UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE POSGRADOS

Characterization of intestinal microbiota from indigenous people with metabolic syndrome in the Highlands of Ecuador

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Trabajo de titulación de posgrado presentado como requisito para la obtención del título de Magister en Microbiología

Quito, 27 de diciembre de 2019

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HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

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Quito, 27 de diciembre 2019

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DEDICATORIA

A mis padres y hermanas, por toda paciencia y amor a lo largo de toda mi vida profesional.

A Dr. Enrique Terán porque sin el nada de esto hubiese sido posible.

AGRADECIMIENTOS

Agradezco a mis padres por permitirme seguir con mi vida profesional y por su infinita paciencia a lo largo de estos años de estudio. A mis hermanas por su apoyo y paciencia incondicional. A Mariangel por sus locuras que hacían siempre que tenga una sonrisa en mi rostro.

Al Dr. Enrique Terán, por su apoyo incondicional y por compartirme las ganas y amor por la investigación

Al Instituto de microbiología por la oportunidad de estudio dentro de su programa de Maestría en Microbiología.

A la Escuela de Medicina de la Universidad San Francisco, en especial a su Decana Dra. Michelle Grunauer por permitirme continuar con mi labor en el Laboratorio de Investigaciones durante el periodo de estudio de la maestría.

A mis amigos Estefania, Daniela, Lázaro y Natalia por hacer que esta experiencia de estudio sea una de las mejores. A todos Infinitos Gracias!

RESUMEN

Antecedentes: el síndrome metabólico (EM) es una constelación de factores fisiológicos, bioquímicos, clínicos y metabólicos interconectados que aumentan el riesgo de enfermedades cardiovasculares. Estudios previos demostraron que los cambios en la composición de la microbiota gastrointestinal por factores externos contribuyen a la etiología de varias enfermedades cardio-metabólicas. Según la Encuesta Nacional de Salud y Nutrición (ENSANUT) en 2012, la prevalencia de EM en la población ecuatoriana, en general, es del 27%, mientras que en la población indígena estuvo presente en el 15,7%. Luego, el objetivo de este estudio fue caracterizar la microbiota intestinal de los pueblos indígenas de las tierras altas de Ecuador con EM y sus respectivos controles.

Métodos: en un grupo aleatorio de personas indígenas de la provincia de Imbabura con síndrome metabólico y sus controles, se obtuvieron muestras de heces y se analizó la microbiota intestinal secuenciando el gen que codifica el 16S rRNA con tecnología MiSeq Illumina y analizó los diferentes factores que podría influir en la microbiota intestinal con el programa de bioinformática Qiime2.org versión 2018.11.

Resultados: La diversidad alfa y beta en sujetos con síndrome metabólico no mostró diferencias significativas en comparación con sus controles. El análisis de abundancia mostró que Bacteroides, Clostridia y miembros del phylum Proteobacteria eran los más prevalentes. Además, los análisis de composición revelaron la presencia de *Lachnospiraceae*, considerada como un marcador de metabolismo alterado de lípidos y glucosa. Sin embargo, en sujetos obesos la diversidad alfa fue significativamente diferente en comparación con los controles. Además, el nivel taxonómico familiar (L5) mostró que hay grupos de Prevotella, Faecalibacterium, Bacteroidetes, *Alistipes* en una porción más significativa presente en el grupo de obesos.

Conclusiones: este constituye el primer estudio de caracterización de la microbiota intestinal en población indígena de las tierras altas de Ecuador. La composición de microbiota de sujetos indígenas con síndrome metabólico en las tierras altas de Ecuador se caracteriza por una presencia significativa de Bacteroides, Clostridia y miembros del filo Proteobacteria, pero se pueden encontrar resultados similares en el control de la misma área.

Palabras clave: Síndrome metabólico, microbiota gastrointestinal, Otavalo, Cotacachi, diversidad.

ABSTRACT

Background: Metabolic syndrome (MS) is a constellation of interconnected physiological, biochemical, clinical, and metabolic factors that increase the risk of cardiovascular diseases. Previous studies demonstrated that changes in the composition of gastrointestinal microbiota by external factors, contribute to the etiology of several cardio metabolic diseases. According to the National Health and Nutrition Survey (ENSANUT) in 2012, the prevalence of MS in the Ecuadorian population, in general, is 27%, while in the indigenous population was present in 15.7%. Then, the objective of this study was to characterize the intestinal microbiota of indigenous people from the highlands in Ecuador with MS and their respective controls.

Methods: In a randomized group of indigenous people from the Imbabura province with metabolic syndrome and their controls, stool samples were obtained and the intestinal microbiota was analyzed by sequencing the gene that code for the 16S rRNA with MiSeq Illumina technology and analyzed the different factors that could influence the intestinal microbiota with the bioinformatics program Qiime2.org version 2018.11.

Results: Alpha and beta diversity in subjects with metabolic syndrome did no show significant differences compared to their controls. Abundance analysis showed that *Bacteroides*, *Clostridia*, and members of the phylum *Proteobacteria* were the morst prevalent. In addition, composition analyzes revealed the presence of *Lachnospiraceae*, considered as a marker of lipid and glucose altered metabolism. However, in obese subjects alpha diversity was significantly different compared to controls. Moreover, family taxonomic level (L5), showed there are *Prevotella, Faecalibacterium, Bacteroidetes, Alistipes* groups in more significant portion present in the obese group.

Conclusions: This constitutes the first study of characterization of intestinal microbiota in indigenous population in the highlands of Ecuador. The microbiota composition of indigenous subjects with Metabolic Syndrome in the highlands of Ecuador, is characterized by a significant presence of *Bacteroides*, *Clostridia*, and members of the phylum *Proteobacteria*, but similar results can be found in the control from the same area.

Key words: Metabolic syndrome, gastrointestinal microbiota, Otavalo, Cotacachi, diversity

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PART I

BIBLIOGRAPHIC REVIEW

Incidence of Metabolic Syndrome

Metabolic syndrome (MS) is a clinical disorder characterized by abdominal obesity, hypertension, dyslipidemia, and insulin resistance. It is a state of chronic low-grade inflammation with profound systemic effects. MS increases the risk of type 2 diabetes and cardiovascular disease. The risk for adverse health outcomes is substantially increased with the accumulation of components of MS. In the last decade, several organizations have proposed different definitions using a different methodology (Carvajal, 2014).

This syndrome is a complex disorder with a high socioeconomic cost and is considered a public health problem since it constitutes an epidemic (Kassi, Pervanidou, Kaltsas, & Chrousos, 2014).

The metabolic syndrome comprises a group of risk factors, including abnormal obesity, dyslipidemia, increased fasting blood pressure, and plasma glucose, which does not increase the risk of type 2 diabetes and cardiovascular disease (Babio, et al., 2015).

Intestinal microbiota (gut microbiome)

The human intestine has a great microbial variety, which plays an important role both in the modulation of the immune system and in the metabolism of the individual. When there is a suspicion of the possible implication that the microbial can present in the development of some disease, different comparative studies are carried out between samples of adjacent neoplastic and normal tissue, as well as stool samples from healthy and diseased patients, to perform an analysis and compare the existing microbial profiles in these individuals by sequencing the bacterial gene (Turner, Ritchie, Brealier, & Chapkin, 2014).

Composition

In the human large intestine, there are approximately 100 billion bacteria with 3.3 million microbial genes, 150 times more than the human genome. Bacteria constitute ecological niches that are in equilibrium and constitute what is called microbiota - the intestinal microbiome. The microbial density found throughout the digestive system is 101-103 microorganisms per gram in stomach and duodenum, 104-107 microorganisms per gram in jejunum or ileum, and finally 1013-1014 microorganisms per gram in colon and feces (Wu, et al.., 2014).

Some studies related to metagenomics on the intestinal microbiota show the existing phylogenetic variety. Thus, the existence of more than 1000 species-level filo types is estimated, with at least 160 prevalent species per individual. Much of the endogenous bacteria concerning species in healthy adults are part of two edges, Firmicutes and

Bacteroides, representing more than 90% of the phylogenetic categories found in the intestine of the human being. The edges Actinobacteria, Proteobacteria, Fusobacteria, *Verrucomicrobia* , *Spirochaete*s and *Lentisphaerae* have a lower abundance (< 1% - 15%) (Consortium, 2014) .

There is a great variety among individuals in the microbial composition, and this is due to various environmental and genetic factors such as geographical location or lifestyle and also the alterations caused by different diseases. Some research determined how, despite the existence of a characteristic microbial profile of each individual, a set of about 50 taxa are common in half of the individuals. Individuals share a series of microbial genes associated with the most important metabolic pathways so that the functional deviation of this group of genes would be related to an elevation of the physiological state. However, specific genetic diversity per individual is essential and remains largely unallocated (Schloissing, Arumugam, Sunagawa, Miterva, Tap, & Zhu, 2014).

Role in intestinal physiology

These microorganisms influence both physiology and human metabolism. Several microorganisms of the intestine are related to different metabolic pathways, such as the production of essential vitamins and trace elements. Thus, they play a crucial role in the nutrition and energy balance of the host (Bonomini, Rodella, & Rezzani, 2015).

The union between the microbiota and the human being is the result of evolution. One more of its functions is the strong resistance they have against enteropathogens due to

their colonization. The effects related to this are the competition for food resources, the inhibition of the growth of pathogens through the production of acetate the synthesis of bacteriocins, or the stimulation of the immune response (Mogre, Salifu, & Abedandi, 2014).

The intestinal microbiota is also considered as one of the components of the human immune system, in this way, calibrating the immunological potential and the immunological responses (Consortium, 2014). The relationship between the intestinal microbiota and the mucosal immune system is a significant factor for the development of the immune system since childhood of the human being, as well as for the maintenance of immunological homeostasis throughout the adult stage (Babio, et al., 2015).

It is essential to highlight the definition of a microbiota-intestine-brain axis, which plays a very important role in the normality of anxiety, cognition, pain and behavior, in addition, in a possible affectation to the pathophysiology of a disorder in the central nervous system (McCoy, Araujo, Azcarate, Yeh, & Sandler, 2014).

Dynamism

It has been shown today that the plasticity of these microorganisms associated with diet, the environment, and physiological changes have influenced the composition and functionality of the intestinal microbiota (Zhao, Wang, Jiaojiao, & Wang, 2015).

The plasticity of the intestinal microbiota in different healthy individuals has been investigated; what has been shown is that 40% of the microbiota is transformed for five years (Consortium, 2014).

Dietary changes in humans involve a simple presence to demonstrate the ability to have the microbiota to adapt its bio architectural in response to environmental stimuli, with the speed and efficiency necessary to maintain a correct symbiosis between host-microbiota (Putignani, Del Chierico, Petrucca, Vernocchi, & Dallapiccola, 2014).

The fluctuations presented can be taken as an essential characteristic of the intestinal microbial ecosystem, which adapts very quickly to the requirements and minimizes the efficiency of nutrient extraction and guarantees good health (Bonomini, Rodella, & Rezzani, 2015). At present, it has been shown that the presence or absence of different bacterial taxa is related to the intake of different nutrients (Babio, et al., 2015).

It has been observed that with the influence of the diet there is a certain plasticity of the intestinal ecosystem of the human being in response to different environmental stress factors, such as climate and geography, the degree of exposure to environmental microorganisms; being the last of the utmost importance for the adequate maintenance of the immune system functions from childhood to adulthood (Bonomini, Rodella, & Rezzani, 2015).

On the other hand, the intake of drugs, antibiotics or anti-inflammatory drugs, influence the microbial structure of the intestine and give rise to different configurations of the microbiota, where there is an increase or reduction in the ability to promote or reduce metabolization and efficacy. of drugs (Bonomini, Rodella, & Rezzani, 2015).

While in adulthood, different physiological transformations occur naturally and join the list of related agents in the modification of the microbial structure, both temporarily (pregnancy or lactation) and permanently (the process of aging) (Consortium, 2014).

The elderly can have a direct impact on the structure of the intestinal microbiota through physiological processes associated with age since they involve local and systemic inflammation and indirectly causes alterations in people's eating habits and lifestyles. At the time that the threshold for taste and smell increases, together with problems in chewing caused by the loss of dentures and muscle mass, there may be reduced consumption of fiber and protein diet (Aguilar, Bhuket, & Torres, 2015).

The intestinal microbiota with advanced age is mainly characterized by a decrease in biodiversity, a large number of opportunistic facultative anaerobes, and a reduction in the abundance of species with anti-inflammatory properties, that is, microorganisms producing butyrate (Putignani, Del Chierico, Petrucca, Vernocchi, & Dallapiccola, 2014).

The variation of the microbiota in healthy adulthood is accompanied by different chronic systemic inflammatory disorders, such as obesity, metabolic syndrome, and inflammatory bowel diseases (Putignani, Del Chierico, Petrucca, Vernocchi, & Dallapiccola, 2014).

Influence of genetic and environmental factors on the intestinal microbiota

In each of the people, in the skin, in the mucous membranes, and the gastrointestinal tract live microorganisms next to which the number of cells and genes looks very small. While several of these microbes are pathogenic, many of them are harmless or even beneficial. The majority of microorganisms in the body, in a group called the microbiota, are similar to an organ in that it performs essential functions for the survival of human beings. Several microbes produce vitamins and other essential nutrients. Some metabolize foods that cannot be digested by humans. Also, they break down drugs and toxins and regulate multiple aspects of innate and acquired immunity, protecting the host organism from various infections and chronic inflammation and many immune-based disorders (Siew, et al., 2015).

Implications of intestinal microbiota on health

Diet and lifestyle are recognized as critical elements in the prevention and treatment of the metabolic syndrome. In recent years, an increasing amount of evidence has shown that the consumption of different foods can have beneficial but at the same time harmful effects on the risk factors that define the metabolic syndrome, including atherogenic dyslipidemia, hyperglycemia, resistance to insulin, diabetes, blood pressure and abdominal obesity (Babio, et al., 2015).

Metabolic syndrome contributes to cardiovascular morbidity and mortality. Updated prevalence trends may be significant, given the potential effect of metabolic syndrome and health complications on the aging of the world population (Aguilar, Bhuket, & Torres, 2015).

Obesity and overweight

At present, few know that the intestinal microbiota plays a vital role in the development of overweight and obesity. Daily mechanisms theory microbiome obese are more defined, these include regulation of the immune system, competition with enterocyte by calorie diet, their role in regulating the endocrine function of the gastrointestinal system that controls appetite and also very fascinating mediation at the epigenetic level (González, González, & Padilla, 2017).

Development and functions of the intestinal flora

Food is fundamental during the first two years of a person's life since it is fundamental in the balance of bacterial flora, which results from a predominance of Bifidobacteria and Lactobacilli over species that can be harmful such as Clostridia, Staphylococcus aureus or certain Bacteroides.

The intestinal flora of humans is significant in the healthy development of the gastrointestinal tract, and each of the functions of the Immune system ITY is constituted by four bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Gonzalez Gonzalez, & Padilla, 2017).

In the adulthood of people, the density of colonization can reach 1014 microorganisms per gram of intestinal content and is constituted by more than 1000 different species that present innocuous bacteria and potential pathogens. The microbiome (microbiota genome) is twice the size of the human nuclear genome and exceeds it 15 times in the number of genes (Angelakis, et al., 2015).

The intestinal microbiota has excellent influence on the intestinal mucosa through the expression of genes, especially those related to defense, in the regulation of barrier function, vascularization, digestion, and absorption processes, and in the glycosylation of proteins and lipids of cell membranes d and the enterocytes and colonocytes, also involved in the bio transformation of xenobiotic (Valsecchi, Tagliacarne, & Castellazzi, 2016).

In the course of the life of the intestinal microbiota, it can be altered by different factors, such as diet, chronic systemic and intestinal diseases, and antibiotic therapy (Valsecchi, Tagliacarne, & Castellazzi, 2016).

Changes in the intestinal microbiota and excess weight

The transformations in the microbiota are related to various clinical aspects such as obesity, diabetes, gastrointestinal, autoimmune diseases, and cancer (González, González, & Padilla, 2017).

A diet high in saturated fats, in trans fatty acids and sugars, causes a decline in the microbiome, which conditions a pro-inflammatory environment and disruption of the intestinal barrier function with low-grade inflammation and metabolic endotoxemia (Everard, et al., 2014).

The intestinal microbiota plays a vital role in obesity, since various investigations have proven that the microbiota may be associated with obesity by means of several mechanisms as would control the permeability and intestinal inflammation, metabolism alteration acid biliary and intestinal hormone release, all this increasing the ability of the

digestive tract to draw energy from diet, and to regulate appetite (Valsecchi, Tagliacarne, & Castellazzi, 2016).

Obesity is related to the bacteria found in the small intestine (Angelakis, et al., 2015). Several of them are more frequently related to adiposity, including Lactobacillus (Drissi, Raoult, & Merhej, 2016).

Theory of the obese microbiome

The intestinal microbiome of obese people has a higher percentage of Firmicutes and lower Bacteroidetes compared to humans with healthy weight and often lower genetic diversity (Angelakis, et al., 2015).

This microbiome is characterized by an increase in the genera Proteobacteria, Bacteroides, Campylobacter, and Shigella, as well as a reduction in Akkermansia muciniphilae. The result of this is associated with the loss of the integrity of the mucosal barrier, degradation of the intestinal mucus layer, and increased oxidative stress (Valsecchi, Tagliacarne, & Castellazzi, 2016).

It is related to an increase in the ability to extract energy from the diet and a reduction to create internal factors, mainly hormones that inhibit fat storage. Also, it is known that the dysbiosis conditions slow intestinal transit and increased insulin resistance (Gonzalez Gonzalez, & Padilla, 2017).

These mechanisms for obesity include the degradation of polysaccharides to monosaccharides that are absorbed and provide extra calories, which results in lipid synthesis and storage of triglycerides in the liver and adipose tissue; Also, there is an

elimination of the adiposity factor caused by fasting, which increases the activity of lipoprotein lipase and an increase in the storage of fatty acids in adipocytes (Valsecchi, Tagliacarne, & Castellazzi, 2016).

Indigenous population from Highland of Ecuador

According to UNICEF (2014), the type of food that a person sustains in their first years of life largely determines their quality of life in adulthood. Therefore, it is crucial to ensure adequate nutrition from pregnancy, encourage breastfeeding, regulate the foods children eat, and promote healthy habits in families throughout their lives.

Concerning the above, UNICEF mentions that policies aimed at the most vulnerable sectors should be improved, as is the case of indigenous people in the country's highlands, who have the least economic resources and are the ones with the highest rates of overweight and malnutrition (UNICEF Ecuador, 2014).

Indigenous households in the Ecuadorian highlands have characteristics that make them more vulnerable to the development of malnutrition caused by the lack of information, among which are: location in rural areas or moors, lack of access to essential services, food high in hydrates carbon and insufficient in proteins and fats, overcrowding and low economic income (Córdova, 2016).

PART II

SCIENTIFIC ARTICLE

Characterization of intestinal microbiota from indigenous people with metabolic syndrome in the Highlands of Ecuador

INTRODUCTION

The human intestine has about 100 trillion microorganisms containing about 1000 different species of bacteria, yeasts, and parasites. These microorganisms have an essential role in the intestinal growth and general health of individuals due to their establishment as symbionts that in turn, protect against the colonization of pathogenic microorganisms (Parekh, Balart, & Johnson, 2015).

Microbiota is considered a set of microorganisms that live in a relationship of mutualism or symbiosis. This microbiota is dispersed differently and mainly in quantity and diversity throughout the human body. It is composed of fungal bacteria, archaea yeasts, and viruses that are necessary and in some cases, indispensable for life. It is estimated that around 80% of these cannot be cultivated since their nature is strict anaerobic (Icaza-Chavez, 2013).

With the emergence of molecular methodologies based on the sequencing of the 16S rRNA, the revolution of the omic sciences (genomic meta, meta-transcriptomic and

metabolic) have provided a piece of extensive knowledge on the subject since they provide information about the bacterial composition of the human intestine and reveal some of its functional properties. At present, the intestinal microbiota has been considered as accompanying commensal microorganisms, as supposing that it is a metabolic organ, which fulfills several functions in nutrition, regulation of immunity and systemic inflammation (Icaza-Chávez, 2013)

The changes that can occur in the microbiota are based on three fundamental aspects which are: extrinsic variables of the individual (diet, habits), genetics of the individual, and environmental factors (Schmidt, Raes, & Bork, 2018). These changes alter the structure of the microbial community in factors such as species richness, community equality, and diversity. Evenness is a measure of the skew in abundance of community members, richness refers to the number of different types of organism present in a microbial community and diversity is considered a combination of richness and evenness and also the statistical summary of the structure of the community as abundance and evenness is taken in account (Cox, Cookson, & Moffatt, 2013).

The association of these parameters with measurements of medical and environmental conditions can assess disease states or of being in health fields. An increase in wealth, equality, and diversity may be related to stable ecosystems, stabilized long ago, or of low activity. The stability of a microbial community, resistance to environmental pressures such as diet and the use of antibiotics, and resistance to the invasion of pathogens are essential factors in disease states in humans affecting bowel, mouth, lungs, skin and sexual organs (Cox et al., 2013)

The gastrointestinal microbiota has been shown to contribute to the etiology of several cardiometabolic diseases (Mazidi, Rezaie, Kengne, Mobarhan, & Ferns, 2016). The change in the composition of the microbiota caused by external factors can lead to a dramatic alteration in the symbiotic relationship established by the intestinal bacteria and the host, which promotes the development of metabolic diseases. In particular, it is believed that the intestinal microbiota contributes to the onset of metabolic diseases by the stimulation of low-grade inflammation (Boulangé, Neves, Chilloux, Nicholson, & Dumas, 2016).

Metabolic syndrome (MS) is a clinical disorder characterized by abdominal obesity, hypertension, dyslipidemia, and insulin resistance (Carvajal, 2014). Similarly, it is characterized by being a constellation of interconnected physiological, biochemical, clinical, and metabolic factors, and these factors directly increase the risk of cardiovascular diseases, type 2 diabetes mellitus (T2DM), and all causes of death. Its prevalence is conditioned by variables such as age, sex, ethnic origin, and the rural or urban environment of the population under study (Kaur, 2014).

Within the Ecuadorian territory, the indigenous ethnic group represents 7.3% of the total population, is distributed mostly throughout the Ecuadorian highlands, and that its economic activities are based on agriculture and handicrafts. Therefore, this type of population is one of the most neglected in quality of health and is a group to which urbanization and modernization affect their ancestral customs (Moossavi & Bishehsari, 2019).

One of the effects of urbanization is reflected in the change in eating habits, which leads to the appearance of diseases and disorders related to food intake (Chee et al., 2019). Among these are obesity, overweight, metabolic disorders, metabolic syndrome, type 2 diabetes, hypertension, among others (Moossavi & Bishehsari, 2019).

According to the National Health and Nutrition Survey (ENSANUT) in 2012, the prevalence of Metabolic Syndrome in the Ecuadorian population, in general, is 27%, while in the indigenous population, it was presented in 15.7% (Mendieta MJ. & Gómez LF., 2015). Similarly, the prevalence of obesity in general Ecuadorian population is 50%, while in the indigenous population, it is 10.8%. However, the prevalence of overweight at a general level did not show a more significant difference with the indigenous population (40.6% vs. 41.3%) (Chee et al., 2019).

The present study focused on indigenous communities in the Cotacachi canton and San Juan de Ilumán, a rural parish in the Otavalo canton. According to the data of the National Institute of Statistics and Census (INEC), the degree of poverty within the places where the indigenous population in Ibarra, Otavalo, and Cotacachi is concentrated, ranges between 67 and 73% (INEC, 2010). This high poverty rate primarily affects traditional food production, which leads the mentioned populations to obtain processed foods or junk food, foods that do not contain a nutritional value (Chee et al., 2019).

According to (Moossavi & Bishehsari, 2019), having these changes in ancestral eating habits due to the socio-economic conditions of the individuals of the aforementioned indigenous populations, the presence of dysbiosis in the intestinal microbiota in these

individuals can lead to a change in the prevalence of metabolic diseases. Therefore, it is imperative to show the presence of an imbalance in the intestinal bacterial communities of the indigenous individuals and if these are related to the lower presence of Metabolic Syndrome in the study population.

Based on the previous, it is stated that the scientific problem is the description of the structure of intestinal microbiota of indigenous people with metabolic syndrome that allow showing a relationship with the prevalence of this condition.

From this, the general objective of this work was to identify the differences between the intestinal microbiota profiles of indigenous people of Otavalo and Cotacachi with Metabolic Syndrome and their respective controls.

We worked on the hypothesis that the changes in the composition of the intestinal microbiota profiles of the indigenous people are related to the lower prevalence of Metabolic Syndrome in the study population.

METHODOLOGY

STUDY DESIGN, PARTICIPANTS AND ETHICAL APPROVAL

The present study was part of the project entitled “Identification of protective factors against metabolic syndrome in the indigenous population of northern Ecuador highlands during 2016-2017”. This component was an observational, descriptive-analytical and cross-sectional study carried out in the indigenous communities of El Cercado, Perafan,

Morochos and Turuco in the Cotacachi canton and the rural towns of San Jose de Iluman and Karabuela, in the province of Imbabura, at the north highlands area from Quito, Ecuador during the second semester of 2016 and the first semester of 2017.

After mapping all people living on those communities, from each sector a certain number of houses were selected in a random manner and then a family member was chosen under the following inclusion criteria were (1) voluntary participation, and (2) minimum age of 18; while the exclusion criteria were: (1) mental incapacity, (2) intake of antibiotics, and (3) being in a state of intoxication by alcohol or other substances.

Sample size for the main study, based on the reported rate of metabolic syndrome was set up in 242 participants. From those, before any analysis a sub-sample of forty-five subjects were selected also in a random manner to provide a fecal sample for microbiome analysis. This study was approved by the Bioethics Committee at Universidad San Francisco de Quito (2016-127IN) and all participants signed an informed consent form.

PARTICIPANTS INFORMATION

The group of participants in this sub-study was composed by thirty-one women and fourteen men (n=45) and the following information was taken: anthropometry consisting of the measurement of weight and height, to determine the body mass index (BMI), abdominal perimeter, blood pressure, a blood sample for lipids plus glucose analysis and two stool samples, one for parasites analysis and the other for the microbiota analysis. Subsequently, each of the participants was given a: global

questionnaire on risk factors for chronic diseases (STEPwise) in which risk factors for metabolic syndrome disease (age, obesity, smoking, alcoholism and family history) are analyzed.; global questionnaire on physical activity (GPAQ) which collects information on physical activity and sedimentary behavior. Finally, a dietary survey of two components, a frequency questionnaire for regular consumption of food and a three-day diet diary.

SAMPLE COLLECTION AND DNA EXTRACTION

Fecal material for microbiota analysis was collected in OMR-200 OMNIGENE-GUT® vials following the instructions of the fabricant and kept at room temperature during transfer (Doukhanine et al., 2014; Penington et al., 2018).

Once in the laboratory facilities in the School of Medicine at the Universidad San Francisco de Quito all samples were refrigerated at 4°C until the DNA was extracted. For the DNA extraction, the FastDNA® Spin Kit for Soil extraction kit (MP Biomedicals, Solon, OH, USA) was used following the manufacturer's instructions. In brief, up to 350 uL of stool sample from the collection vials was added to a Lysing Matrix E tube, add 978 µl Sodium Phosphate Buffer to sample in Lysing Matrix E tube and add 122 µl MT Buffer, then homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6. After that centrifuged at 14,000g for 5-10 minutes to pellet. Transfer supernatant to a clean 2.0 ml microcentrifuge tube and Add 250 µl PPS (Protein Precipitation Solution) and mix by shaking the tube by hand ten times. Then centrifuge at 14,000g for 5 minutes to pellet precipitate. Also, transfer supernatant to a clean 15 ml tube and while that resuspend Binding Matrix suspension and add 1.0 ml to

the supernatant in 15 ml tube. Invert by hand for 2 minutes to allow binding of DNA. Place the tube in a rack for 2 minutes to allow settling of the silica matrix. Transfer approximately 600 µl of the mixture to a SPIN® Filter and centrifuge at 14,000 g for 1 minute.

Empty the catch tube and add the remaining mixture to the SPIN® Filter and centrifuge as before. Empty the catch tube again. Add 500 µl prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip. centrifuge at 14,000 g for 1 minute. Empty the catch tube and replace. Without any addition of liquid, centrifuge a second time at 14,000 g for 2 minutes to "dry" the matrix of residual wash solution. Discard the catch tube and replace with a new, clean catch tube. Air dry the SPIN® Filter for 5 minutes at room temperature then gently resuspend Binding Matrix (above the SPIN filter) in 50-100 µl of DES (DNase/Pyrogen-Free Water). Finally, centrifuge at 14,000g for 1 minute to bring eluted DNA into the clean catch tube. Discard the SPIN filter. DNA is now ready for PCR and other downstream applications. Store at -20°C for extended periods or 4°C until use.

The quantity (absorbance at 260 nm) and quality (absorbance ratio 260/230 and 260/280) of extracted DNA were examined on Take3 Micro-volume plates for an EPOCH (BioTek, VT, USA) spectrophotometer. All 49 samples were lyophilized prior shipment to be sequenced.

GENOMIC LIBRARIES AND SEQUENCING

The quantified and lyophilized DNA was sent to the Genomics Facility in University of North Carolina at Chapel Hill in the United States for the preparation of genomic

libraries by amplifying the V3 and V4 regions of the 16S rRNA gene (Lippert et al., 2017; Qin et al., 2010) using PCR with barcoded primers for subsequent sequencing using Illumina MiSeq technology yielding a total of 35099 readings. Then, the 16S microbiome gene was analyzed from the raw sequences with the software Quantitative Insights into Microbial Ecology (QIIME2) version 2019.4.

DATA ANALYSIS

For the estimation of alpha diversity within the study groups, Faith's phylogenetic diversity metric that measures the biodiversity of the samples incorporating phylogenetic differences between species was used. For statistical analysis of these results, the paired Kruskal-Wallis test was used. Among the groups, Pielou's Evenness test that measures the ratio of the observed diversity to the maximum possible in a collection having the same number of species was used.

Beta diversity was analyzed using: Bray-Curtis index and Jaccard distance that shows a qualitative measure of community dissimilarity, unweighted and weighted UniFrac that represent a qualitative measure of community dissimilarity that incorporates phylogenetic relationships between the features

For the visualization of the patterns based on the length of beta diversity between the samples, principal coordinate analysis (PCoA) was used (ter Braak, 1983). Also, for measuring the significance of the analysis of weighted and unweighted UniFrac used PERMANOVA statistical analysis (Lozupone, Lladser, Knights, Stombaugh, & Knight, 2011).

The taxonomic classification was made using the Greengenes database (Almeida, Mitchell, Tarkowska, & Finn, 2018), which provides information on 16S region sequences. Relative abundance was analyzed with "Analysis of the composition of microbiomes" (ANCOM) (Mandal et al., 2015), which is used to compare the composition of microbiomes in two or more populations and GNEISS. These tests were carried out within QIIME2 (Almeida et al., 2018; Bunyavanich et al., 2016; Cardenas et al., 2019) performing their respective statistical validation with PERMANOVA metrics (Lozupone et al., 2011).

Taxa Bar plots were made based on the taxonomic assignment of the frequency table, in which the relative frequency of the different taxa found in the samples is represented, at a particular taxonomic level. The purpose of these graphs is the visual inquiry of the taxonomic structure of each sample in the study.

RESULTS

From the 45 initial samples, 5 subjects were positive for Metabolic Syndrome (11%), while 17 subject had overweight (37,8%), and 14 volunteers were obese (31,1%) (table 1). It means that only 9 subjects were considered as normal (20%).

CONDITION/GENDER	FEMALE	MALE	TOTAL
METABOLIC SYNDROME	3	2	5
OBESITY	13	1	14
OVERWEIGHT	12	5	17

Table 1 Summary of total samples classified by Metabolic Syndrome, obesity and overweight conditions

With this population, we performed the analysis of the composition of intestinal microbiome taking the variables described above (gender, presence of metabolic syndrome, obesity, overweight), the following results were obtained:

Gender

Alpha diversity

Alpha diversity by gender was very similar (figure 1) and, Faith's Phylogenetic Diversity metric did not show statistical difference (p=0.676).

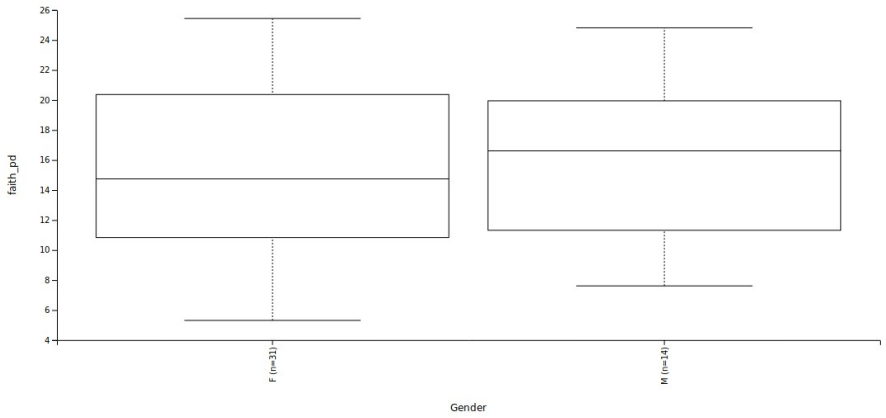

Figure 1 Faith index plot (alpha diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands. e

In the same manner, Pielou's Evenness analysis did not show statistical difference (p=0.523), therefore the frequency of OTUs in the two genders was similar.

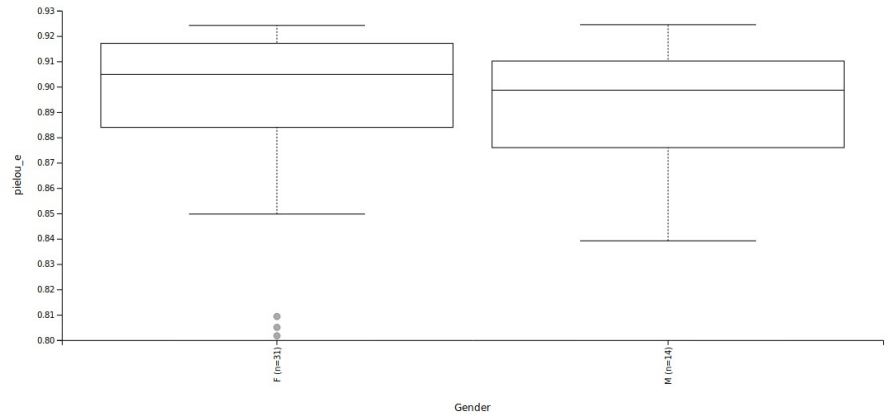

Figure 2 Pielou's evenness index plot (alpha diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands.

Beta diversity

After measured of the dissimilarity of the groups by gender, the results obtained in the PCoA of unweighted (figure 3) and weighted (figure 4) UniFrac did not show any cluster formation within the subjects.

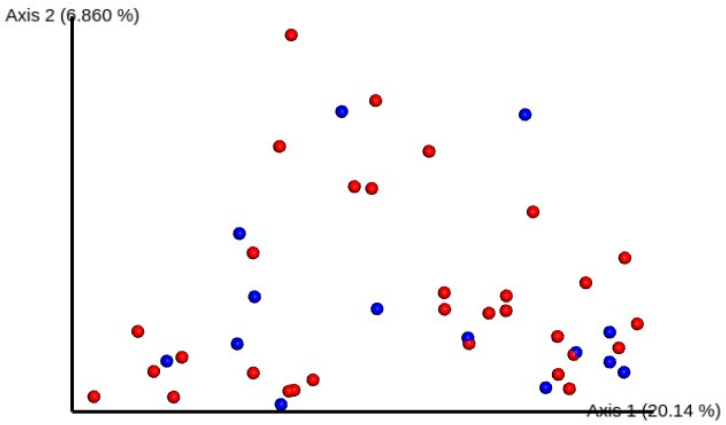

Figure 3 PCoA of weighted UniFrac distances (beta diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands. The red dots correspond to female; the blue dots correspond to male.

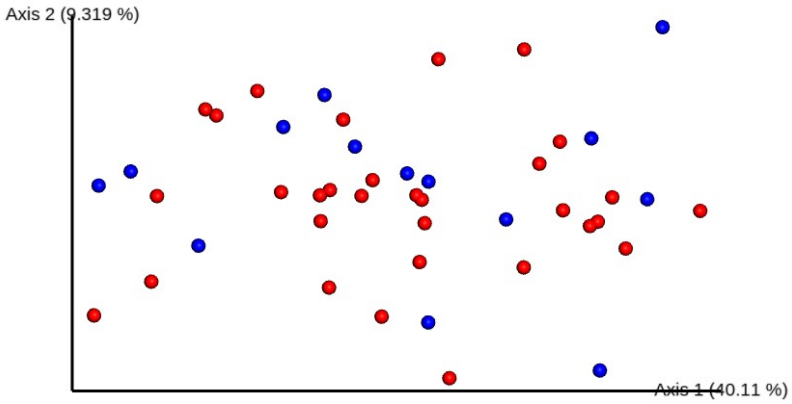

Figure 4 PCoA of unweighted UniFrac distances (beta diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands. The red dots correspond to female; the blue dots correspond to male.

Similarly, the distance between females and males compared with the PERMANOVA analysis did not show significant differences in either unweighted UniFrac (figure 5) (p=0.921) or weighted UniFrac (figure 6) (p=0.868).

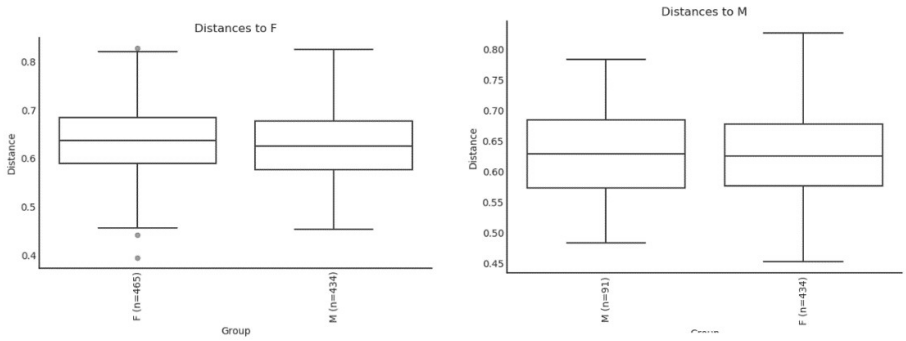

Figure 5 PERMANOVA analysis plots of Unweighted UniFrac (beta diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands.

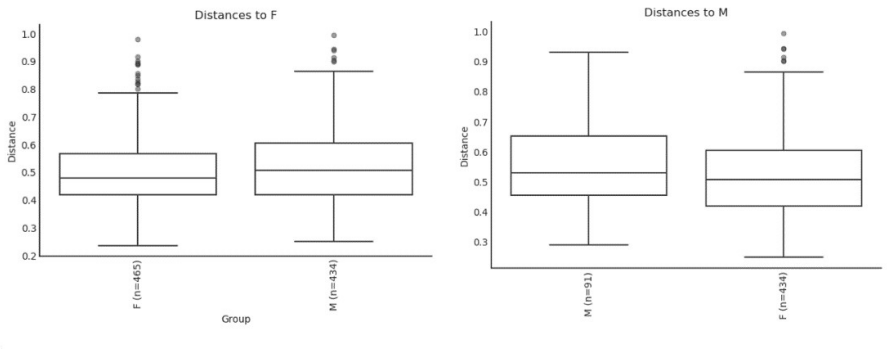

Figure 6 PERMANOVA analysis plots of weighted UniFrac (beta diversity) of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands.

Differential abundance analysis

Taxa abundancy with the GNEISS tool (figure 7) showed a difference in the microbiota composition at phylogenetic level 5, where bacteria of the genera *Prevotella*, *Succinivibrio*, *Faecalibacterium*, *Bacteroidetes*, and *Proteobacteria* were the most abundant in males than in females.

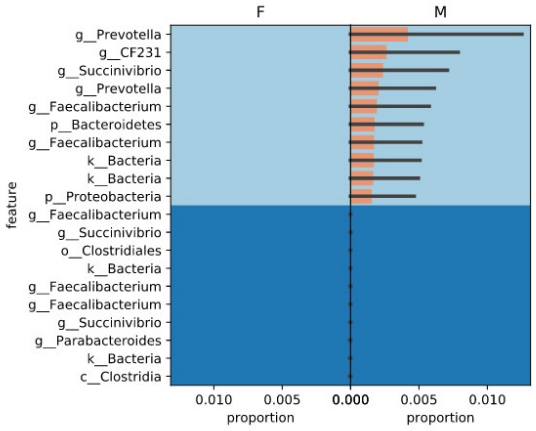

Figure 7 Proportion Plot at taxonomic level 5 of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands.

Also, to identify differences in abundance across sample groups with the ANCOM test. This test takes into account the compositional restrictions and allows the reduction of false-positive discoveries in the detection of differentially abundant taxa. no gender highlighted within the variable.

Taxonomic composition

The analysis of the taxonomic composition by gender, using Taxa Bar plots (figure 8), showed that the abundance of the found OTUS were similar in both men and women.

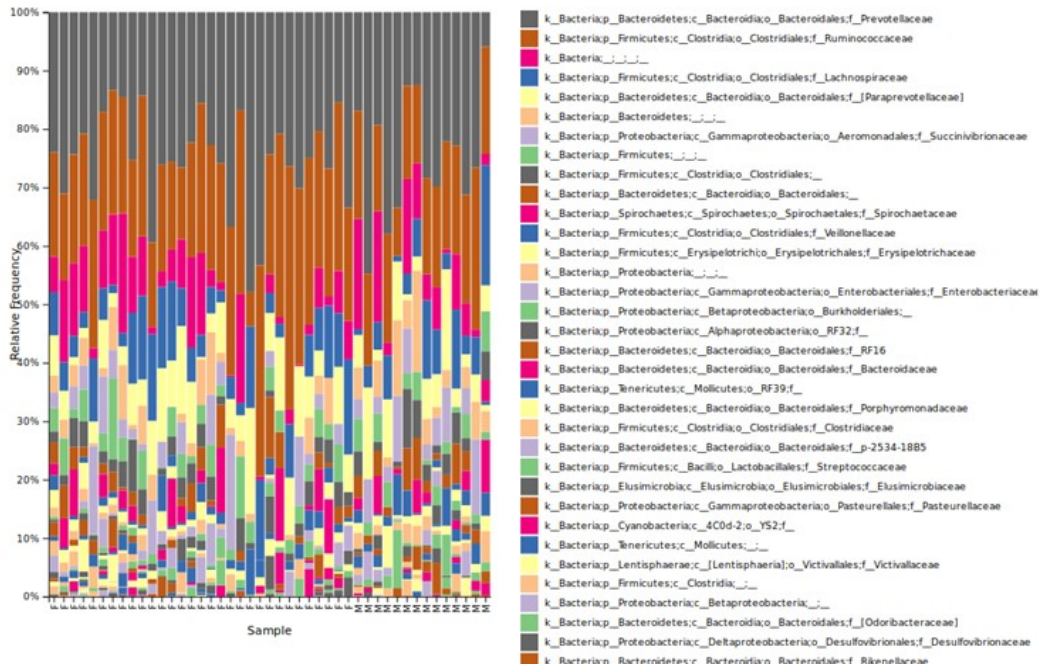

Figure 8. Family taxonomic composition graph of the intestinal microbiota by gender in a sample of indigenous people from the Ecuador highlands.

Metabolic syndrome

Alpha diversity

Faith's Phylogenetic Diversity analysis (figure 9) showed no statistical difference (p=0.772) between subjects with Metabolic Syndrome and their controls. Similarly, Pielou`s evenness (figure 10) analyzes did not show a statistically significant difference between the two groups (p=0.262).

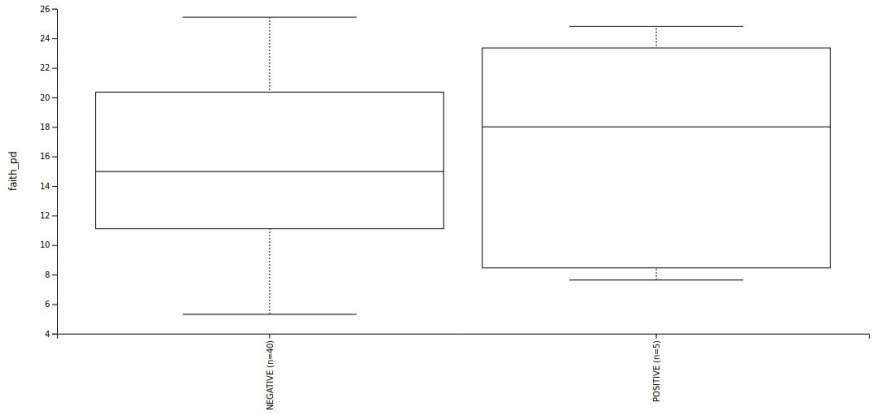

Figure 9 Faith index plot (alpha diversity) of the intestinal microbiota by metabolic syndrome presence in a sample of indigenous people from the Ecuador highlands.

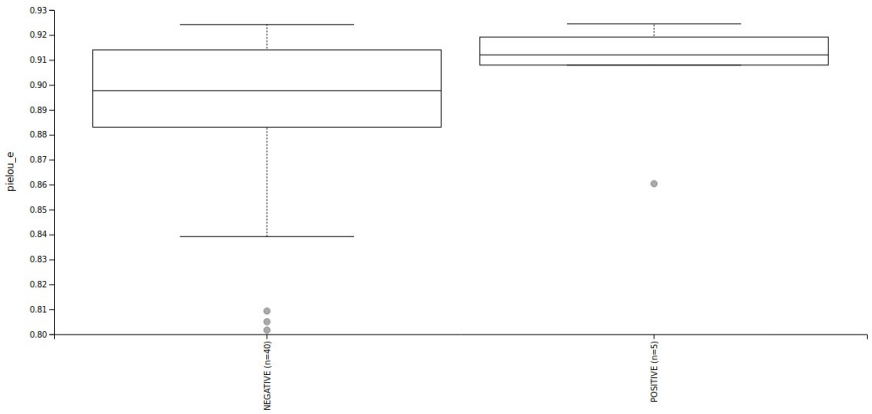

Figure 10 Pielou's evenness index plot (alpha diversity) of the intestinal microbiota by metabolic syndrome presence in a sample of indigenous people from the Ecuador highlands.

Subsequently, once subjects with metabolic syndrome were classified by gender, as expected no significant differences were found with the Faith's Phylogenetic Diversity (p=0.248) nor the Pielou`s evenness analyzes (p=0.563).

Beta diversity

In the analysis of weighted (figure 11) UniFrac PCoA in metabolic syndrome showed no clustering with their controls (variance = 49.3%) similarly in the unweighted (figure 12) UniFrac analysis PCoA (variance = 26.8%) did not show a clustering differential between subjects.

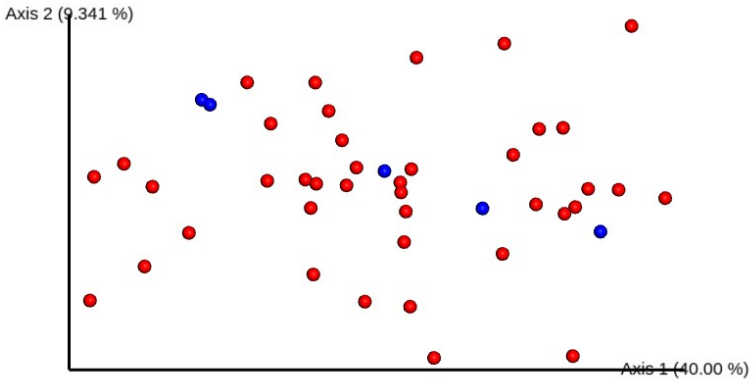

Figure 11 PCoA of Weighted UniFrac distances of the intestinal microbiota , indicating the effect of Metabolic Syndrome in a sample of indigenous people from the Ecuador highlands. The red dots correspond to negative; the blue dots correspond to positive for Metabolic Syndrome.

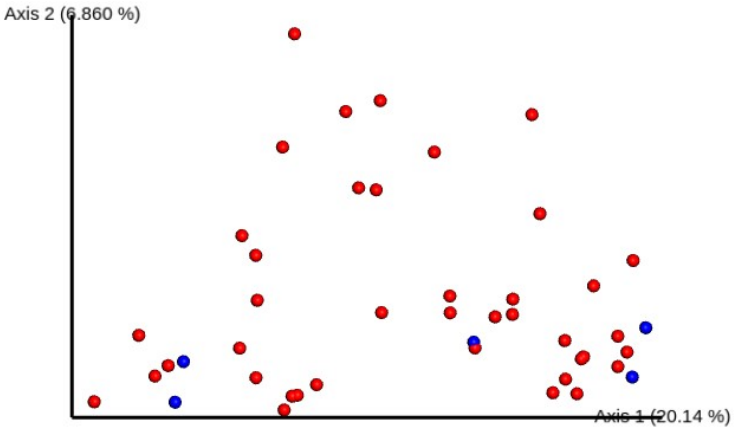

Figure 12 PCoA of Unweighted UniFrac distances of the intestinal microbiota, indicating the effect of Metabolic Syndrome in a sample of indigenous people from the Ecuador highlands. . The red dots correspond to negative; the blue dots correspond to positive for Metabolic Syndrome.

These results were then verified with PERMANOVA analysis (figures 13 and 14) confirming the lack of significance in the weight ($p=0.9$) and unweighted ($p=0.975$) UniFrac analysis.

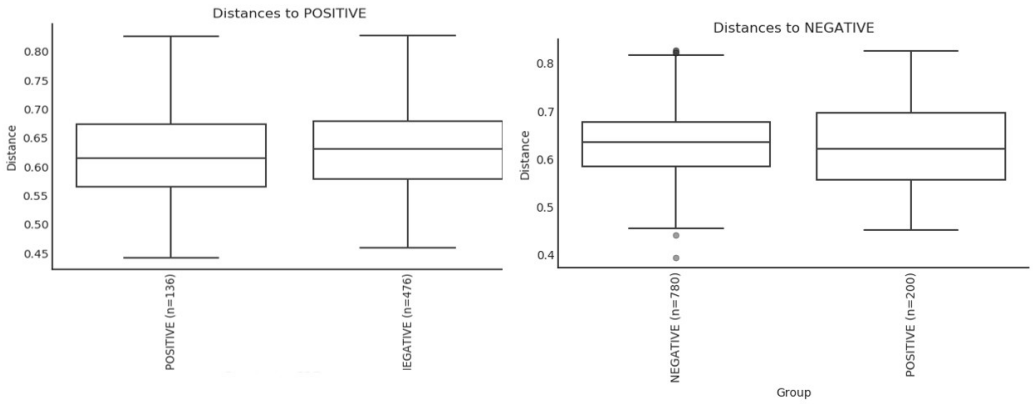

Figure 13 Plots of PERMANOVA analysis significance of Unweighted UniFrac analysis for Metabolic Syndrome in a sample of indigenous people from the Ecuador highlands.

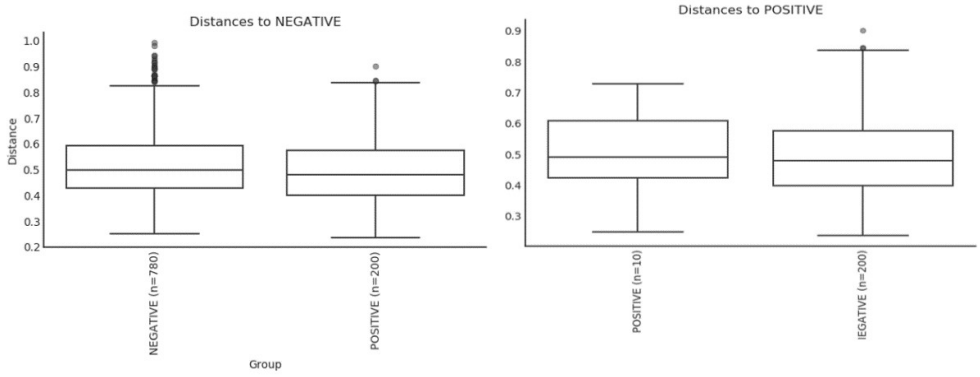

Figure 14 Plots of PERMANOVA analysis of significance of Weighted UniFrac analysis for Metabolic Syndrome in a sample of indigenous people from the Ecuador highlands.

The beta diversity analysis performed using weighted and unweighted UniFrac PCoA in those subjects with metabolic syndrome did not show statistical differences by gender (p=0.387 and p=0.303), overweight (p=0.395 and p=0.262) or obesity (p=0.411 and p=0.307).

Differential abundance analysis

In the plots of proportion with a phylogenetic level 5 (figure 15) the OTUs genus *Faecalibacterium*, *Bacteroidetes*, *Bacteroidales*, *Prevotella*, *Oscillospira,* and *Proteobacteria* were in higher proportion in the group with Metabolic Syndrome compared to their controls.

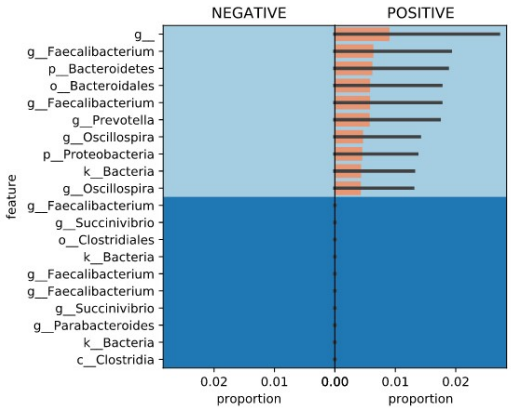

Figure 15 Proportion Plot at taxonomic level 5 of the intestinal microbiota by metabolic syndrome in a sample of indigenous people from the Ecuador highlands.

After the analysis with ANCOM Volcano plot, a prominent OTU from the *Lachnospiraceae* family was found, which was not present in one of the subjects with Metabolic Syndrome (figure 16).

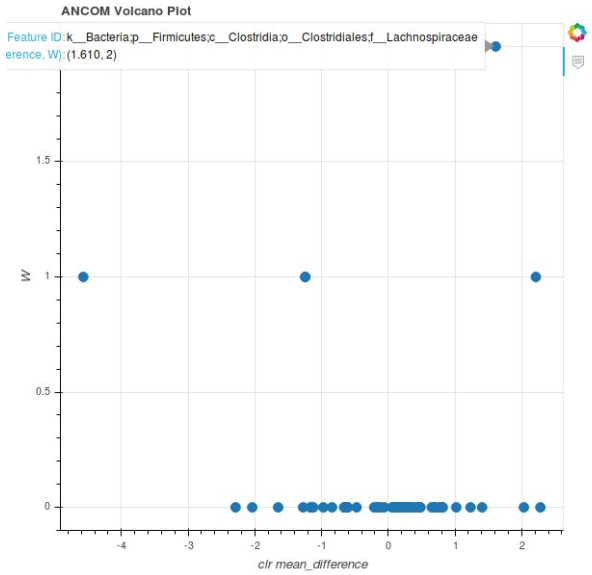

Figure 16 Volcano plot generated of the ANCOM test where the statistical value W is represented in the test for each family against the difference of means of the Metabolic Syndrome in a sample of indigenous people from the Ecuador highlands.

Taxonomic composition

Taxa bar plots at a taxonomic level of phylum for subjects with metabolic syndrome have a similar proportion of *Firmicutes*, *Bacteroidetes*, P*roteobacteria* within their taxonomic structure, compared to the control subjects (figure 17).

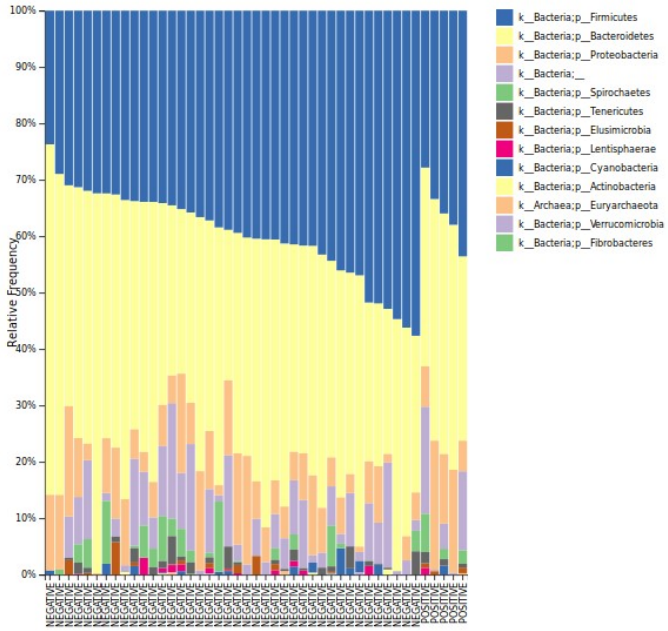

Figure 17 Taxa bar Plot for the Metabolic Syndrome variable at phylum taxonomic level in a sample of indigenous people from the Ecuador highlands.

At the taxonomic level of family, taxa bar plots present almost the same proportions in persons with metabolic syndrome vs. controls. In figure 18, it is notable that the presence of *Prevotellaceae*, *Ruminococcaceae* is similar in both cases. Also, we can notice the absence of *Lachnospiraceae* in one of the samples positive for Metabolic Syndrome, described before in ANCOM analysis.

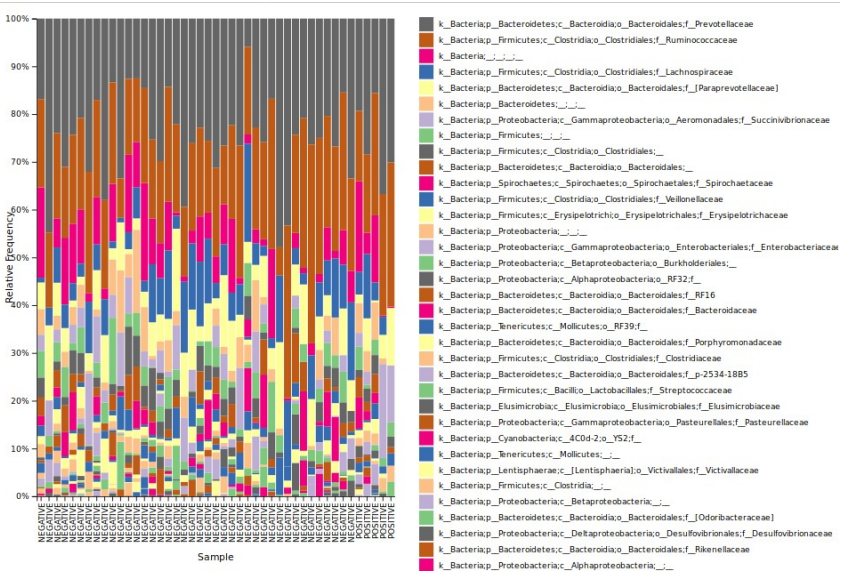

Figure 18 Taxa bar Plot for the Metabolic Syndrome variable at family taxonomic level in a sample of indigenous people from the Ecuador highlands.

The analysis of taxonomic proportions at a phylos level was performed on the group of subjects with metabolic syndrome. Both men and women have similar proportions of *Bacteroidetes* and *Firmicures*. Also, bearing evidence of the taxa bar plot (figure 19), a difference is observed in the phylum *Spirochaetes*, this is present in two of the samples of men and one of the three of women.

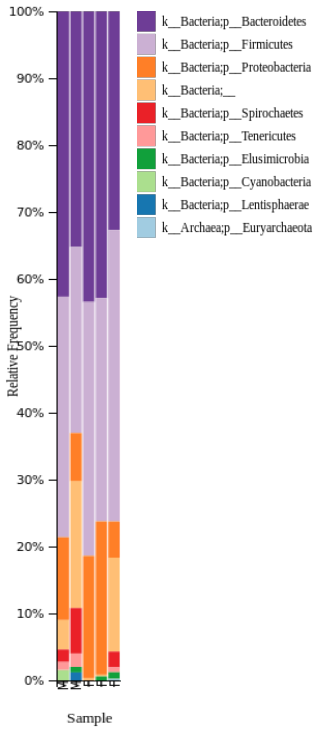

Figure 19 Taxa bar Plot at phylum level for the group with Metabolic Syndrome by gender in a sample of indigenous people from the Ecuador highlands..

Taking the same conditions as above, a taxa bar plot was carried out at a family taxonomic level (L5) (figure 20), where it is observed that the families with the highest proportion in men and women are the bacteria belonging to the *Prevotellaceae* and *Ruminococcaceae* family. Similarly, it is observed that bacteria of the *Succinivibrionaceae* family is absent in one of the three samples of women with metabolic syndrome.

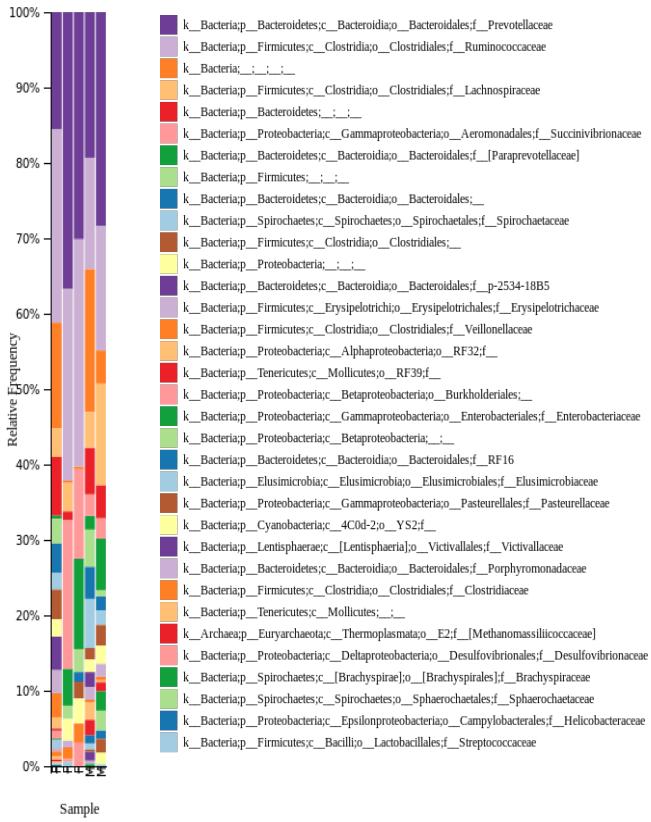

Figure 20 Taxa bar Plot at family level for the group with Metabolic Syndrome by genera in a sample of indigenous people from the Ecuador highlands.

In subjects with metabolic syndrome, taxonomic proportions were analyzed in the presence of obesity. The taxa bar plot at the phylum level (figure 21) reveals that the proportions of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are similar between the groups. Also, it was found that in two of the three samples from obese subjects the proportion of *Spirochaetes* was reduced compared to the control samples.

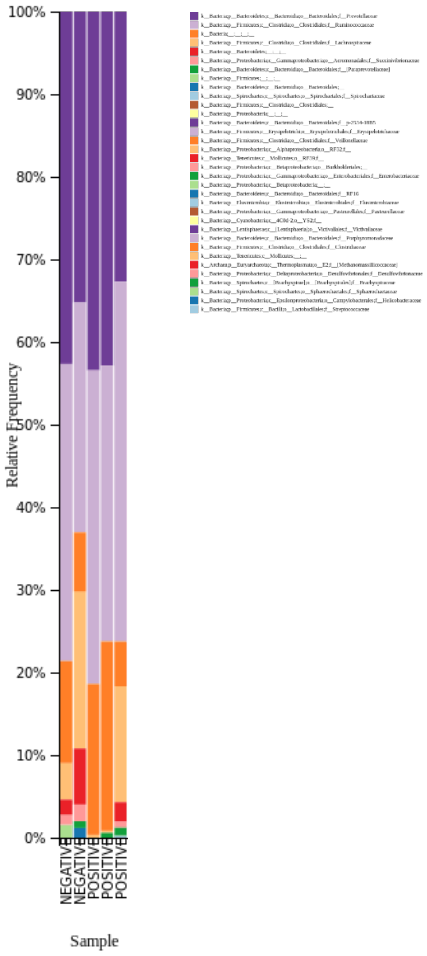

Figure 21 Taxa bar Plot at phylum level for the group with Metabolic Syndrome taking Obesity as variable.

A taxa bar plot with a family taxonomic level was performed in subject with obesity. As show in figure 22 that the families with the highest proportion in obese people compared to their controls are bacteria from the family of *Prevotellaceae* and *Ruminococcaceae*. It was observed that in one of the three positive samples from obese subjects, the proportion of the *Lachnospiraceae* family was minimal, almost nil. Likewise, in one of the three samples from obese subjects the family *Succinivibrionaceae* was not observed.

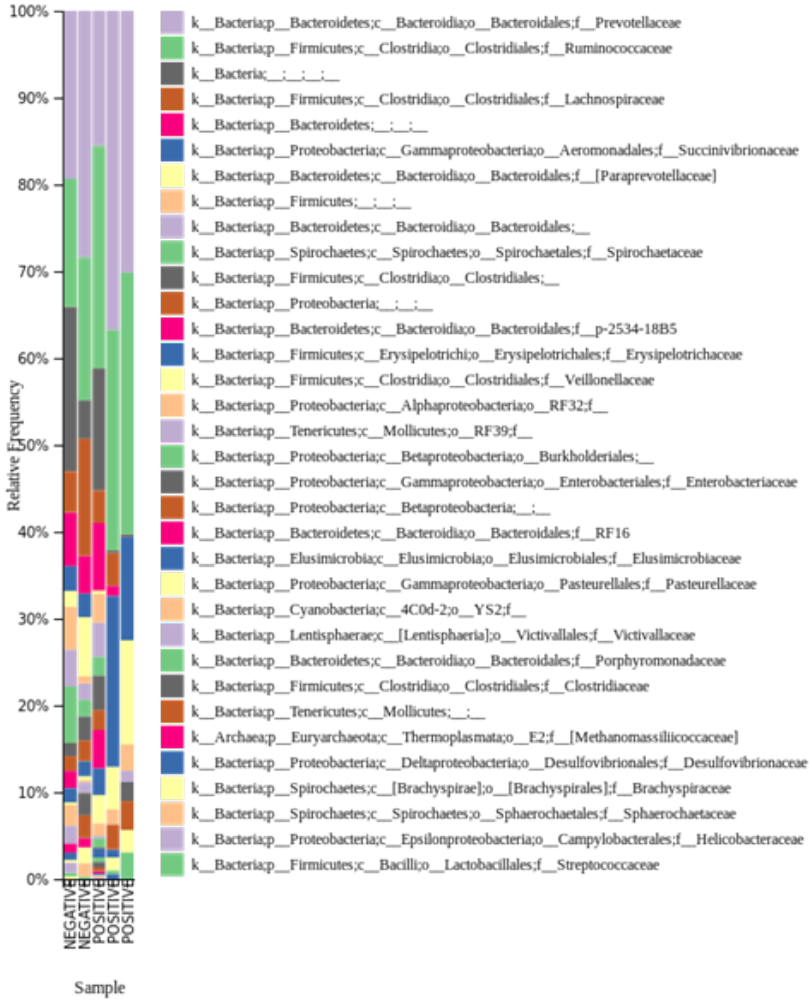

Figure 22 Taxa bar Plot at phylum level for Metabolic Syndrome and Obesity in a sample of indigenous people from the Ecuador highlands.

Overweight

Alpha diversity

Faith's Phylogenetic Diversity (figure 23) analyzes for subjects with overweight showed no significant difference (p=146) with the control group. Similarly, Pielou`s evenness (figure 24) analyzes did not show a statistically significant difference (p=0.232) between the two groups.

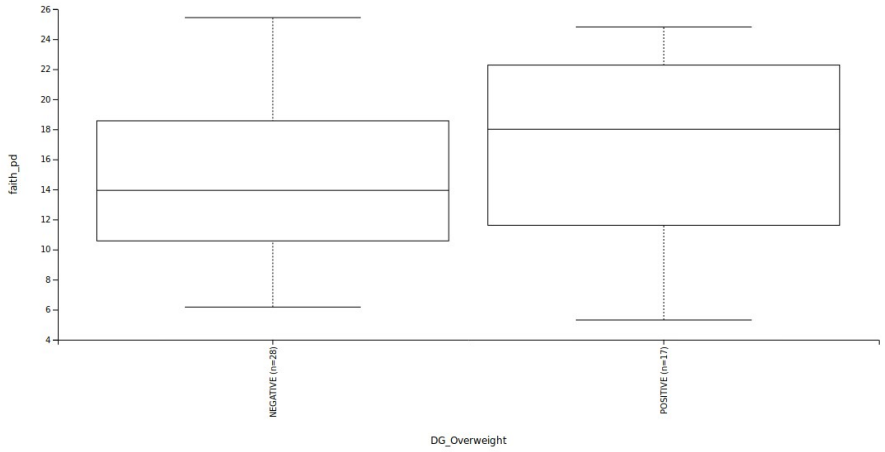

Figure 23 Faith index plot (alpha diversity) by the presence of Overweight in a sample of indigenous people from the Ecuador highlands.

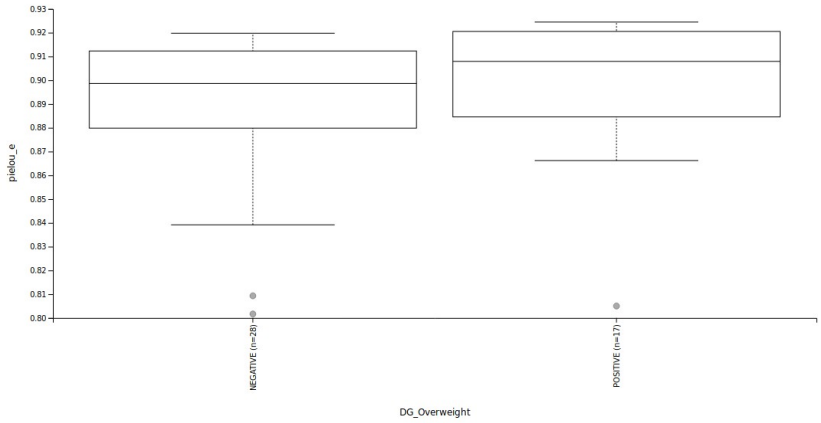

Figure 24 Pielou's evenness (alpha diversity) index plot by presence of Overweight in a sample of indigenous people from the Ecuador highlands.

Beta diversity

The PERMANOVA validation of the results of weighted UniFrac and unweighted UniFrac (in the analysis of the overweight variable do not present a significant difference (p=0.689 and p=0.358, respectively).

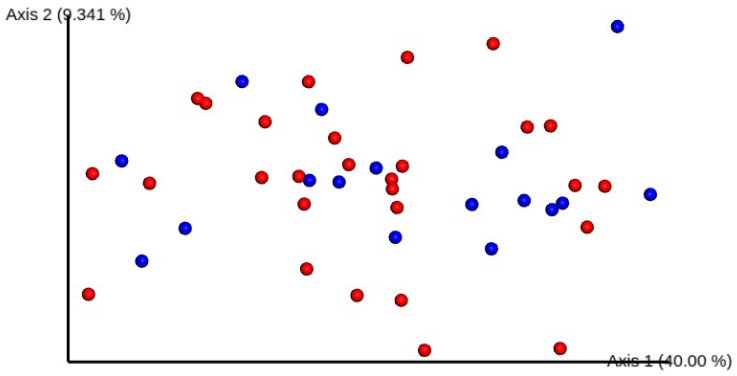

Figure 25 PCoA of Weighted UniFrac distances for the intestinal microbiota in a sample of indigenous people from the Ecuador highlands, indicating the effect of Overweight. The red dots correspond to absence; the blue dots correspond to presence of Overweight.

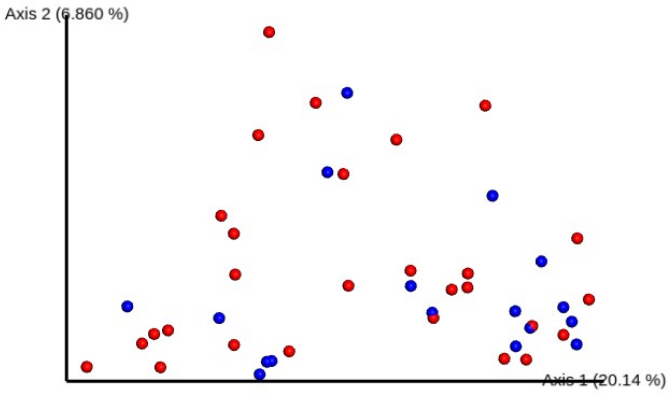

Figure 26 PCoA of Unweighted UniFrac distances for the intestinal microbiota in a sample of indigenous people from the Ecuador highlands, indicating the effect of Overweight. The red dots correspond to absence; the blue dots correspond to presence of Overweight.

Differential abundance analysis

In the plots of proportion with a phylogenetic level 5 in the group of overweight people vs. the control group. The OTUS that occur in a higher percentage for overweight people is *Oscillospira*, *Prevotella*, *Clostridiales*, *Roseburia*, *Ruminococcus*.

In the same way, the results of the ANCOM tests do not show any prominent OTU at volcano plot for taxonomic levels of phylum and family (figure 27).

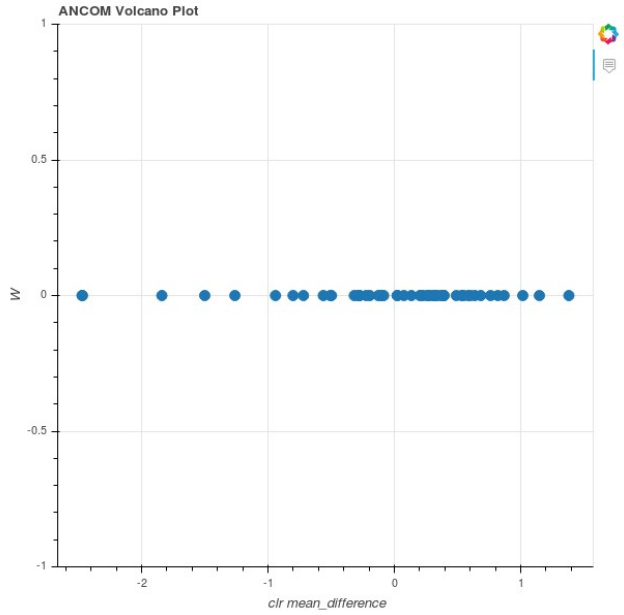

Figure 27 Volcano plot of the ANCOM test where the statistical value W is represented in the test for each family against the difference of means of the Overweight variable in a sample of indigenous people from the Ecuador highlands.

Taxonomic composition

Taxa bar plots at phyla level (figure 28) showed that the proportion of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* is similar in people overweight and their controls.

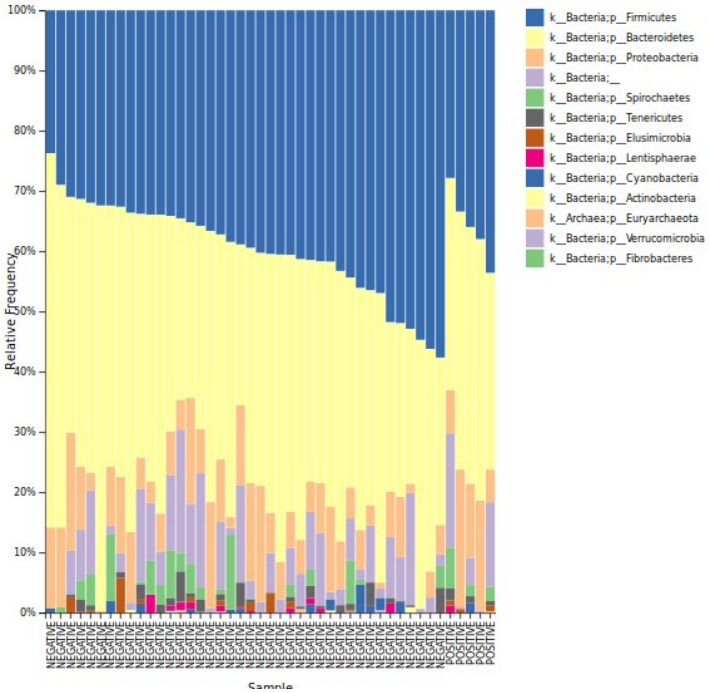

Figure 28 Taxa bar Plot at phylum taxonomic level for the intestinal microbiota in a sample of indigenous people from the Ecuador highlands, indicating the effect of Overweight.

Obesity

Alpha diversity

Phylogenetic Diversity of Faith showed a significant difference between obese and control groups (p=0.05). On the other hand, Pielou's Evenness did not show statistical differences (p= 0.303).

Once the obese subject was classified by obesity and gender, alpha diversity between women and men did no show differences.

Beta diversity

Using the PERMANOVA test, weighted UniFrac showed no difference (p=0.23) while in the unweighted UniFrac analysis a significant difference (p=0.03) between groups was found (figure 31). Within the analysis of PCoA weighted UniFrac (figure 29) and unweighted UniFrac (figure 30) for the obesity variable, the formation of clusters between the groups was not visualized, in the same way, there is no evidence of a difference between these within the explanatory percentage of the analysis.

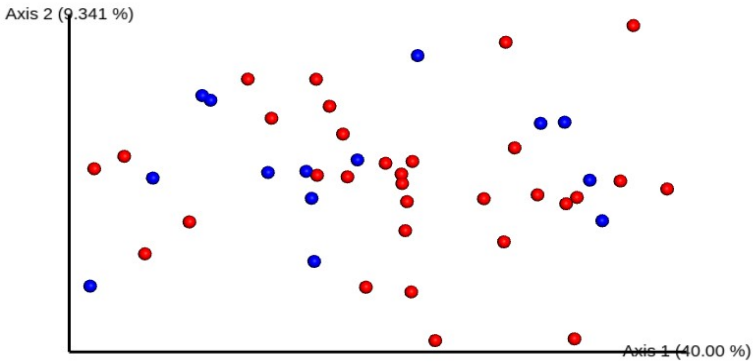

Figure 29 PCoA of weighted UniFrac distances for the intestinal microbiota in a sample of indigenous people from the Ecuador highlands, indicating the effect of obesity. The red dots correspond to negative; the blue dots correspond to positive for obesity.

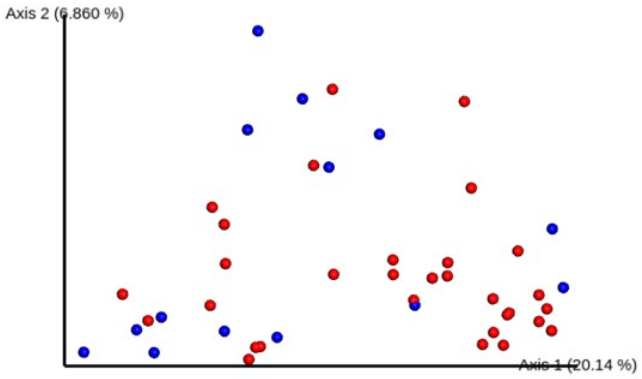

Figure 30 PCoA of unweighted UniFrac distances for the intestinal microbiota in a sample of indigenous people from the Ecuador highlands, indicating the effect of obesity in microbiome composition. The red dots correspond to negative; the blue dots correspond to positive for obesity.

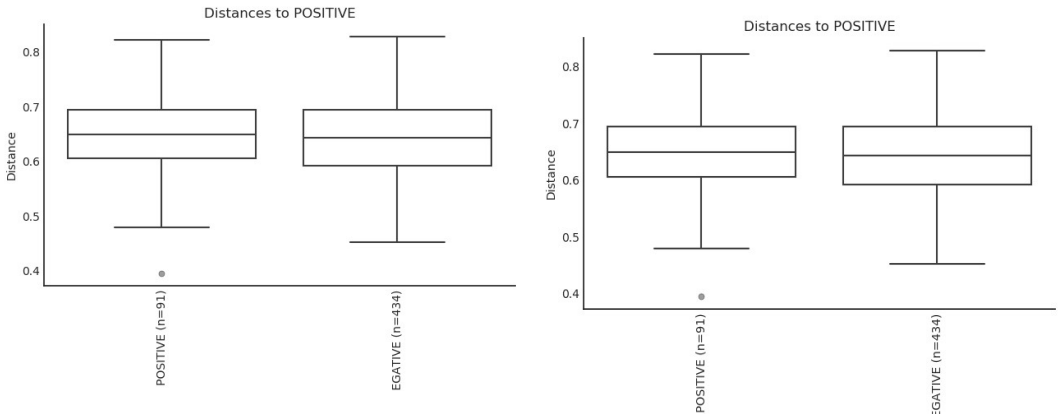

Figure 31 Plots of analysis of Significance of Unweighted UniFrac analysis for the Obesity variable using PERMANOVA analysis for intestinal microbiota in a sample of indigenous people from the Ecuador highlands.

Beta diversity analysis by gender and Metabolic Syndrome in obese subjects was performed using weighted and unweighted UniFrac PCoA, and the plots of this analysis did not show any cluster formation. These results also were confirmed by PERMANOVA analysis, showing no statistical significance.

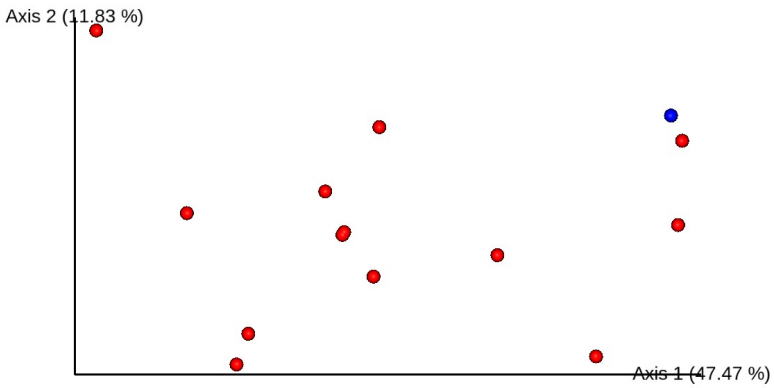

Figure 32 PCoA analysis of the gut microbiota intestinal microbiota in a sample of indigenous people from the Ecuador highlands. Weighted Unifrac represents division of the group of subject's positive for Obesity in relation by the Gender. Male (blue) and Female (red).

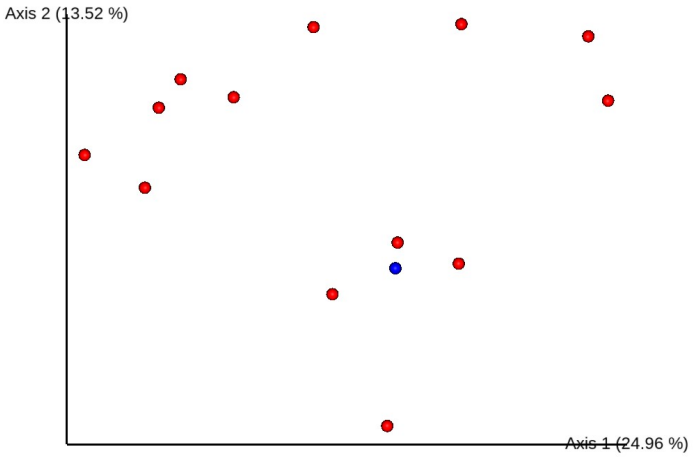

Figure 33 PCoA analysis of the gut microbiota intestinal microbiota in a sample of indigenous people from the Ecuador highlands. Unweighted Unifrac represents division of the group of subject's positive for Obesity in relation by the Gender. Male (blue) and Female (red).

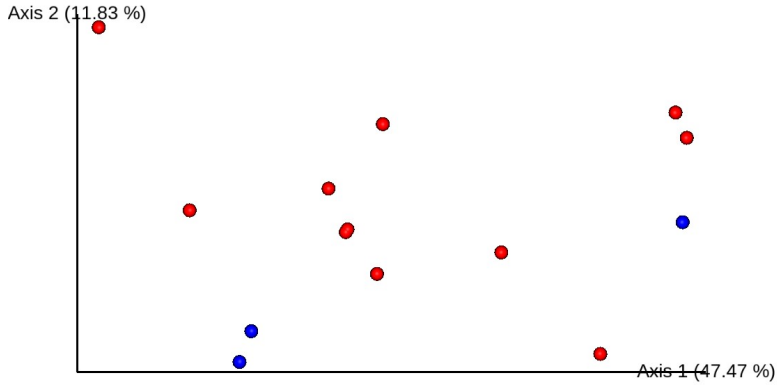

Figure 34 PCoA analysis of the gut microbiota intestinal microbiota in a sample of indigenous people from the Ecuador highlands. Weighted Unifrac represents division of the group of subjects positive for Obesity in relation by the Metabolic Syndrome. Metabolic Syndrome positive (blue) and Metabolic Syndrome negative(red).

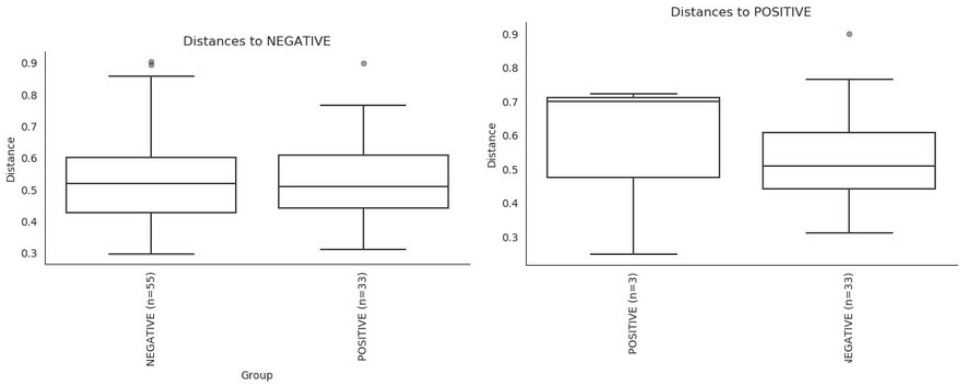

Figure 35 Plots of analysis of Significance of Weighted UniFrac analysis for the group of subject positive for Obesity with Metabolic Syndrome using PERMANOVA analysis in intestinal microbiota in a sample of indigenous people from the Ecuador highlands.

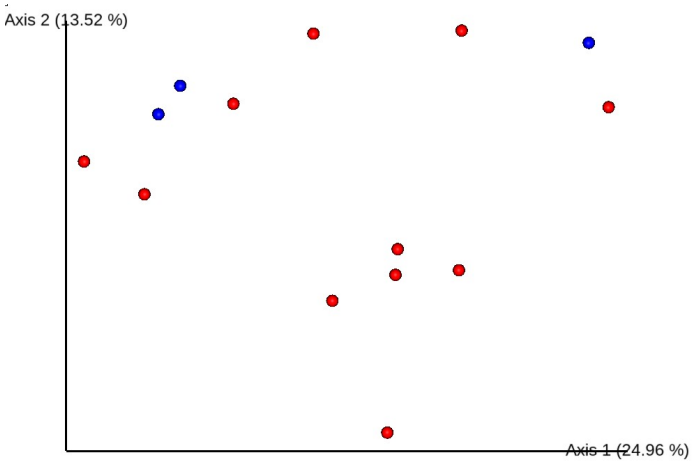

Figure 36 PCoA analysis of the gut microbiota intestinal microbiota in a sample of indigenous people from the Ecuador highlands. Unweighted Unifrac represents division of the group of subjects positive for Obesity in relation by the Metabolic Syndrome. Metabolic Syndrome positive (blue) and Metabolic Syndrome negative (red).

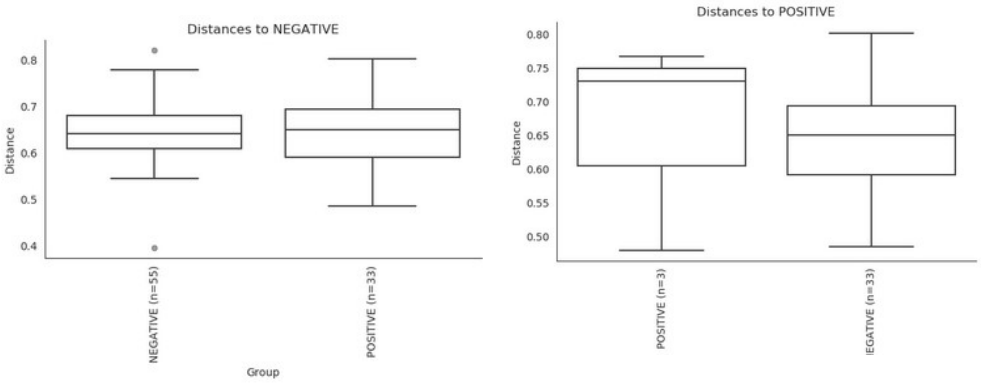

Figure 37 Plots of analysis of Significance of Unweighted UniFrac analysis for the group of subject positive for Obesity with Metabolic Syndrome using PERMANOVA analysis in intestinal microbiota in a sample of indigenous people from the Ecuador highlands

Differential abundance analysis

The proportion plots, at a family taxonomic level (L5), showed a difference of relative abundance in the composition of microbiota between obesity and control groups (figure 38), where the OTUS of the genus *Oscillospira, Prevotella, Clostridiales, Roseburia, Ruminococcus, Ruminococcaceae* in the control group were found in higher proportion. Similarly, there are *Prevotella, Faecalibacterium, Bacteroidetes, Alistipes* groups in more significant portion present in the obese group.

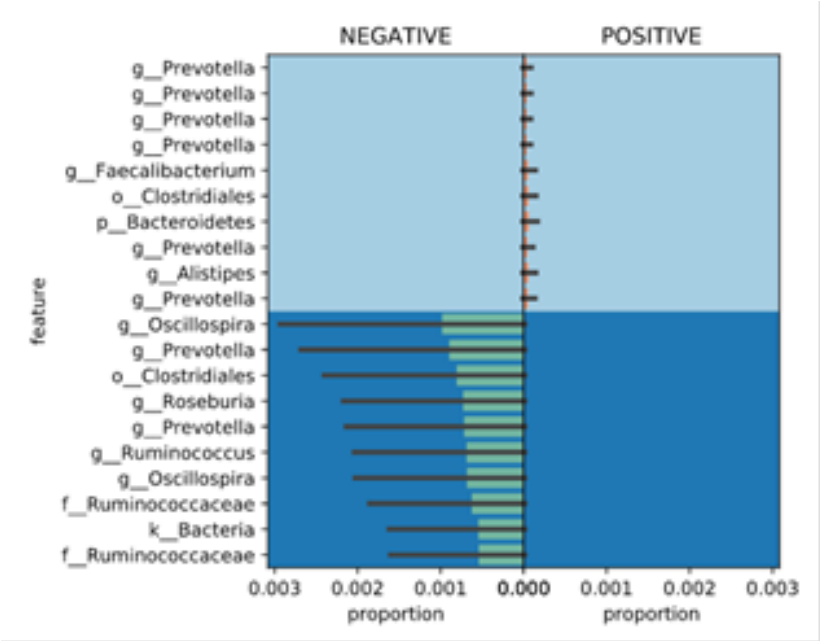

Figure 38 Proportion Plot of the variable presence of Obesity at the taxonomic level 5 of intestinal microbiota *in a sample of indigenous people from the Ecuador highlands*

After the analysis with ANCOM, it shows the presence of two different OTUSs between the groups, *Bacteroidales*, and *Clostridiaceae*, these are visualized in the volcano plots (figure 39).

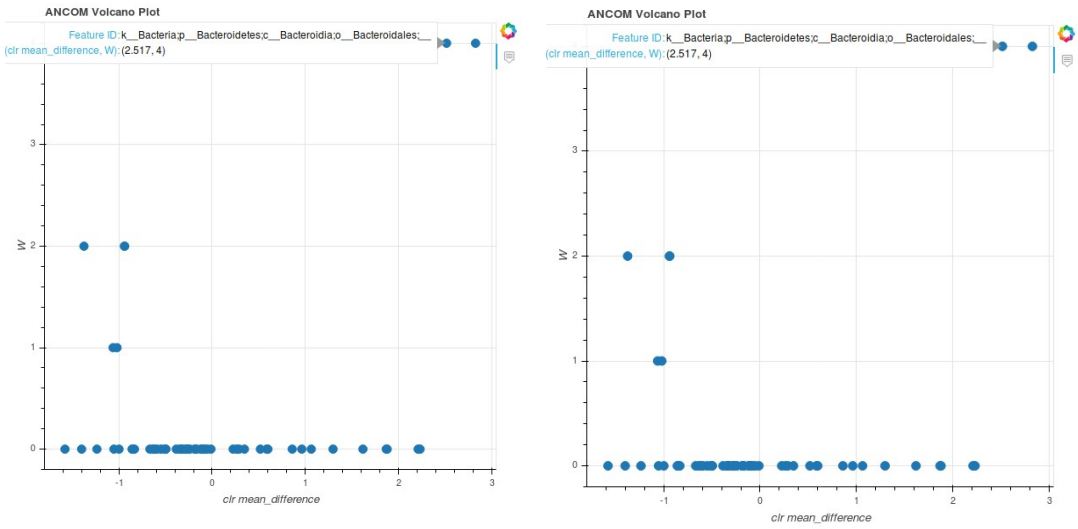

Figure 39 Volcano plot from ANCOM test where the statistical value W is represented in the test for each family against the difference of means of the Obesity intestinal microbiota in a sample of indigenous people from the Ecuador highlands

For these analyses, subjects were also regrouped based on the presence of obesity. With this new group, the variables of gender and Metabolic Syndrome were analyzed, and the following results were obtained.

For the gender variable, at a taxonomic level of phylum, the organisms belonging to the phylum *Clostridia*, *Bacteroidia*, have a higher proportion in women with obesity compared to men with the same condition (figure 40).

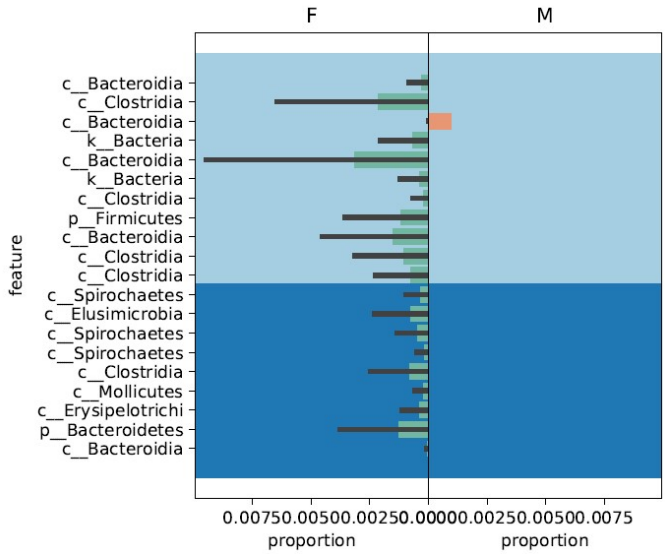

Figure 40 Proportion Plot by gender and Obesity at Phylum taxonomic level intestinal microbiota in a sample of indigenous people from the Ecuador highlands

In the same manner, the analysis of variance was carried out with the variable of presence or absence of Metabolic Syndrome. The figure 41 shows a difference in the proportion of the organisms present in the positive and negative subjects to Metabolic Syndrome. It is denoted that *Prevotella*, *Faecalibacterium*, *Clostridial*es organisms are present in greater abundance in obese subjects but without the presence of Metabolic Syndrome, on the contrary, there are organisms belonging to the *Prevotella* genus, but different from those previously mentioned, that are found in greater abundance in people with Obesity and Metabolic Syndrome, in addition to organisms belonging to the genus *Escherichia*.

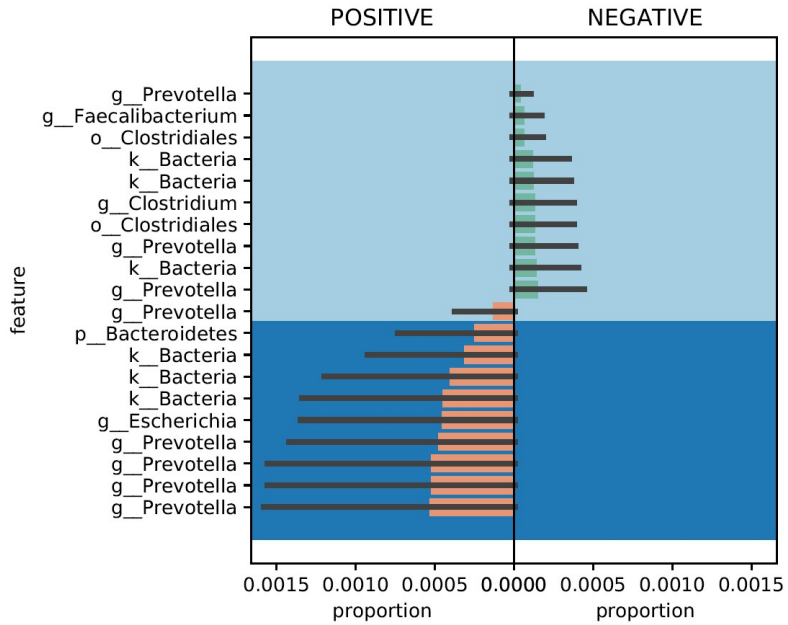

Figure 41 Proportion Plot by Metabolic Syndrome and Obesity at Family taxonomic level intestinal microbiota in a sample of indigenous people from the Ecuador highlands

Taxonomic composition

Taxa bar graphs based on the taxonomic allocation (figure 42) for this variable were made at taxonomic levels of phylum and family. At phyla level, a similar proportion of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* is observed. Taxa bar Plot for Obesity at phylum taxonomic level intestinal microbiota in a sample of indigenous people from the Ecuador highlands

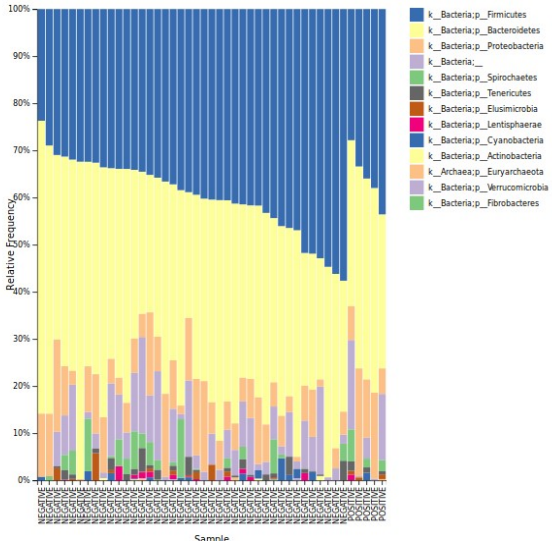

Figure 42 Taxa bar Plot for Obesity at phylum taxonomic level intestinal microbiota in a sample of indigenous people from the Ecuador highlands

In the same way, the taxonomic composition analysis of the group of obese subjects was performed, maintaining the same variables, gender and Metabolic Syndrome.

Taxa bar plot at graph 43 shows the taxonomic composition of the group of obese subjects classified by gender and it shows, at the phylum level, that the proportions of *Bacteroidetes*, *Firmicutes* are similar between the groups. Also, we can see a slightest difference in the proportion of *Proteobacteria* between the groups.

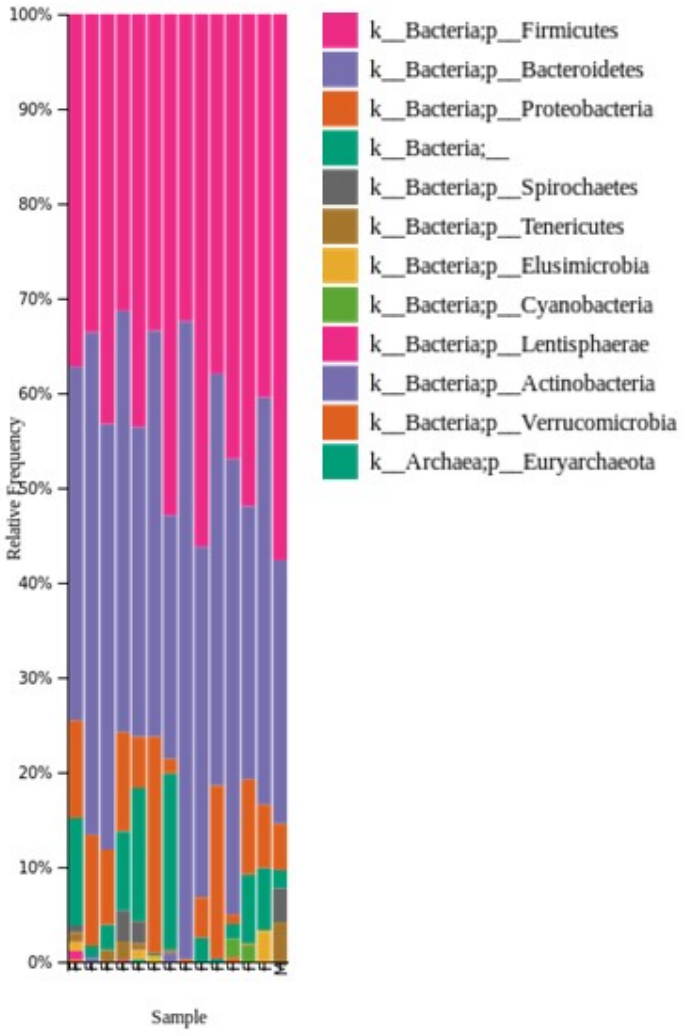

Figure 43 Taxa bar Plot at phylum level for the group with Obesity and Gender intestinal microbiota in a sample of indigenous people from the Ecuador highlands.

Based on the metabolic syndrome variable for obese subjects, it is observed in the bar plot taxa of Fig. 44 that the proportion of *Firmicutes* and *Bacteroidetes* is similar in the two groups. A difference in the proportion of *Proteobacteria* is denoted both among

obese subjects negative to Metabolic Syndrome and among obese subjects positive to Metabolic Syndrome.

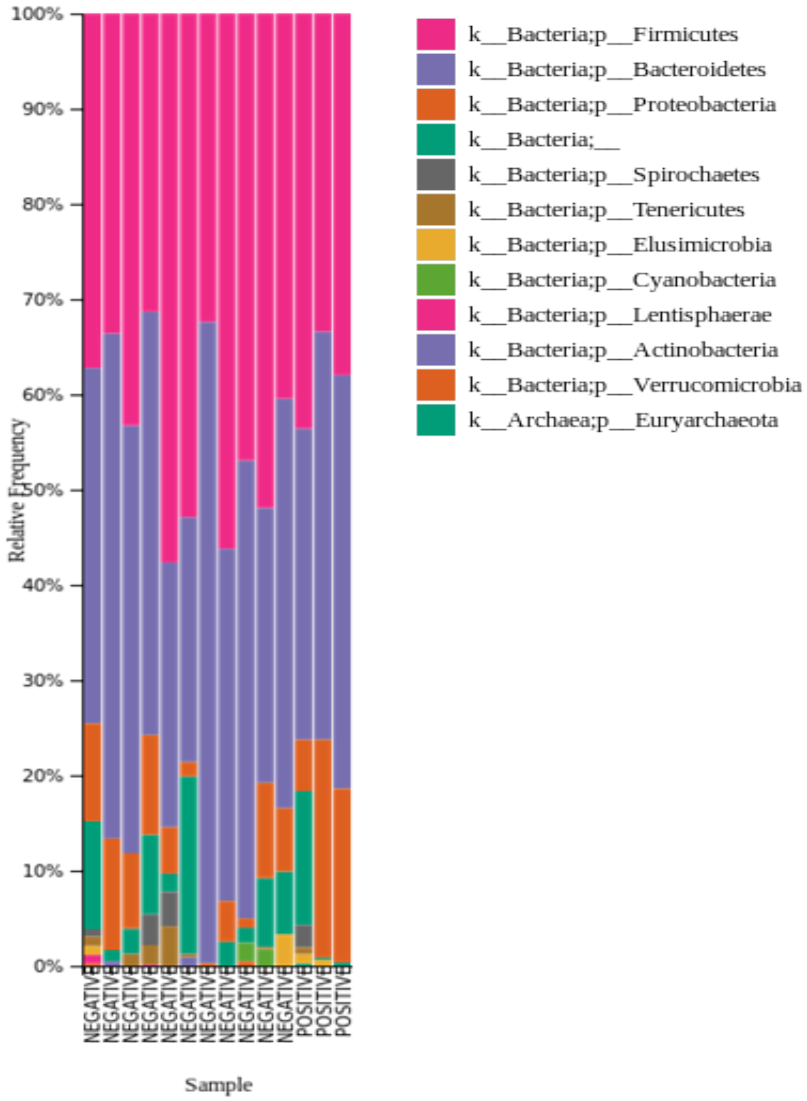

Figure 44 Taxa bar Plot at phylum level for the group with Obesity and Metabolic Syndrome intestinal microbiota in a sample of indigenous people from the Ecuador highlands.

DISCUSSION

This study focus on indigenous people from the Ecuador highlands found no differences in the intestinal microbiota, either by alpha nor beta diversity analyzes, between males and females. It is similar to the lack of differences by gender reported previously in Spanish, Chinese and Japanese populations (Haro, Rangel-Zúñiga, et al., 2016; Gao, et al., 2018; Takagi, et al., 2019).

However, the abundance analyzes, showed that OTUS assigned to the genus *Prevotella*, *Succinivibrio*, *Faecalibacterium*, *Bacteroidetes*, and *Proteobacteria* occurred more abundantly in indigenous males, as previously reported for some of those genus in Caucasian population (Ding & Schloss, 2014; Borgo et al., 2018).

Taxonomic composition analyses showed that the most abundant taxa were *Bacteroidetes* and *Firmicutes* with a higher percenter of *Bacteroidetes* in males (40%) compared to females (38%) also females show a higher composition *Firmicutes* (41%) compare with males (36%).This finding is similar with previous reports in Caucasian population showing that *Bacteroidetes*-content and the *Bacteroidetes*/*Firmicutes*-ratio were significantly higher in men as compared to women (Most et al., 2017). Also, Dominianni report that women tend to have a low abundance of *Bacteroidetes* compared to men (Dominianni et al., 2015).

Regarding metabolic syndrome in this sample of indigenous people from the Ecuador highlands, it was found in 5 out 45 subject (11.1%) that is slightly lower to the rate reported (15.1%) population recruited into the biggest study which this component

belongs (Hernandez, 2019), and means that, although samples were taking random even before diagnose of metabolic syndrome was done; they are representative for the studied population.

Initial assessment for alpha and beta diversity in those with metabolic syndrome, showed no significant differences compared to their controls. Further analyzes with the group positive for metabolic syndrome showed no differences for alpha and beta diversity by gender, overweight, and obesity. These findings counteract, somehow, with a recently study in Mexican women showing that obese subjects and metabolic syndrome had greater diversity based on Shannon and Simpson indices (Chávez-Carbajal et al., 2019).

Abundance analyzes for indigenous from Ecuador highlands with metabolic syndrome showed that the genera with the highest presence were *Bacteroides*, *Clostridia*, and members of the phylum *Proteobacteria*. These findings, however, differ from the Spanish population where abundance of *Bacteroides*, *Eubacterium*, and *Lactobacillus* was reported in metabolic syndrome (Haro, Garcia-Carpintero, et al., 2016) and also from Thai population with metabolic syndrome where the phylos of *Firmicutes* and *Bacteroidetes* are the most abundant among individuals (Org et al., 2017).

Furthermore, ANCOM composition analyzes showed that four of the five indigenous subject from highlands in Ecuador and with metabolic syndrome had *Lachnospiraceae,* a microorganism of the genus *Firmicutes* and considered as a marker of lipid and glucose metabolism altered (Lippert et al., 2017). It is consistent with the same finding

in the Mexican population with obesity and metabolic syndrome (Chavez-Carbajal et al., 2019).

In the same sense, when only indigenous subject with overweight were analyzed, alpha and beta diversity did not show significant differences, a result similar to Mexican children with overweight (Murugesan et al., 2015). However, abundance analysis showed that overweight individuals had a higher amount of *Clostridia* and *Bacteroidea*, as previously reported in German population (Schwiertz et al., 2010).

On the other hand, in those indigenous subjects with obesity, a significant difference in the alpha diversity was found, similarly to a previous report but in obese Japanese population (Kasai et al., 2015), although it was no different in Chinese population (Lin et al., 2015). Regarding beta diversity, no difference was found in the Ecuadorian indigenous with obesity, a similar result to communities of obese Mexican children (Murugesan et al., 2015), but discordant with Chinese obese people (Lin et al., 2015).

In Colombians, similar to Americans, it found a tendency in *Firmicutes* to diminish with increasing BMI, whereas no change was observed in *Bacteroidetes*. A more detailed inspection of the Colombian dataset revealed that five fiber-degrading bacteria, including *Akkermansia*, *Dialister*, *Oscillospira*, *Ruminococcaceae* and *Clostridiales*, became less abundant in obese subjects (Escobar, Klotz, Valdes, & Agudelo, 2014; Goodrich et al., 2014; Tims et al., 2013). In the present study, indigenous living at the Ecuador highlands and with obesity showed a greater abundance of *Prevotella*, *Bacteroides*, and *Faecalibacterum*.

Abundance analyses showed that in indigenous obese females differ in the genera *Clostridia*, *Bacteroidea*, and *Firmicutes*. Similarly, in obese individuals with metabolic syndrome, the abundance is more significant for *Gammaproteobacteria*, *Escherichia*, *Prevotella*, and *Proteobacteria*. In contrast, in obese individuals without metabolic syndrome, abundance is higher in the genera *Prevotella*, *Faecalibaterium*, and *Clostridiales*.

Taxonomic analyses showed that the composition between obese indigenous subjects and their controls was similar to phylums *Firmicutes* and *Bacteroidetes*. This result is similar as the results of Caucasian from Netherlands, where the abundance of the phylum *Bacteroidetes* was lower in obese people and phylum *Firmicutes* was greater (Verdam et al., 2013).

Then, as mentioned in the introduction, several studies have suggested that the gastrointestinal microbiota contributes to the etiology of several cardio-metabolic diseases, and its modulation is of great importance as a therapeutic target for the treatment of metabolic syndrome and related components, like overweight and obesity (Mazidi et al., 2016). However, changes in the composition of the microbiota caused by external factors can lead to a dramatic alteration in the symbiotic relationship established by the intestinal bacteria and the host, which promotes the development of metabolic diseases (Boulangé et al., 2016).

In Ecuador, there is only one previous study about the intestinal microbiota, and it was done in mestizo children from the urban highlands in Ecuador. It showed no difference in alpha diversity. However, the geographical location increases genera such as

Prevotella or order as *Clostridiales* and in the same way in the infection with Entamoeba increases the relative abundance of families like *Ruminococcaceae*, or order as *Bacteroidale*s in the gut microbiota of these children. (Fiallos L & P. Cardenas, 2019)

Therefore, this report constitutes the first characterization of intestinal microbiota in indigenous population with metabolic syndrome in the highlands of Ecuador.

CONCLUSIONS AND RECOMMENDATIONS

After analyzing the intestinal microbiota in the adult indigenous population of Cotacachi and Iluman, in the highlands of Ecuador, the most prevalent agents were *Prevotella*, *Succinivibrio*, *Faecalibacterium*, *Bacteroidetes*, and *Proteobacteria,* with no differences in terms of diversity by gender.

The microbiota composition of indigenous individuals with Metabolic Syndrome from Cotacachi and Iluman, in the highlands of Ecuador, is characterized by a significant presence of *Bacteroides*, *Clostridia*, and members of the phylum *Proteobacteria*, but is similar results can be found in the control from the same area.

The presence of *Lachnospiraceae* in the microbiota composition of indigenous individuals with metabolic syndrome is a marker of abnormal lipid and glucose metabolism.

Obesity was related to a change in the diversity of the intestinal microbiota in indigenous individuals, with high abundance of *Oscillospira*, *Prevotella*, *Clostridiales*, and *Ruminococcus* in indigenous non-obese individuals.

The diversity of the intestinal microbiota might be influence by environmental, genetic, and nutritional factors, therefore to properly evaluate the relationship between overweight and metabolic syndrome and intestinal microbiota it is desirable to consider another bigger sample size study.

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