMC superior a 30, li demanarem una nova mostra a la segona visita (un any després de la primera)

3. La realització d'una exploració física amb mesures del pes, talla, freqüència cardíaca i tensió arterial.

4. La realització d'una ressonància magnètica cerebral i corporal. Aquesta és una prova d'imatge que es realitza per estudiar les característiques del cervell, el múscul i el teixit adipós. És una prova no invasiva que es realitza mitjançant un aparell de Ressonància Magnètica, que no produeix radiació i no origina cap molèstia a la persona explorada. Es tracta d'una prova d'uns 30 minuts de durada, en els quals vostè haurà de romandre estirat, mentre un aparell realitza els estudis d'imatge. És una prova innòcua i no existeixen contraindicacions, llevat que vostè sigui portador d'algun element metàl·lic, sigui al·lèrgic al contrast o estigui embarassada.

5. La realització d'una exploració neuropsicològica per part d'un neuropsicòleg

6. Realització d'un clamp hiperinsulinèmic-euglucèmic

Si vostè està d'acord en donar les mostres de sang, femta i orina i sotmetre's a les exploracions que s'han detallat, cal que signi aquest formulari de consentiment. Després que vostè signi aquest formulari li prendrem la mostra de sang.

La mostra serà enviada al nostre laboratori, on serà processada i emmagatzemat el plasma, sèrum, limfòcits i ADN resultant de la mostra de sang.La mostra de femta la recollirà vostè mateix en el seu domicili després de rebre unes senzilles instruccions, i se li facilitarà el material per recollir-la i guardar-la fins que la pugui portar a l'hospital. Aquesta mostra serà guardada al Biobanc fins el seu processat.

Se li realitzarà també una prova per estudiar el metabolisme de la glucosa i la seva resistència a la insulina. Per aquesta prova haurà de venir en dejú. És aconsellable que consumeixi aliments rics en sucres, farines i

almidons (patates, pasta, arrós, pa) durant els 3 dies previs, així com no realitzi exercici físic intens i no consumeixi cafeïna o alcohol en aquests dies. Se li col·locarà una via en una vena del braç i se li administrarà de forma continuada una sol·lució de glucosa endovenosa d'insulina realitzant extraccions de sang a través d'una via col·locada a l'altra braç cada 5 minuts fins el final de l'estudi (2 hores). Durant tota la prova se li realitzarà un control estricte dels nivells de sucre en sang.

L'emmagatzematge de totes les mostres està previst realitzar-lo a les instal·lacions del Biobanc IDIBGI (B. 0000872). Una vegada finalitzat l'estudi, si vostè ho autoritza, l'excedent de les mostres que no hagi estat utilitzat passarà a formar part del Biobanc per poder ser utilitzat en estudis posteriors. És per això que se li facilita un segon full d'informació i consentiment específic que haurà de signar i que serà custodiat pel coordinador del biobanc del seu Hospital.

En el cas que es produís un desenvolupament comercial dels coneixements generats, els possibles beneficis que es poguessin rebre seran íntegrament destinats a satisfer els objectius científics del grup de recerca al seu manteniment. Signant aquest formulari de consentiment, vostè renuncia als seus drets sobre qualsevol ús comercial relacionat amb la informació o les mostres que vostè ha cedit.

Excepcionalment, i sempre que vostè així ho autoritzi, es podria tornar a contactar amb vostè per a sol·licitar informació addicional.

3.- US DE LES SEVES MOSTRES BIOLÒGIQUES

Les seves mostres biològiques i la seva informació personal recollida com a part del projecte ***Estudi del contingut de ferro en el teixit adipós, múscul i cervell en subjectes obesos. Interacció amb la composició de la microbiota i efectes de la pèrdua de pes*** seran utilitzades únicament per a la realització del mateix.
El tractament i ús de les mostres es realitzarà seguint el que especifica la Llei de Recerca Biomèdica (14/2007), i el RD 1716/2011.

4.- INFORMACIÓ DELS RESULTATS DE LA INVESTIGACIÓ

Els estudis que es puguin incloure en el plà de recerca del nostre projecte no es fan per diagnosticar una determinada malaltia en una persona concreta; l'objectiu és fonamentalment epidemiològic i no tenen una utilitat clínica immediata. De fet, depenent del plà de recerca pot passar força temps des que es realitzi l'extracció fins que es realitzi l'estudi. És a dir, els temps de la investigació dependran de les preguntes científiques que es vagin produint i no es corresponen amb els temps de l'atenció clínica, generalment més curts.

Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per la qual cosa no han de ser considerats amb valor clínic per a vostè. No obstant això, en el cas que aquestes investigacions proporcionin dades que puguin ser clínica o genèticament rellevants per a vostè i interessar la seva salut o la de la seva família, li seran comunicats si així ho estima oportú. Així mateix, podria donar-se el cas d'obtenir informació rellevant per a la seva família; si es produeix aquesta situació, li correspondrà a vostè decidir si vol o no comunicar. Si vostè vol que se li comuniqui aquesta informació rellevant, ha de marcar la casella corresponent al final d'aquest document.

Si vostè no desitja rebre aquesta informació, tingui en compte que la llei estableix que, quan la informació obtinguda sigui necessària per evitar un greu perjudici per a la salut dels seus familiars biològics, un comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar als afectats o als seus representants legals.
Si per alguna raó vostè volgués conèixer els resultats de les investigacions que s'hagin produït com a conseqüència de la seva col·laboració, podrà posar-se en contacte amb els responsables del projecte, que l'informaran degudament.

5.- PARTICIPACIÓ VOLUNTARIA

La seva participació en aquesta investigació és voluntària i l'alternativa és no participar. Si decideix no participar, aquesta decisió no afectarà la seva assistència mèdica.

Així mateix, vostè pot retirar-se de l'estudi en qualsevol moment, sense donar explicacions. En aquest cas, cap dada nova serà afegida a la base de dades, i es procedirà a la destrucció de les mostres identificables, per evitar la realització de noves anàlisis.

Per sol·licitar la destrucció de les seves mostres haurà de contactar amb el Dr. José Manuel Fernández-Real al telèfon 972.940.200 ext. 2325.

6.- COST, RISCS I PROTECCIÓ DAVANT DE DANYS

L'extracció de la mostra no suposarà cap cost econòmic per a vostè.

El risc per a la salut d'una extracció de sang és molt petit, però pot incloure les molèsties habituals: lleu dolor local, pell contusionada, sagnat per on entra l'agulla, o l'ansietat davant les agulles. Es prendran precaucions per evitar aquests inconvenients.

La Ressonància Magnètica no produeix irradiació i no suposa cap risc afegit per a la seva salut. El radiòleg li preguntarà per possibles al·lèrgies al contrast abans de la seva administració. En el cas de tenir al·lèrgia a aquest, no s'administrarà i es realitzarà la prova sense contrast.

La principal complicació que podria ocórrer durant la realització del clamp hiperinsulinèmic-euglucèmic és la hipoglucèmia (disminució dels nivells de glucosa en sang), podent donar lloc a símptomes com sudoració, nerviosisme, tremolor, debilitat, palpitacions i/o sensació de gana. Donat el control de glucosa realitzat cada 5 minuts, aquesta complicació és poc freqüent i habitualment lleu.

7.- BENEFICIS

Encara que aquest projecte no li prometi cap avantatge directe, vostè contribuirà a una major comprensió de la patologia estudiada. Els resultats de la investigació amb la seva mostra biològica i material genètic poden ajudar a pacients amb la mateixa malaltia, i a la població en general.

8.- PRIVACITAT I CONFIDENCIALITAT

La seva privacitat està protegida per la llei nacional (LO15 / 1999, RD 1720/2007) i europea (95/46 / CE). Durant aquest estudi es generarà informació sensible i aquestes lleis volen ajudar a evitar accessos involuntaris a aquesta informació que podrien exposar als pacients, i a les seves famílies, a efectes adversos econòmics, legals, psicològics, i / o socials.D'acord a la LO15 / 1999, vostè pot exercir els drets d'accés, rectificació, oposició i cancel·lació de les dades; per això, haurà de contactar amb l'Investigador Principal del projecte. Així mateix, pot sol·licitar la destrucció de les mostres.

Per protegir la seva privacitat, les seves mostres de les seves mostres de sang, i teixit adipós tindran només un codi alfanumèric perquè no puguin identificar pel seu nom. Només els investigadors responsables del projecte podran connectar-los amb el seu nom. En cap moment el seu nom, adreça, o qualsevol altra informació que l'identifiqui serà utilitzada per als propòsits de la investigació.

Igualment, les seves dades personals seran identificades mitjançant un codi, mantenint així la confidencialitat de les mateixes. Només els investigadors responsables del projecte podran vincular-los amb el seu nom. En cap moment el seu nom, adreça, o qualsevol altra informació que l'identifiqui serà donada per als propòsits de la investigació

L'accés a la seva informació personal quedarà restringit als investigadors del projecte, les autoritats sanitàries i al Comitè Ètic d'Investigació Clínica, quan ho necessitin per comprovar les dades i procediments de l'estudi, però sempre mantenint la confidencialitat dels mateixos d'acord amb la legislació vigent.

ANNEX 2

CONSENTIMIENT INFORMAT

He estat informat dels riscos o inconvenients i de les possibles avantatges de la meva participació en el projecte: **Estudi del contingut de ferro en el teixit adipós, múscul i cervell en subjectes obesos. Interacció amb la composició de la microbiota i efectes de la pèrdua de pes**. Entenc:

1. Que no tinc obligació de participar en aquest projecte, i que la meva negativa a fer-ho no implicarà cap pena o pèrdua dels drets que em corresponen.

2. Que puc retirar meus mostres en qualsevol moment sense donar explicacions.

3. Que els resultats de la investigació que utilitza la meva mostra biològica podran ser publicats, però les meves dades personals no seran revelats llevat que siguin requerits per llei.

4. En cas que hi hagi problemes mèdics o preguntes, m'han comunicat que puc trucar al Dr. José Manuel Fernández-Real al telèfon 972.940.200 ext. 2325.

5. Que puc fer qualsevol pregunta en qualsevol moment sobre aquest projecte.

6. El propòsit del projecte i com el meu material biològic i genètic seran utilitzats.

7. Els meus drets com a pacient de la investigació, i voluntàriament consenteixo en cedir el meu material biològic i genètic.

- Desitjo ser informat dels resultats clínicament rellevants

☐ Si ☐ No

- Accedeixo a què es em contacti en un futur en el cas que es consideri oportú afegir noves dades als recollits inicialment, o mostres biològiques addicionals

☐ Si ☐ No

DESTINACIÓ MOSTRA DESPRÉS DE LA SEVA UTILITZACIÓ EN AQUEST PROJECTE DE RECERCA

Un cop finalitzada la investigació, és possible que hi hagi mostres sobrants. En relació a aquestes, se li ofereixen les següents opcions:

A) La destrucció de la mostra sobrant.

B) La introducció de l'excedent de la mostra a un Biobanc

En aquest cas, se li facilitarà full d'informació al pacient i consentiment específic que haurà de signar i serà custodiat pel coordinador del biobanc del seu Hospital.

Si hagués excedent de la mostra, afirmo haver estat advertit sobre les opcions de destinació en finalitzar el projecte de recerca.

En aquest sentit:

Sol·licito la destrucció de la mostra excedent ☐

Permeto que les meves mostres siguin introduïdes en el biobanc de l'hospital.

☐ Sí ☐ No

LA SEVA SIGNATURA INDICA QUE HA LLEGIT I ENTÉN LA INFORMACIÓ esmentada, QUE HA DISCUTIT AQUEST ESTUDI AMB LA PERSONA QUE OBTÉ AQUEST CONSENTIMENT, QUE HA DECIDIT PARTICIPAR BASAT EN LA INFORMACIÓ FACILITADA, I QUE LI HA DONAT A VOSTÈ UNA CÒPIA D'AQUEST FORMULARI.

	o	
Nom i firma del pacient		Nom i firma del familiar o Tutor (en cas necessari)

Data: _____ / _____ / _____

PER FAVOR, GUARDI UNA CÒPIA ÍNTEGRA D'AQUEST FORMULARI

Persona que obté el consentiment

Nom:

Declaro que els requisits per al consentiment informat per al projecte d'investigació mèdica descrit en aquest formulari han estat satisfets, que he proporcionat al participant una còpia d'aquest formulari, que he discutit el projecte de recerca amb el participant i li he explicat a ell o ella i en termes no tècnics, tota la informació continguda en aquest formulari de consentiment informat, incloent qualsevol risc i reacció adversa que raonablement es pugui esperar que passi. Igualment, faig constar que vaig animar al participant que fes preguntes i que totes les preguntes fetes van ser contestades.

Firma de la persona que obté el consentiment

Data: _____ / _____ / _____

ANNEX 3

VISIT 1 **IRONMET**

PATIENT NUMBER: _____ **INITIALS ___ ___ ___**

INCLUSION CRITERIA

1. **Aged 30 to 65 years** Yes ☐ No ☐
2. **Ability to understand study procedure** Yes ☐ No ☐
3. **Written informed consent obtained from the patient** Yes ☐ No ☐

EXCLUSION CRITERIA

1. **Serious systemic diseases not related with obesity, such as cancer, severe kidney disease or liver disease, type 1 or type 2 diabetes** Yes ☐ No ☐
2. **Systemic diseases with inflammatory activity, such as rheumatoid arthritis, Crohn's disease, asthma, chronic infection (HIV, TBC)** Yes ☐ No ☐
3. **Pregnancy and lactation** Yes ☐ No ☐
4. **Severe disorder of eating behavior** Yes ☐ No ☐
5. **Clinical symptoms and signs of infection in the previous month** Yes ☐ No ☐
6. **Antibiotic, antifungal and antiviral treatment for the last 3 months** Yes ☐ No ☐
7. **Anti-inflammatory cronic treatment (with steroidal and/or non-steroidal anti-inflammatory drugs) or insulin treatment** Yes ☐ No ☐
8. **Major psychiatric antecedents** Yes ☐ No ☐
9. **Serum liver enzymes (ALT and AST) activity over twice the upper limit of normal** Yes ☐ No ☐
10. **History of disturbances in iron balance (e.g. genetic hemochromatosis)** Yes ☐ No ☐
11. **Excessive alcohol intake (40g a day (women) or 80gr/day (men) or drug abuse** Yes ☐ No ☐

VISIT DATE: _____ / _____ / _____

DEMOGRAPHY

RACE:
- ☐ **Caucasic**
- ☐ **Asiatic**
- ☐ **Subsaharian**
- ☐ **Arabic**
- ☐ **Amerindian**
- ☐ **Others**

Date of birth: |_____|_____|_____|

(DD/MMM/YYYY)

Sex ☐ **Male**

☐ **Female**

MEDICAL HISTORY

DESCRIPTION	Onset date	End date	Current?
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N
			Y N

Period: ☐Y ☐N

DLP (menopause): _____ / _____ / _____

FAMILY HISTORY OF OBESITY

Father ☐Y ☐N ☐Unk

Mother ☐ Y ☐N ☐Unk

Brothers ☐ Y ☐N ☐Unk

TOBACCO

☐ Current smoker ☐ Ex-smoker ☐ Non-smoker

VITAL SIGNS

Height: _____ cm Weight: _____ kg

BMI: _____ Kg/m^2

Waist circumference: _____ cm Hip circumference: _____cm

BP (seated): _____/_____ mmHg Pulse: _____ bpm

BODY COMPOSITION

Fat mass: _____ Fat free mass: _____

Performed by:

☐ bioelectric impedance ☐ air plethysmography

PHYSICAL EXAMINATION

Is there any abnormality? ☐ Yes ☐ No

Specify abnormalities:

1. _____

2. _____

3. _____

4. _____

CONCOMITANT MEDICATION

Trade name	Daily dose/Units	Route*	Start date	End date	Ongoing? Y /N	Indication

*1=Oral; 2= Subcutaneous ; 3=Intravenous; 4=Topic; 5=Inhalate; 6=Intramuscular; 7=Rectal; 8=Transdermic; 9=Other

LABORATORY

¿A blood sample has been collected in this visit? ☐ Y ☐ N *

*Reason:_____

¿Fasting?**:** ☐**Yes** ☐**No** Hours of fasting: _____

Last intake: Date: _____/_____/_____ Time: _____:_____

(DD/MMM/YYYY) (00:00-23:59)

Sample date: _____/_____/_____ Sample time: _____:_____

(DD/MMM/YYYY) (00:00-23:59)

¿A faeces sample has been collected in this visit? ☐ Y ☐ N *

*Reason:_____

Sample date: _____/_____/_____ Sample time: _____:_____

(DD/MMM/YYYY) (00:00-23:59)

HEMATOLOGY

Red blood cells:	_____	Units**:** _____
Hemoglobin:	_____	Units**:** _____
Hematocrit:	_____	Units**:** _____
Platelets:	_____	Units**:** _____
Total WBC:	_____	Units**:** _____
Neutrophils:	_____	Units**:** _____
Eosinophils:	_____	Units**:** _____
Basophils:	_____	Units**:** _____
Lymphocytes:	_____	Units**:** _____
Monocytes:	_____	Units**:** _____

CHEMISTRY

HbA1c:	_____	Units**:** _____
Glucose	_____	Units**:** _____
Cholesterol:	_____	Units**:** _____
HDL Cholesterol:	_____	Units**:** _____
LDL Cholesterol:	_____	Units**:** _____
Triglycerides:	_____	Units**:** _____
Uric acid:	_____	Units**:** _____
Creatinine:	_____	Units**:** _____

GOT: _____ Units**:** _____

GPT: _____ Units**:** _____

GGT: _____ Units**:** _____

Alcaline Phosphatase: _____ Units**:** _____

Bilirrubin: _____ Units**:** _____

Total proteins: _____ Units**:** _____

Albumin: _____ Units**:** _____

Calcium: _____ Units**:** _____

Na: _____ Units**:** _____

K: _____ Units**:** _____

Ferritin: _____ Units**:** _____

Transferrin: _____ Units**:** _____

PCR ultrasensitive: _____ Units**:** _____

Erythrocyte sedimentation: _____ Units**:** _____

Fibrinogen: _____ Units**:** _____

TSH free: _____ Units**:** _____

T4 (tiroxine) free: _____ Units**:** _____

T3 free: _____ Units**:** _____

Cortisol: _____ Units**:** _____

24 hours diet recall

Time	Meal	Food and Cooked mode	g/plate size
	Breakfast		
	Second Breakfast		
	Lunch (Incluiding first, second, dessert, drink, oil, bread, coffe, sugar and snacking)		
	Afternoon		
	Diner (Incluiding first, second, dessert, drink, oil, bread, coffe, sugar and snacking)		
	Snacking between meals (incluiding at night)		

Do you get up at night to eat? What do you eat?.....

Yes No .....

Drink	Measure	Volume	Measure / week	**ml. OH / week**	**% OH**	**x 0,8 /100 (gr. OH)**	Average/dia (/ 7)
Wine /champagne	Wineglass	125 ml			12		
	Bottle	750 ml					
	glass	100ml					
Beer	"Mitjana/tercio"	330 ml			5		
	"Quinto/botellin"	200 ml					
	Tin	330 ml					
Distillates	"Cubata"	40 ml			40		
	"Chupito"	20 ml					
	"Carajillo"	10 ml					

ANNEX 4

QÜESTIONARI DE FREQÜÈNCIA DE CONSUM D'ALIMENTS

Nº història _____

Nom _____

Data _____

LEER LAS INSTRUCCIONES APARECIDAS EN EL CUESTIONARIO !!

Para cada alimento, consignar <u>cuantas veves como media</u> ha tomado la cantidad que se indica durante el año pasado. Tenga en cuenta las veces que lo toma solo y las que lo añade a otros alimentos o platos (Ej.: La leche del café, huevos en las tortillas, etc)

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+ al día
1. Leche entera (1 vaso o taza, 200 cc)									
2. Leche descremada (1 vaso, 200cc)									
3. Leche condensada (1 cucharada)									
4. Yogurt (Uno, 125 gramos)									
5. Requesón, cuajada, queso blanco o fresco (100g)									
6. Queso cremoso o en porciones (Una porción)									
7. Queso curado o semicurado: Manchego (1 trozo, 50 g)									
8. Natillas, flan, puding (uno)									
9. Helados (1 cucurucho, vasito o bola)									
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
10. Huevos de gallina (uno)									
11. Pollo con piel (1 plato o pieza)									
12. Pollo sin piel (1 plato o pieza)									
13. Carne de ternera, cerdo, cordero como plato principal (1 plato o pieza)									
14. Carne de caza: conejo, codorniz, pato (1 plato)									
15. Higado de ternera, cerdo o pollo (1 plato)									
16. Visceras: callos, sesos, mollejas (1 ración, 100 g)									
17. Embutidos: jamón, salchichón, salami, mortadela (1 ración, 50g)									
18. Salchichas y similares (una mediana)									
19. Patés, foie-gras (media ración, 50 g)									
20. Hamburguesa (una, 100 g)									
21. Tocino, bacon, panceta (2 lonchas, 50 g)									
22. Pescado frito variado (un plato o ración)									
23. Pescado hervido o plancha: merluza, lenguado, sardinas, atún. (1 ración)									
24. Pescados en salazón: bacalao, anchoas (media ración, 50 g)									
25. Pescados en conservas: atún, sardinas, arenques (1 lata)									
26. Almejas, mejillones, ostras (1 ración, 100 g)									
27. Calamares, pulpo (1 ración, 100 g)									
28. Marisco: gambas, langosta y similares (1 ración, 100 g)									

(Si no se especifica, los platos para carnes y pescado son de tamaño mediano)

¡Error! Marcador no definido. **III. VERDURAS Y 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+ al día
29. Espinacas cocinadas (1 plato)									
30. Col, coliflor, brocoles cocinadas (1 plato)									
31. Lechuga, endivias, escarola (1 plato)									
32. Tomates (uno mediano)									

Para alimentos que se consumen por temporadas, calcular el consumo medio para todo el año. Por ejemplo, si un alimento como la sandía se come 4 veces a la semana durante todo el verano (3 meses), entonces el consumo medio al año se marcaría en "1 vez por semana".

¡Error! Marcador no definido. **III. VERDURAS Y LEGUMBRES (Continuación)**	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+ al día
33. Cebolla (una mediana)									
34. Zanahoria, calabaza (una o plato pequeño)									
35. Judias verdes cocinadas (1 plato)									
36. Berenjenas, calabacines, pepinos (uno)									
37. Pimientos (uno)									
38. Espárragos (una ración o plato)									
39. Champiñones, setas (1 plato)									
40. Legumbres cocinadas: lentejas, garbanzos, judías pintas o blancas (1 plato mediano)									
41. Guisantes cocinados (1 plato)									
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
42. Naranjas, pomelo, mandarinas (Una)									
43. Zumo de naranja natural (un vaso pequeño, 125 cc)									
44. Plátano (uno)									
45. Manzana, pera (una mediana)									
46. Fresas (1 plato o taza de postre)									
47. Cerezas (1 plato o taza de postre)									
48. Melocotón, albaricoques (uno mediano)									
49. Higos frescos (uno)									
50. Sandía, melón (1 tajada o cala, mediana)									
51. Uvas (un racimo mediano o plato de postre)									
52. Aceitunas (tapa o plato pequeño, aprox. 15 unidades pequeñas)									
53. Frutas en almíbar: melocotón, peras, piña (2 mitades o rodajas)									
54. Frutos secos: piñones, almendras, cacahuetes, avellanas (1 plato o bolsita pequeña)									
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
55. Pan blanco (Una pieza pequeña o 3 rodajas de molde, 60 g)									
56. Pan integral (Pieza pequeña o 3 rodajas de molde)									
57. Picos, roscos y similares (una unidad, 3,5 g)									

58. Patatas fritas (1 ración, 100 g)									
59. Patatas cocidas, asadas (1 patata mediana)									
60. Bolsa de patatas fritas (1 bolsa pequeña, 25-30 g)									
61. Arroz cocinado (1 plato mediano)									
62. Pastas: espagueti, macarrones y similares (1 plato)									
VI. ACEITES Y GRASAS	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
63. Aceite de oliva (1 cucharada)									
64. Otros aceites vegetales: girasol, maíz, soja (1 cucharada)									
65. Margarina añadida al pan o la comida (1 cucharada o untada)									
66. Mantequilla añadida al pan o la comida (1 cucharada o untada)									
67. Manteca (de cerdo) añadida al pan o la comida (1 cucharada o untada)									

Para cada alimento, marcar la casilla apropiada para cu consumo medio durante el año pasado. Por ejemplo si toma una cucharada de mermelada cada dos días, entonces debe marcar la casilla "2-4 veces por semana"

¡Error! Marcador no definido. **VII. DULCES Y PASTELESRES**	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+ al día
68. Galletas tipo María (1 galleta)									
69. Galletas con chocolate (1 galleta doble)									
70. Croissant, donuts (uno)									
71. Magdalena, bizcocho (uno)									
72. Pasteles, tarta (unidad o trozo mediano)									
73 Churros (masa frita), 1 ración									
74. Chocolate, bombones (una barrita o dos bombones, 30 g)									
75. Chocolate en polvo y similares (1 cucharada)									
VIII. BEBIDAS	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 blanco, tinto o rosado (1 vaso, 125 cc)									
77. Cerveza (una caña o botellín 1/5, 200 cc)									
78. Brandy, ginebra, ron, wiskey, vodka, aguardientes 40º (1 copa, 50 cc)									
79. Refrescos con gas: cola, naranja, limón (ej. cocacola, fanta, etc) (Uno, 250 cc)									
80. Zumo de frutas envasado (1 lata pequeña o vaso, 200 cc)									
81. Café (1 taza)									
82. Café descafeinado (1 taza)									
83. Té (1 taza)									
IX. PRECOCINADOS, PREELABORADOS 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
84. Croquetas (una)									
85. Palitos o delicias de pescado fritos (una unidad)									
86. Sopas y cremas de sobre (1 plato)									
87. Mayonesa (1 cucharada)									
88. Salsa de tomate (media taza)									
89. Picantes: tabasco, pimienta, guindilla (1/2 cucharadita)									

90. Sal (1 pizca o pellizco con dos dedos)									
91. Ajo (1 diente)									
92. Mermeladas, miel (1 cucharada)									
93. Azucar (ej. en el café, postres, etc.) (1 cucharadita)									

1. ¿Qué hace Vd. con la grasa visible cuando come carne?
   1. La quito toda 2. Quito la mayoría 3. Quito un poco 4. No quito nada
2. ¿Cada cuanto tiempo come comidas fritas, fuera o dentro de casa?
   1. A diario 2. 4-6 veces/sem 3. 1-3 veces/sem 4. < 1 vez/sem
3. ¿Qué clase de grasa o aceite usa para:

	Manteca/Mantequilla	Margarina	Aceite oliva	Otros ac. vegetales
ALIÑAR	_____	_____	_____	_____
COCINAR/FREIR	_____	_____	_____	_____

4. ¿Toma Vd. algún producto de vitaminas? 1. Sí 2. No ¿Cual? _____
5. ¿Ha tomado alguno en el año pasado? 1. Sí 2. No ¿Cual? _____
6. ¿Hace algún tipo de dieta? 1. Sí 2. No ¿Cual? _____
7. ¿Ha cambiado su dieta durante el año pasado? 1. Sí 2. No
8. **¿Ha cambiado su peso en el último año? 1. Igual 2. Aumentado 3. Disminuido**

ANNEX 5

Table 3. Differences in microbiota composition in males and females with and without metabolic syndrome

	Male			Female		
Bacteria	**without MS (n=19)**	**with MS (n=23)**	**P value**	**without M (n=47)**	**with MS (n=42)**	**P value**
Acidobacteria	$6.17x10^{-6}$ ($4.25x10^{-6}$, $7.70x10^{-6}$)	$3.72x10^{-6}$ ($2.83x10^{-6}$, $4.51x10^{-6}$)	0.002	$5.28x10^{-6}$ ($2.68x10^{-6}$, $8.19x10^{-6}$)	$4.30x10^{-6}$ ($3.06x10^{-6}$, $6.91x10^{-6}$)	0.373
Candidatus Aminicenantes	$4.50x10^{-7}$ (0, $8.56x10^{-7}$)	0 (0, $4.05x10^{-7}$)	0.032	$4.17x10^{-7}$ (0, $1.02x10^{-6}$)	$1.63x10^{-7}$ (0, $7.06x10^{-7}$)	0.303
Candidatus Atribacteria	$9.46x10^{-7}$ ($3.62x10^{-7}$, $1.35x10^{-6}$)	0 (0, $5.53x10^{-7}$)	0.008	$6.40x10^{-7}$ (0, $1.28x10^{-6}$)	$4x10^{-7}$ (0, $9.57x10^{-7}$)	0.116
Candidatus Desantisbacteria	$6.69x10^{-7}$ ($3.62x10^{-7}$, $1.12x10^{-6}$)	0 (0, $5.43x10^{-7}$)	0.023	0 (0, $6.95x10^{-7}$)	0 (0, $4.87x10^{-7}$)	0.242
Candidatus Gottesmanbacteria	$4.73x10^{-7}$ (0, $6.79x10^{-7}$)	0 (0, $4.82x10^{-7}$)	0.024	$3.73x10^{-7}$ (0, $6.10x10^{-7}$)	0 (0, $4.88x10^{-7}$)	0.140
Candidatus Magasanikbacteria	$5.50x10^{-7}$ (0, $1.09x10^{-6}$)	$4.24x10^{-7}$ (0, $8.75x10^{-7}$)	0.370	$6.41x10^{-7}$ ($3.68x10^{-7}$, $1.60x10^{-6}$)	$3.95x10^{-7}$ (0, $8.45x10^{-7}$)	0.031
Candidatus Omnitrophica	$3.35x10^{-6}$ ($2.18x10^{-6}$, $5.72x10^{-6}$)	$1.89x10^{-6}$ ($1.46x10^{-6}$, $2.74x10^{-6}$)	0.004	$2.39x10^{-6}$ ($1.48x10^{-6}$, $5.90x10^{-6}$)	$1.93x10^{-6}$ ($1.36x10^{-6}$, $3.96x10^{-6}$)	0.406
Candidatus Rokubacteria	$1.12x10^{-6}$ ($5.95x10^{-7}$, $2.36x10^{-6}$)	$5.29x10^{-7}$ (0, $1.05x10^{-6}$)	0.017	$8.17x10^{-7}$ ($4.58x10^{-7}$, $1.36x10^{-6}$)	$9.85x10^{-7}$ ($4.43x10^{-7}$, $1.50x10^{-6}$)	0.493
Chloroflexi	$3.02x10^{-5}$ ($1.99x10^{-5}$, $4.40x10^{-5}$)	$1.91x10^{-5}$ ($1.32x10^{-5}$, $2.96x10^{-5}$)	0.022	$2.28x10^{-5}$ ($1.45x10^{-5}$, $4.83x10^{-5}$)	$1.99x10^{-5}$ ($1.43x10^{-5}$, $3.20x10^{-5}$)	0.340
Chrysiogenetes	$3.94x10^{-7}$ (0, $1.35x10^{-6}$)	0 (0, 0)	0.031	$4.19x10^{-7}$ (0, $1.11x10^{-6}$)	0 (0, $4.80x10^{-7}$)	0.058
Cyanobacteria	$1.19x10^{-5}$ ($8.93x10^{-6}$, $1.70x10^{-5}$)	$8.34x10^{-6}$ ($6.56x10^{-6}$, $1.19x10^{-5}$)	0.045	$1.16x10^{-5}$ ($6.92x10^{-6}$, $1.45x10^{-5}$)	$1.09x10^{-5}$ ($7.24x10^{-6}$, $1.41x10^{-5}$)	0.605
Fusobacteria	$5.74x10^{-5}$ ($4.58x10^{-5}$, $1.09x10^{-4}$)	$3.77x10^{-5}$ ($3.04x10^{-5}$, $6.82x10^{-5}$)	0.029	$6.58x10^{-5}$ ($4.30x10^{-5}$, $1.12x10^{-4}$)	$5.15x10^{-5}$ ($4.02x10^{-5}$, $7.12x10^{-5}$)	0.353
Gemmatimonadetes	$1.45x10^{-6}$ ($8.56x10^{-7}$, $2.04x10^{-6}$)	$5.43x10^{-7}$ ($4.24x10^{-7}$, $1.62x10^{-6}$)	0.025	$1.33x10^{-6}$ ($4.92x10^{-7}$, $2.24x10^{-6}$)	$1.34x10^{-6}$ ($7.01x10^{-7}$, $2.09x10^{-6}$)	0.850
Ignavibacteria	$2.03x10^{-6}$ ($1.23x10^{-6}$, $4.37x10^{-6}$)	$1.09x10^{-6}$ ($5.47x10^{-7}$, $2.15x10^{-6}$)	0.018	$1.95x10^{-6}$ ($9.51x10^{-7}$, $3.60x10^{-6}$)	$1.96x10^{-6}$ ($9.73x10^{-7}$, $2.65x10^{-6}$)	0.559
Kiritimatiellaeota	0 (0, $9.45x10^{-7}$)	0 (0, 0)	0.019	0 (0, $5.60x10^{-7}$)	0 (0, $6.87x10^{-7}$)	0.782
Lentisphaerae	$1.04x10^{-5}$ ($4.50x10^{-6}$, $6.36x10^{-5}$)	$2.77x10^{-6}$ ($1.41x10^{-6}$, $4.85x10^{-6}$)	0.001	$7.94x10^{-6}$ ($2.07x10^{-6}$, $3.43x10^{-5}$)	$6.10x10^{-6}$ ($1.30x10^{-6}$, $1.32x10^{-5}$)	0.167
Nitrospirae	$5.46x10^{-6}$ ($3.15x10^{-6}$, $6.15x10^{-6}$)	$2.94x10^{-6}$ ($1.75x10^{-6}$, $4.71x10^{-6}$)	0.014	$3.66x10^{-6}$ ($1.97x10^{-6}$, $7.40x10^{-6}$)	$3.11x10^{-6}$ ($1.41x10^{-6}$, $5.29x10^{-6}$)	0.362
Planctomycetes	$8.69x10^{-6}$ ($6.31x10^{-6}$, $1.18x10^{-5}$)	$4.14x10^{-6}$ ($2.69x10^{-6}$, $5.93x10^{-6}$)	<0.001	$6.99x10^{-6}$ ($4.14x10^{-6}$, $1.33x10^{-5}$)	$6.34x10^{-6}$ ($2.91x10^{-6}$, $1.08x10^{-5}$)	0.312
Spirochaetes	$1.20x10^{-4}$ ($8.93x10^{-5}$, $1.55x10^{-4}$)	$8.27x10^{-5}$ ($6.81x10^{-5}$, $1.08x10^{-4}$)	0.018	$1.12x10^{-4}$ ($6.84x10^{-5}$, $2.35x10^{-4}$)	$9.36x10^{-5}$ ($5.38x10^{-5}$, $1.35x10^{-4}$)	0.116
Tenericutes	$4.57x10^{-5}$ ($2.63x10^{-5}$, $1.30x10^{-4}$)	$1.96x10^{-5}$ ($1.50x10^{-5}$, $2.74x10^{-5}$)	0.001	$2.98x10^{-5}$ ($1.63x10^{-5}$, $5.24x10^{-4}$)	$2.16x10^{-5}$ ($1.19x10^{-5}$, $7.91x10^{-5}$)	0.221
Candidate division Zixibacteria	$9 x10^{-7}$ (0, $1.76x10^{-6}$)	$3.84x10^{-7}$ (0, $5.01x10^{-7}$)	0.040	$5.84x10^{-7}$ (0, $1.39x10^{-6}$)	$4.62x10^{-7}$ (0, $1.15x10^{-6}$)	0.285
Eukaryota Na	$1.21x10^{-5}$ ($4.37x10^{-6}$, $4.33x10^{-4}$)	$3.48x10^{-6}$ ($2.36x10^{-6}$, $1.33x10^{-5}$)	0.002	$5.63x10^{-6}$ ($2.06x10^{-6}$, $1.72x10^{-4}$)	$4.18x10^{-6}$ ($1.80x10^{-6}$, $8.53x10^{-6}$)	0.269
Neocallimastigomycota	$1.55x10^{-6}$ ($9.45x10^{-7}$, $2.93x10^{-6}$)	$6.56x10^{-7}$ ($4.37x10^{-7}$, $1.50x10^{-6}$)	0.033	$9.56x10^{-7}$ ($4.96x10^{-7}$, $2.08x10^{-6}$)	$7.17x10^{-7}$ (0, $1.30x10^{-6}$)	0.068

	Female (n= 89)					
	Pre-Menopause (n=45)			Post-Menopause (n=44)		
Bacteria	**without MS (n=27)**	**with MS(n=18)**	**P value**	**without MS (n=20)**	**with MS (n=24)**	**P value**
Fibrobacteres	$3.32x10^{-5}$ ($2.63x10^{-5}$, $5.53x10^{-5}$)	$2.36x10^{-5}$ ($1.78x10^{-5}$, $3.82x10^{-5}$)	0.029	$2.65x10^{-5}$ ($1.95x10^{-5}$, 4.08 $x10^{-5}$)	$3.28x10^{-5}$ ($2.31x10^{-5}$, $4.15x10^{-5}$)	0.480
Candidatus Magasanikbacteria	$8.19x10^{-7}$ ($3.68x10^{-7}$, $1.74x10^{-6}$)	$7 x10^{-7}$ ($2.64x10^{-7}$, $1.41x10^{-6}$)	0.514	$5.05x10^{-7}$ ($9.32x10^{-8}$, $1.48x10^{-6}$)	0 (0, $6.37x10^{-7}$)	0.035
Candidatus Marinimicrobia	0 (0, $5.68x10^{-7}$)	0 (0, $6.43x10^{-7}$)	0.754	$5.58x10^{-7}$ (0, $1.15x10^{-6}$)	0 (0, $4.79x10^{-7}$)	0.014
Candidatus Woesebacteria	0 (0, $7.51x10^{-7}$)	$4.07x10^{-7}$ (0, $6.54x10^{-7}$)	0.680	$5.15x10^{-7}$ (0, $9.82x10^{-7}$)	0 (0, $4.73x10^{-7}$)	0.027

Table 4. Differences in microbiote composition in subjects with and without metabolic syndrome depending on the presence of obesity

	without MS			with MS		
Bacterias	**without obesity**	**with obesity**	**P value**	**without obesity**	**with obesity**	**P value**
Euryarchaeota	$1.80x10^{-3}$ ($1.76x10^{-5}$, $6.58x10^{-3}$)	$6.25x10^{-5}$ ($1.15x10^{-5}$, $2.80x10^{-3}$)	0.043	$1,72x10^{-3}$ ($2.47x10^{-5}$, $9.02x10^{-3}$)	$2.35x10^{-5}$ ($1.09x10^{-5}$, $1.71x10^{-3}$)	0.041
Thaumarchaeota	0 (0, $1.04x10^{-6}$)	0 (0, $3.94x10^{-7}$)	0.030	0 (0, $5.01x10^{-7}$)	0 (0, $4.71x10^{-7}$)	0.419
Acidobacteria	$6.15x10^{-6}$ ($4.16x10^{-6}$, $9.56x10^{-6}$)	$4.49x10^{-6}$ ($2.34x10^{-6}$, $6.60x10^{-6}$)	0.023	$6.37x10^{-6}$ ($3.52x10^{-6}$, $8.62x10^{-6}$)	$3.74x10^{-6}$ ($2.91x10^{-6}$, $5.53x10^{-6}$)	0.034
Aquificae	$1.75x10^{-6}$ ($1.02x10^{-6}$, $3.15x10^{-6}$)	$6.79x10^{-7}$ ($3.18x10^{-7}$, $2.04x10^{-6}$)	0.008	$2.33x10^{-6}$ ($5.01x10^{-7}$, $2.97x10^{-6}$)	$6.68x10^{-7}$ ($3.77x10^{-7}$, $1.76x10^{-6}$)	0.079
Armatimonadetes	$2.38x10^{-6}$ ($9.16x10^{-7}$, $3.42x10^{-6}$)	$9.55x10^{-7}$ (0, $2.84x10^{-6}$)	0.035	$2.36x10^{-6}$ ($1.05x10^{-6}$, $4.11x10^{-6}$)	$1.32x10^{-6}$ ($5.32x10^{-7}$, $2.66x10^{-6}$)	0.104
Bacteria Na	$1.88x10^{-3}$ ($1.46x10^{-3}$, $2.58x10^{-3}$)	$1.92x10^{-3}$ ($1.39x10^{-3}$, $2.49x10^{-3}$)	0.952	$2.41x10^{-3}$ ($1.90x10^{-3}$, $3.12x10^{-3}$)	$1.66x10^{-3}$ ($1.22x10^{-3}$, $2.15x10^{-3}$)	0.005
Calditrichaeota	$4.09x10^{-7}$ (0, $7.36x10^{-7}$)	0 (0, $3.94x10^{-7}$)	0.015	0 (0, $1.01x10^{-6}$)	0 (0, $4.31x10^{-7}$)	0.332
Candidatus Falkowbacteria	$7.64x10^{-7}$ (0, $1.69x10^{-6}$)	$9.49x10^{-7}$ ($4.04x10^{-7}$, $1.43x10^{-6}$)	0.627	$1.01x10^{-6}$ ($5.29x10^{-7}$, $1.70x10^{-6}$)	$4.79x10^{-7}$ (0, 0)	0.013
Candidatus Moranbacteria	$8.89x10^{-7}$ ($5.15x10^{-7}$, $1.81x10^{-6}$)	$3.48x10^{-7}$ (0, $5.50x10^{-7}$)	0.001	$4.66x10^{-7}$ (0, $1.08x10^{-6}$)	$6.40x10^{-7}$ (0, $1.45x10^{-6}$)	0.456
Candidatus Nomurabacteria	0 (0, $6.41x10^{-7}$)	0 (0, $4.76x10^{-7}$)	0.328	$4.71x10^{-7}$ (0, $1.08x10^{-6}$)	0 (0, $4.17x10^{-7}$)	0.024
Candidatus Omnitrophica	$3.42x10^{-6}$ ($1.78x10^{-6}$, $6.78x10^{-6}$)	$2.09x10^{-6}$ ($9.49x10^{-7}$, $3.35x10^{-6}$)	0.007	$2.33x10^{-6}$ ($1.57x10^{-6}$, $5.01x10^{-6}$)	$1.88x10^{-6}$ ($1.31x10^{-6}$, $3.15x10^{-6}$)	0.142
Candidatus Peregrinibacteria	$9.51x10^{-7}$ (0, $2.24x10^{-6}$)	$3.68x10^{-7}$ (0, $5.95x10^{-7}$)	0.013	$1.89x10^{-6}$ ($5.24x10^{-7}$, $2.64x10^{-6}$)	$5.42x10^{-7}$ ($2.64x10^{-7}$, $1.02x10^{-6}$)	0.003
Candidatus Uhrbacteria	$9.92x10^{-7}$ (0, $2.34x10^{-6}$)	$4.09x10^{-7}$ (0, $1.38x10^{-6}$)	0.093	$1.62x10^{-6}$ ($5.18x10^{-7}$, $3.13x10^{-6}$)	$6.15x10^{-7}$ (0, $1.46x10^{-6}$)	0.014
Candidatus Woesebacteria	$5.17x10^{-7}$ (0, $9.92x10^{-7}$)	0 (0, $5.68x10^{-7}$)	0.015	0 (0, $4.40x10^{-7}$)	0 (0, $4.85x10^{-7}$)	0.628
Chlamydiae	$7.96x10^{-4}$ ($1.88x10^{-4}$, $1.17x10^{-3}$)	$1.70x10^{-3}$ ($2.35x10^{-4}$, $4.62x10^{-3}$)	0.046	$6.52x10^{-4}$ ($2.66x10^{-4}$, $2.49x10^{-3}$)	$4.60x10^{-4}$ ($1.54x10^{-4}$, $1.59x10^{-3}$)	0.319
Chlorobi	$2.66x10^{-6}$ ($1.71x10^{-6}$, $4.67x10^{-6}$)	$2.38x10^{-6}$ ($9.55x10^{-7}$, $3.70x10^{-6}$)	0.172	$3.77x10^{-6}$ ($2.65x10^{-6}$, $5.76x10^{-6}$)	$1.92x10^{-6}$ ($1.12x10^{-6}$, $3.25x10^{-6}$)	0.009
Chloroflexi	$2.93x10^{-5}$ ($1.88x10^{-5}$, $5.06x10^{-5}$)	$2.10x10^{-5}$ ($1.19x10^{-5}$, $3x10^{-5}$)	0.027	$2.91x10^{-5}$ ($1.81x10^{-5}$, $6.33x10^{-5}$)	$1.91x10^{-5}$ ($1.28x10^{-5}$, $2.82x10^{-5}$)	0.030
Cyanobacteria	$1.24x10^{-5}$ ($8.81x10^{-6}$, $1.61x10^{-5}$)	$8.93x10^{-6}$ ($5.09x10^{-6}$, $1.23x10^{-5}$)	0.029	$1.21x10^{-5}$ ($8.96x10^{-6}$, $1.45x10^{-5}$)	$9.16x10^{-6}$ ($6.44x10^{-6}$, $1.23x10^{-5}$)	0.086
Deinococcus Thermus	$2.53x10^{-6}$ ($1.77x10^{-6}$, $4.75x10^{-6}$)	$1.67x10^{-6}$ ($9.49x10^{-7}$, $2.86x10^{-6}$)	0.046	$4.71x10^{-6}$ ($2.50x10^{-6}$, $5.29x10^{-6}$)	$1.97x10^{-6}$ ($9.61x10^{-7}$, $3.48x10^{-6}$)	0.002
Elusimicrobia	$4.69x10^{-6}$ ($2.34x10^{-6}$, $6.69x10^{-6}$)	$1.67x10^{-6}$ ($8.07x10^{-7}$, $4.22x10^{-6}$)	0.023	$4.67x10^{-6}$ ($2.01x10^{-6}$, $5.92x10^{-6}$)	$2.31x10^{-6}$ ($1.22x10^{-6}$, $4.88x10^{-6}$)	0.077
Fibrobacteres	$3.31x10^{-5}$ ($2.21x10^{-5}$, $5.42x10^{-5}$)	$3.58x10^{-5}$ ($3.02x10^{-5}$, $5.19x10^{-5}$)	0.350	$5.52x10^{-5}$ ($2.91x10^{-5}$, $7.31x10^{-5}$)	$3.22x10^{-5}$ ($2.21x10^{-5}$, $4.21x10^{-5}$)	0.034
Ignavibacteriae	$2.17x10^{-6}$ ($1.23x10^{-6}$, $4.45x10^{-6}$)	$1.63x10^{-6}$ ($6.37x10^{-7}$, $3.27x10^{-6}$)	0.130	$2.15x10^{-6}$ ($1.70x10^{-6}$, $4.50x10^{-6}$)	$1.50x10^{-6}$ ($5.66x10^{-7}$, $2.48x10^{-6}$)	0.044
Lentisphaerae	$1.16x10^{-5}$ ($3.83x10^{-6}$, $6.16x10^{-5}$)	$3.27x10^{-6}$ ($1.23x10^{-6}$, $2.67x10^{-5}$)	0.046	$4.85x10^{-6}$ ($2.51x10^{-6}$, $3.46x10^{-5}$)	$3.41x10^{-6}$ ($1.24x10^{-6}$, $8.64x10^{-6}$)	0.132
Nitrospinae	$6.78x10^{-7}$ (0, $1.44x10^{-6}$)	$4.09x10^{-7}$ (0, $6.79x10^{-7}$)	0.010	$8.23x10^{-7}$ ($5.02x10^{-7}$, $1.27x10^{-6}$)	$5.50x10^{-7}$ (0, $9.47x10^{-7}$)	0.168
Nitrospirae	$5.49x10^{-6}$ ($2.68x10^{-6}$, $8.45x10^{-6}$)	$2.86x10^{-6}$ ($1.79x10^{-6}$, $5.45x10^{-6}$)	0.025	$5.76x10^{-6}$ ($1.50x10^{-6}$, $7x10^{-6}$)	$2.68x10^{-6}$ ($1.46x10^{-6}$, $4.92x10^{-6}$)	0.044
Planctomycetes	$8.25x10^{-6}$ ($6.50x10^{-6}$, $1.34x10^{-5}$)	$5.95x10^{-6}$ ($4.14x10^{-6}$, $8.17x10^{-6}$)	0.014	$6.51x10^{-6}$ ($4.24x10^{-6}$, $1.31x10^{-5}$)	$4.94x10^{-6}$ ($2.68x10^{-6}$, $8.25x10^{-6}$)	0.142
Spirochaetes	$1.27x10^{-4}$ ($9.06x10^{-5}$, $2.41x10^{-4}$)	$8.29x10^{-5}$ ($6.22x10^{-5}$, $1.28x10^{-4}$)	0.001	$1.28x10^{-4}$ ($7.92x10^{-5}$, $2.84x10^{-4}$)	$8.54x10^{-5}$ ($5.38x10^{-5}$, $1.19x10^{-4}$)	0.023
Synergistetes	$4.82x10^{-5}$ ($3.44x10^{-5}$, $1.60x10^{-4}$)	$3.27x10^{-5}$ ($1.71x10^{-5}$, $5.30x10^{-5}$)	0.015	$7.16x10^{-5}$ ($3.97x10^{-5}$, $9x10^{-5}$)	$3.40x10^{-5}$ ($2.20x10^{-5}$, $8.24x10^{-5}$)	0.077
Tenericutes	$1.10x10^{-4}$ ($2.27x10^{-5}$, $1.09x10^{-3}$)	$2.25x10^{-5}$ ($1.63x10^{-5}$, $4.57x10^{-5}$)	0.003	$4.20x10^{-5}$ ($1.71x10^{-5}$, $1.34x10^{-4}$)	$1.96x10^{-5}$ ($1.19x10^{-5}$, $3.28x10^{-5}$)	0.025
Thermotogae	$5.14x10^{-6}$ ($3.79x10^{-6}$, $8.25x10^{-6}$)	$3.52x10^{-6}$ ($1.90x10^{-6}$, $5.36x10^{-6}$)	0.006	$5.52x10^{-6}$ ($3.77x10^{-6}$, $6.60x10^{-6}$)	$3.82x10^{-6}$ ($2.65x10^{-6}$, $4.99x10^{-6}$)	0.066
Candidate division WWE3	0 (0, $6.100x10^{-7}$)	0 (0,0)	0.049	$4.40x10^{-7}$ (0, $8.49x10^{-7}$)	0 (0, $4.64x10^{-7}$)	0.151
Candidate division Zixibacteria	$1.13x10^{-6}$ (0, $1.84x10^{-6}$)	$4.04x10^{-7}$ (0, $8.38x10^{-7}$)	0.019	$4.71x10^{-7}$ (0, $8.23x10^{-7}$)	$4.13x10^{-7}$ (0, $7.10x10^{-7}$)	0.663
Ascomycota	$4.75x10^{-6}$ ($2.90x10^{-6}$, $1.37x10^{-5}$)	$3.13x10^{-6}$ ($2.04x10^{-6}$, $4.54x10^{-6}$)	0.006	$3.71x10^{-6}$ ($1.76x10^{-6}$, $7.40x10^{-6}$)	$3.50x10^{-6}$ ($1.93x10^{-6}$, $8.18x10^{-6}$)	0.834
Chlorophyta	$2.07x10^{-6}$ ($9.92x10^{-7}$, $2.86x10^{-6}$)	$1.09x10^{-6}$ (0, $1.78x10^{-6}$)	0.013	$2.50x10^{-6}$ ($1.40x10^{-6}$, $3.67x10^{-6}$)	$8.59x10^{-7}$ ($4x10^{-7}$, $1.28x10^{-6}$)	0.001
Eukaryota Na	$1.11x10^{-5}$ ($4.59x10^{-6}$, $3.49x10^{-4}$)	$3.77x10^{-6}$ ($1.58x10^{-6}$, $1.22x10^{-5}$)	0.012	$7.49x10^{-6}$ ($4.02x10^{-6}$, $9.69x10^{-5}$)	$3.18x10^{-6}$ ($1.80x10^{-6}$, $6.43x10^{-6}$)	0.007
Eukaryota Uc	$4.61x10^{-7}$ (0, $1.83x10^{-6}$)	0 (0, $5.84x10^{-7}$)	0.188	$5.02x10^{-7}$ (0, $1.65x10^{-6}$)	0 (0, $4.68x10^{-7}$)	0.004

Table 5. Differences in microbiote composition in subjects with and without metabolic syndrome regarding the grade of inflammation

	without MS			with MS		
Bacterias	**without inflammation**	**with inflammation**	**P value**	**without inflammation**	**with inflammation**	**P value**
Archaea na	$5.50x10^{-7}$ (0, $1.22x10^{-6}$)	$4.99x10^{-7}$ (0, $7.97x10^{-7}$)	0.571	$6.59x10^{-7}$ (0, $1x10^{-6}$)	0 (0, $6.27x10^{-7}$)	0.029
Archaea uc	$4.73x10^{-7}$ (0, $1.84x10^{-6}$)	0 (0, $5.88x10^{-7}$)	0.034	$3.85x10^{-7}$ (0, $1.36x10^{-6}$)	0 (0, $6.26x10^{-7}$)	0.194
Euryarchaeota	$1.80x10^{-3}$ ($1.78x10^{-5}$, $6.68x10^{-3}$)	$2.48x10^{-5}$ ($8.22x10^{-6}$, $1.56x10^{-3}$)	0.013	$1.29x10^{-3}$ ($1.26x10^{-5}$, $8.84x10^{-3}$)	$2.16x10^{-5}$ ($1.09x10^{-5}$, $7.16x10^{-4}$)	0.070
Acidobacteria	$5.95x10^{-6}$ ($4.11x10^{-6}$, $9.46x10^{-6}$)	$4.49x10^{-6}$ ($2.03x10^{-6}$, $7.03x10^{-6}$)	0.044	$3.80x10^{-6}$ ($2.96x10^{-6}$, $7.40x10^{-6}$)	$3.79x10^{-6}$ ($2.98x10^{-6}$, $5.53x10^{-6}$)	0.468
Aquificae	$1.58x10^{-6}$ ($9.46x10^{-7}$, $3.12x10^{-6}$)	$8.57x10^{-7}$ ($3.41x10^{-7}$, $2.07x10^{-6}$)	0.038	$6.59x10^{-7}$ ($3.85x10^{-7}$, $2.65x10^{-6}$)	$7.77x10^{-7}$ ($4.02x10^{-7}$, $1.99x10^{-6}$)	0.896
Armatimonadetes	$2.38x10^{-6}$ ($9.75x10^{-7}$, $3.40x10^{-6}$)	$6.83x10^{-7}$ (0, $2.42x10^{-6}$)	0.009	$1.31x10^{-6}$ ($8.47x10^{-7}$, $3.52x10^{-6}$)	$1.49x10^{-6}$ ($5.71x10^{-7}$, $2.71x10^{-6}$)	0.921
Bacteria na	$1.86x10^{-3}$ ($1.47x10^{-3}$, $2.53x10^{-3}$)	$2x10^{-3}$ ($1.27x10^{-3}$, $2.55x10^{-3}$)	0.798	$2.11x10^{-3}$ ($1.59x10^{-3}$, $2.74x10^{-3}$)	$1.63x10^{-3}$ ($1.21x10^{-3}$, $2.16x10^{-3}$)	0.046
Balneolaeota	$5.79x10^{-7}$ (0, $1.14x10^{-6}$)	$1.74x10^{-7}$ (0, $6.10x10^{-7}$)	0.029	0 (0, $8.23x10^{-7}$)	$4.13x10^{-7}$ (0, $9.65x10^{-7}$)	0.328
Calditrichaeota	$4.09x10^{-7}$ (0, $8.60x10^{-7}$)	0 (0, 0)	0.007	0 (0, $7.68x10^{-7}$)	0 (0, $4.02x10^{-7}$)	0.208
Candidatus Aminicenantes	$4.96x10^{-7}$ (0, $1.56x10^{-6}$)	0 (0, $4.75x10^{-7}$)	0.015	$4.05x10^{-7}$ (0, $8.49x10^{-7}$)	0 (0, $4.80x10^{-7}$)	0.173
Candidatus Atribacteria	$9.55x10^{-7}$ ($3.79x10^{-7}$, $1.43x10^{-6}$)	$4.93x10^{-7}$ (0, $8.50x10^{-7}$)	0.021	$4.37x10^{-7}$ (0, $7.68x10^{-7}$)	$3.57x10^{-7}$ (0, $9.14x10^{-7}$)	0.498
Candidatus Cloacimonetes	$1.03x10^{-6}$ ($4.67x10^{-7}$, $1.71x10^{-6}$)	$1.59x10^{-7}$ (0, $1.62x10^{-6}$)	0.065	$8.81x10^{-7}$ ($6.56x10^{-7}$, $1.16x10^{-6}$)	$6.26x10^{-7}$ ($3.52x10^{-7}$, $9.14x10^{-7}$)	0.014
Candidatus Marinimicrobia	$5.14x10^{-7}$ (0, $1.13x10^{-6}$)	0 (0, $2.61x10^{-7}$)	0.007	$4.37x10^{-7}$ (0, $1.05x10^{-6}$)	0 (0, $4.73x10^{-7}$)	0.080
Candidatus Moranbacteria	$8.89x10^{-7}$ ($4.76x10^{-7}$, $1.72x10^{-6}$)	$3.76x10^{-7}$ (0, $6.38x10^{-7}$)	0.002	$4.71x10^{-7}$ (0, $1.32x10^{-6}$)	$4.91x10^{-7}$ (0, $1.66x10^{-6}$)	0.664
Candidatus Omnitrophica	$3x10^{-6}$ ($1.81x10^{-6}$, $6.90x10^{-6}$)	$1.72x10^{-6}$ ($6.49x10^{-7}$, $3.41x10^{-6}$)	0.003	$1.93x10^{-6}$ ($1.54x10^{-6}$, $4.11x10^{-6}$)	$1.92x10^{-6}$ ($1.15x10^{-6}$, $3.11x10^{-6}$)	0.332
Candidatus Peregrinibacteria	$9.51x10^{-7}$ ($4.41x10^{-7}$, $1.88x10^{-6}$)	0 (0, $4.08x10^{-7}$)	0.001	$1.08x10^{-6}$ ($4.05x10^{-7}$, $2.12x10^{-6}$)	$6.51x10^{-7}$($3.80x10^{-7}$, $1.09x10^{-6}$)	0.154
Candidatus Uhrbacteria	$1.09x10^{-6}$ ($2.22x10^{-7}$, $2.25x10^{-6}$)	$3.58x10^{-7}$ (0, $7.82x10^{-7}$)	0.047	$1.16x10^{-6}$ ($5.43x10^{-7}$, $2.04x10^{-6}$)	$4.94x10^{-7}$ (0, $1.54x10^{-6}$)	0.020
Candidatus Woesebacteria	$5.10x10^{-7}$ (0, $1.11x10^{-6}$)	0 (0, $5.55x10^{-7}$)	0.019	0 (0, $4.24x10^{-7}$)	0 (0, $4.96x10^{-7}$)	0.203
Chloroflexi	$2.93x10^{-5}$ ($1.88x10^{-5}$, $5.03x10^{-5}$)	$1.83x10^{-5}$ ($1.05x10^{-5}$, $2.88x10^{-5}$)	0.010	$2.50x10^{-5}$ ($1.50x10^{-5}$, $4.02x10^{-5}$)	$1.91x10^{-5}$ ($1.19x10^{-5}$, $2.64x10^{-5}$)	0.086
Cyanobacteria	$1.25x10^{-5}$ ($9.24x10^{-6}$, $1.68x10^{-5}$)	$7.88x10^{-6}$ ($3.60x10^{-6}$, $1.14x10^{-5}$)	0.001	$9x10^{-6}$ ($6.93x10^{-6}$, $1.36x10^{-5}$)	$9.39x10^{-6}$ ($6.09x10^{-6}$, $1.22x10^{-5}$)	0.663
Deinococcus Thermus	$2.55x10^{-6}$ ($1.86x10^{-6}$, $4.57x10^{-6}$)	$1.42x10^{-6}$ ($6.82x10^{-7}$, $2.74x10^{-6}$)	0.001	$2.96x10^{-6}$ ($1.50x10^{-6}$, $4.94x10^{-6}$)	$1.96x10^{-6}$ ($9.63x10^{-7}$, $3.25x10^{-6}$)	0.027
Elusimicrobia	$4.69x10^{-6}$ ($2.35x10^{-6}$, $6.75x10^{-6}$)	$1.62x10^{-6}$ ($8.43x10^{-7}$, $3.75x10^{-6}$)	0.002	$3.81x10^{-6}$ ($2.02x10^{-6}$, $5.43x10^{-6}$)	$2.19x10^{-6}$ ($9.79x10^{-7}$, $4.82x10^{-6}$)	0.028
Fibrobacteres	$3.54x10^{-5}$ ($2.30x10^{-5}$, $5.41x10^{-5}$)	$3.29x10^{-5}$ ($2.74x10^{-5}$, $5.26x10^{-5}$)	0.932	$4.13x10^{-5}$ ($2.91x10^{-5}$, $7.31x10^{-5}$)	$3.12x10^{-5}$ ($1.91x10^{-5}$, $4x10^{-5}$)	0.005
Ignavibacteriae	$2.24x10^{-6}$ ($1.30x10^{-6}$, $4.57x10^{-6}$)	$1.53x10^{-6}$ ($5.15x10^{-7}$, $2.70x10^{-6}$)	0.032	$1.75x10^{-6}$ ($9.43x10^{-7}$, $2.64x10^{-6}$)	$1.78x10^{-6}$ ($5.53x10^{-7}$, $2.48x10^{-6}$)	0.598
Kiritimatiellaeota	$4.10x10^{-7}$ (0, $9.64x10^{-7}$)	0 (0, 0)	0.007	0 (0,0)	0 (0, $4.96x10^{-7}$)	0.219
Lentisphaerae	$1.11x10^{-5}$ ($3.96x10^{-6}$, $7.44x10^{-5}$)	$2.58x10^{-6}$ ($1.23x10^{-6}$, $2.29x10^{-5}$)	0.016	$3.95x10^{-6}$ ($2.31x10^{-6}$, $1.05x10^{-5}$)	$2.80x10^{-6}$ ($1.14x10^{-6}$, $8.85x10^{-6}$)	0.197
Nitrospirae	$5.49x10^{-6}$ ($2.48x10^{-6}$, $7.79x10^{-6}$)	$2.68x10^{-6}$ ($2.04x10^{-6}$, $4.99x10^{-6}$)	0.049	$4.71x10^{-6}$ ($1.75x10^{-6}$, $6.51x10^{-6}$)	$2.75x10^{-6}$ ($1.38x10^{-6}$, $5.01x10^{-6}$)	0.177
Planctomycetes	$8.69x10^{-6}$ ($6.58x10^{-6}$, $1.62x10^{-5}$)	$5.07x10^{-6}$ ($3.59x10^{-6}$, $7.51x10^{-6}$)	0.003	$5.93x10^{-6}$ ($2.83x10^{-6}$, $1.10x10^{-5}$)	$4.90x10^{-6}$ ($2.23x10^{-6}$, $8.06x10^{-6}$)	0.289
Spirochaetes	$1.28x10^{-4}$ ($8.99x10^{-5}$, $2.41x10^{-4}$)	$7.58x10^{-5}$ ($5.70x10^{-5}$, $1.16x10^{-4}$)	0.000	$8.76x10^{-5}$ ($7.11x10^{-5}$, $1.73x10^{-4}$)	$8.34x10^{-5}$ ($5.42x10^{-5}$, $1.23x10^{-4}$)	0.137
Synergistetes	$4.81x10^{-5}$ ($3.49x10^{-5}$, $1.36x10^{-4}$)	$2.81x10^{-5}$ ($1.44x10^{-5}$, $5.12x10^{-5}$)	0.008	$3.97x10^{-5}$ ($2.44x10^{-5}$, $8.15x10^{-5}$)	$3.44x10^{-5}$ ($2.31x10^{-5}$, $7.60x10^{-5}$)	0.675
Tenericutes	$5.40x10^{-5}$ ($2.52x10^{-5}$, $7.50x10^{-4}$)	$2.09x10^{-5}$ ($1.04x10^{-5}$, $4.36x10^{-5}$)	0.003	$2.20x10^{-5}$ ($1.58x10^{-5}$, $6.56x10^{-5}$)	$1.88x10^{-5}$ ($1.19x10^{-5}$, $3.24x10^{-5}$)	0.089
Thermotogae	$5.36x10^{-6}$ ($3.94x10^{-6}$, $7.56x10^{-6}$)	$2.85x10^{-6}$ ($1.32x10^{-6}$, $4.60x10^{-6}$)	0.000	$4.11x10^{-6}$ ($3.28x10^{-6}$, $6.51x10^{-6}$)	$3.75x10^{-6}$ ($2.54x10^{-6}$, $4.96x10^{-6}$)	0.243
Verrucomicrobia	$1.57x10^{-3}$ ($6.64x10^{-5}$, $6.39x10^{-3}$)	$1.46x10^{-4}$ ($1.79x10^{-5}$, $1.36x10^{-3}$)	0.014	$1.46x10^{-4}$ ($2.88x10^{-5}$, $6.11x10^{-3}$)	$1.50x10^{-4}$ ($1.94x10^{-5}$, $1.31x10^{-3}$)	0.557
Candidate division WWE3	0 (0, $6.73x10^{-7}$)	0 (0, 0)	0.016	0 (0, $6.56x10^{-7}$)	0 (0, $4.54x10^{-7}$)	0.544
Candidate division ZIxibacteri	$9.45x10^{-7}$ (0, $1.80x10^{-6}$)	$3.76x10^{-7}$ (0, $1.04x10^{-6}$)	0.043	$4.71x10^{-7}$ (0, $1.27x10^{-6}$)	$4.13x10^{-7}$ (0, $6.27x10^{-7}$)	0.573
Ascomycota	$5.28x10^{-6}$ ($2.91x10^{-6}$, $1.25x10^{-5}$)	$3.17x10^{-6}$ ($1.79x10^{-6}$, $4.44x10^{-6}$)	0.009	$3.01x10^{-6}$ ($1.69x10^{-6}$, $1.90x10^{-5}$)	$3.55x10^{-6}$ ($2.06x10^{-6}$, $5.79x10^{-6}$)	0.957
Chlorophyta	$2.07x10^{-6}$ ($1.16x10^{-6}$, $2.86x10^{-6}$)	$7.84x10^{-7}$ (0, $1.69x10^{-6}$)	0.004	$1.36x10^{-6}$ ($6.59x10^{-7}$, $2.65x10^{-6}$)	$8.75x10^{-7}$ ($3.52x10^{-7}$, $1.40x10^{-6}$)	0.023
Eukaryota na	$9.26x10^{-6}$ ($4.48x10^{-6}$, $3.91x10^{-4}$)	$3.45x10^{-6}$ ($1.12x10^{-6}$, $5.82x10^{-5}$)	0.011	$5.92x10^{-6}$ ($3.07x10^{-6}$, $5.38x10^{-5}$)	$2.81x10^{-6}$ ($1.81x10^{-6}$, $6.35x10^{-6}$)	0.066
Eukaryota uc	$5.50x10^{-7}$ (0, $2.02x10^{-6}$)	0 (0, $3.86x10^{-7}$)	0.010	$4.37x10^{-7}$ (0, $1.57x10^{-6}$)	0 (0, $4.71x10^{-7}$)	0.083
Neocallimastigomycota	$1.43x10^{-6}$ ($8.33x10^{-7}$, $2.58x10^{-6}$)	$4.96x10^{-7}$ ($3.26x10^{-7}$, $1.20x10^{-6}$)	0.001	$8.47x10^{-7}$ ($4.37x10^{-7}$, $1.89x10^{-6}$)	$6.89x10^{-7}$ (0, $1.25x10^{-6}$)	0.241