DOCTORAL THESIS

The dysbiosis of the bacterial population of the digestive system (intestinal microbiota) in patients with metabolic syndrome improves after two models of healthy diets: a diet rich in complex carbohydrates and a Mediterranean diet. CORDIOPREV study.

CARMEN MARIA HARO MARISCAL

Thesis by Compendium of Articles
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TITULO: *The dysbiosis of the bacterial population of the digestive system (intestinal microbiota) in patients with metabolic syndrome improves after two models of healthy diets: a diet rich in complex carbohydrates and a Mediterranean diet. CORDIOPREV study*

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The dysbiosis of the bacterial population of the digestive system (intestinal microbiota) in patients with metabolic syndrome improves after two models of healthy diets: a diet rich in complex carbohydrates and a Mediterranean diet. CORDIOPREV study.

Trabajo presentado por
CARMEN MARIA HARO MARISCAL
Licenciada en Biología, para optar al grado de
Doctor por la Universidad de Córdoba.
Tesis por compendio de Artículos con mención Internacional

Dirigido por
Prof. Dr. Francisco Pérez Jiménez
Dr. Antonio Camargo García

CÓRDOBA, ESPAÑA 2015

TÍTULO DE LA TESIS: La disbiosis de la población bacteriana del Sistema Digestivo (microbiota intestinal) en pacientes con Síndrome Metabólico mejora tras dos modelos de dietas saludables: dieta rica en carbohidratos complejos y dieta Mediterránea. Estudio CORDIOPREV.

DOCTORANDO/A: Carmen María Haro Mariscal

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS:

El trabajo de tesis realizado por Carmen María Haro Mariscal, bajo nuestra dirección en la Unidad de Lípidos y Arteriosclerosis del Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC/Hospital Universitario Reina Sofía de Córdoba/Universidad de Córdoba), se ha basado en la demostración de la disbiosis de la microbiota intestinal en pacientes con Síndrome Metabólico y su mejora tras el consumo a largo plazo de una dieta saludable, dieta baja en grasa o dieta Mediterránea. Los resultados obtenidos responden a los objetivos planteados inicialmente. En cuanto a la difusión de los resultados de la tesis, la doctoranda ha participado en 14 congresos científicos de ámbito Nacional e Internacional, presentando comunicaciones orales y de tipo póster. Asimismo, dichos resultados se han publicado en colaboración con sus tutores de tesis y otros colaboradores de nuestro grupo de investigación, lo que le ha valido a la doctoranda presentar su tesis por compendio de artículos. Las publicaciones derivadas de esta tesis doctoral son las siguientes:

Haro C, Garcia-Carpintero S, Alcala-Diaz JF, Gomez-Delgado F, Delgado-Lista J, Perez-Martinez P, Rangel Zuñiga OA, Quintana-Navarro GM, Landa BB, Clemente JC, Lopez-Miranda J, Camargo A, Perez-Jimenez F. The gut microbial community in metabolic syndrome patients is modified by diet. J Nutr Biochem. 2016 Jan;27:27-31. Impact Factor: 4.668 (Q1).

Haro C, Montes-Borrego M, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Tinahones FJ, Landa BB, López-Miranda J, Camargo A, Pérez-Jiménez F. Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population. J Clin Endocrinol Metab. 2016 Jan; 101(1):233-42. Impact Factor: 5.531 (Q1).

Haro C, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Landa BB, Tena-Sempere M Clemente JC, López-Miranda J, Pérez-Jiménez F, Camargo A. Intestinal microbiota is influenced by gender and body mass index. PLoS One. 2016 May 26;11(5):e0154090. Impact Factor: 3.54 (Q1).

A nuestro juicio, el trabajo realizado por la doctoranda Carmen María Haro Mariscal reúne los méritos suficientes para ser defendido ante el tribunal correspondiente y poder optar al grado de Doctor. Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba a 20 de Octubre de 2016

Firma del/de los director/es

Fdo.: Prof. Dr. Francisco Pérez Jiménez

Fdo.: Dr. Antonio Camargo García

INFORME SOBRE LA CALIDAD DE LAS PUBLICACIONES DE LA TESIS.

- Número de artículos: 3 artículos en el cuartil 1.

1. **Haro C**, Garcia-Carpintero S, Alcala-Diaz JF, Gomez-Delgado F, Delgado-Lista J, Perez-Martinez P, Rangel Zuñiga OA, Quintana-Navarro GM, Landa BB, Clemente JC, Lopez-Miranda J, Camargo A, Perez-Jimenez F. *The gut microbial community in metabolic syndrome patients is modified by diet*. J Nutr Biochem. 2016 Jan;27:27-31. doi: 10.1016/j.jnutbio.2015.08.011. Epub 2015 Aug 20 PMID: 26376027. **Impact Factor: 4.668 (Q1).**

2. **Haro C**, Montes-Borrego M, Rangel-Zuñiga OA, Alcala-Diaz JF, Gomez-Delgado F, Perez-Martinez P, Delgado-Lista J, Quintana-Navarro GM, Tinahones FJ, Landa BB, Lopez-Miranda J, Camargo A, Perez-Jimenez F. *Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population*. J Clin Endocrinol Metab.2016 Jan;101(1):233-42. doi: 10.1210/jc.2015-3351. Epub 2015 Oct 27. PMID: 26505825. **Impact Factor: 5.531 (Q1).**

3. **Haro C**, Rangel-Zuñiga OA, Alcala-Diaz JF, Gomez-Delgado F, Perez-Martinez P, Delgado-Lista J, Quintana-Navarro GM, Landa BB, Tena-Sempere M Clemente JC, Lopez-Miranda J, Perez-Jimenez F, Camargo A. *Intestinal microbiota is influenced by gender and body mass index*. PLoS One. 2016 May 26;11(5):e0154090. doi: 10.1371/journal.pone.0154090. eCollection 2016. **Impact Factor: 3.54 (Q1).**

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“Todas las enfermedades empiezan en el intestino”
Hipocrates

“Cada día sabemos más y entendemos menos”
Albert Einstein

“La investigación de las enfermedades ha avanzado tanto
que cada vez es más difícil encontrar a alguien
que esté completamente sano”
Aldous Huxley

"Sólo una cosa convierte en imposible un sueño:
el miedo a fracasar"
Paulo Coelho

INDEX

ABBREVIATIONS

The most commonly used abbreviations in the text are outlined below:

AACE: American Association of Clinical Endocrinologists

ADA: American Diabetes Association

AHA: American Heart Association

AMPK: Adenosine Monophosphate-activated Protein Kinase

ApoB: Apolipoprotein B

AT: Adipose Tissue

ATP III: Adult Treatment Panel III

BMI: Body Mass Index

CBR-1: Cannobinoid Receptor 1

CD: Crohn´s Disease

CETP: Cholesteryl Ester Transport Protein

CFU: Colony Forming Units

CHD: Coronary Heart Disease

CRC: Colorectal Cancer

CVD: Cardiovascular Disease

DBP: Diastolic Blood Preassure

EGIR: European Group for the study of Insulin Resistance

FDR: False Discovery Rate

FFA: Free Fatty Acids

FIAF: Fasting –induced Adipocyte Factor

GALT: Gut –associated Lymphoid Tissue

GC-MS: Gas Chromatography –Mass Spectroscopy

GLP-1: Glucagon-like Peptide 1

Abbreviations

GPR41: G Protein coupled receptor 41

GPR43: G Protein-coupled receptor 43

GT: Gastrointestinal Tract

HDL (-c): High Density Lipoprotein (-cholesterol)

HPLC: High Performance Liquid Chromatography

IBD: Inflammatory Bowel Disease

IBS: Irritable Bowel Syndrome

IDF: International Diabetes Federation

IFG: Impaired Fasting Glucose

IGT: Impaired Glucose Tolerance

IL-6 : Interleukin 6

IR: Insulin Resistance

ISI: Insulin Sensitivity Index

LDL (-c): Low Density Lipoprotein (-cholesterol)

LFHCC: Low-Fat High-Complex Carbohydrate

LPL: Lipoprotein lipase

LPS: Lipopolysaccharide

Med: Mediterranean

MetS: Metabolic Syndrome

MGWAS: Metagenome-Wide Association Study

NAFLD: Non-Alcoholic Fatty Liver Disease

NCEP ATP III: National Cholesterol Education Programme Adult Treatment Panel III

NGS: Next Generation Sequencing

NHLBI: National Heart, Lung and Blood Institute

OGTT: Oral Glucose Tolerance Tes

OTU(s): Operational Taxonomic Unit(s)

PAI-1: Plasminogen activator inhibitor 1

PCoA: Principal Coordinate Analysis

PD: Phylogenetic Diversity

PRRs: Pattern-recognition receptors

QA/QC: Quality Assurance / Quality Control

QIIME: Quantitative Insight into Microbial Ecology

qPCR: Quantitative Polymerase Chain Reaction

RAS: Renin Angiotensin System

ROS: Reactive Oxygen Species

SBP: Systolic Blood Pressure

SCFA(s): Short Chain Fatty Acid(s)

T2D : Type 2 Diabetes

TC : Total Cholesterol

TG: Triglycerides

TLRs: Toll-like Receptor(s)

TMA: Trimethylamine

TMAO: Trimethylamine

TNF-α: Tumor Necrosis Factor Alpha

UC: Ulcerative Colitis

UPLC-MS/MS: Ultra-high Performance Liquid Chromatography –Tandem Mass Spectroscopy

VLDL: Very Low Density Lipoprotein

WC: Waist Circumference

WHO: World Health Organization

I. ABSTRACT

RESUMEN

I. ABSTRACT

Introduction:

The microbial community harbored in the human intestine, commonly known as the gut microbiota, is considered an organ fully integrated in the host which plays an important role in metabolism, physiology, nutrition and the immune function. The gut microbiota has coevolved with us and the changes in its composition and/or structure can have major consequences for human health and disease. At present, it is known that microbial imbalance or dysbiosis of the gut microbiota is associated with metabolic disorders such as Metabolic Syndrome, Obesity, Type 2 Diabetes and Cardiovascular Disease. In fact, some studies have suggested that changes in the intestinal microbiota may trigger pathogenic mechanisms that promote inflammation, insulin resistance and the development of Metabolic Syndrome. In this context, the relationship between the composition of the intestinal microbiota and human health has led to the design of strategies to promote the prevalence of beneficial bacteria that improve health status. Recent evidence has shown that significant alterations in the intestinal microbiota have been associated with alterations in diet, mainly influenced by the consumption of dietary fiber from fruit and vegetables. Studies are therefore needed to research into the possibility that diet-induced alterations in the intestinal microbiota contribute to an improvement in intestinal disorders and related diseases.

Hypothesis:

Our hypothesis is that the consumption of two healthy diets, Mediterranean diet and Low Fat diet, corrects the dysbiosis of the intestinal microbiota present in patients with Metabolic Syndrome, after a prolonged dietary intervention at least one year. On the other hand, the null hypothesis is that the long-term consumption of two healthy

diets, Mediterranean diet and Low Fat diet, not corrects the dysbiosis of the intestinal microbiota present in patients with Metabolic Syndrome.

Objectives:

To determine whether the Metabolic Syndrome is associated with a dysbiosis of the intestinal microbiota and if this dysbiosis can be modulated by the long-term consumption of two healthy diets, a Mediterranean diet and Low Fat diet. This main objective is divided in three main objectives, which corresponding to the three papers of this Thesis:

1. *Objective 1- Paper 1:* To evaluate the changes in the composition of the intestinal microbiota in Metabolic Syndrome patients compared with a non- Metabolic Syndrome people group and to test the effect of the long-term consumption of two healthy diets: a Mediterranean diet and a Low Fat diet, in restoring the gut microbiota composition analyzing the intestinal microbiota composition by real-time quantitative polymerase chain reaction.

2. *Objective 2- Paper 2:* To analyze the changes in the intestinal microbiota composition in an obese population after of the long-term consumption of a Mediterranean diet and a Low Fat diet by Next Generation Sequencing.

3. *Objective 3- Paper 3:* To identify differential signatures of the gut microbiota for obesity according to gender and changes in body mass index by Next Generation Sequencing.

Secondary objectives:

1. To characterize the dysbiosis of the intestinal microbiota associated with Metabolic Syndrome analyzing the functional capability to specie level, and to test the effect of the long-term consumption of a Mediterranean diet and a Low Fat diet.

2. To study the effect of the one year consumption of a healthy diet, Mediterranean diet and Low Fat diet, in the relationship between specific bacteria and the metabolome of the feces and plasma.

3. To evaluate the contribution of the intestinal microbiota composition to the individual variance in Body Mass Index and plasma lipid levels.

Subjects, design and methodology of the publications:

The current Thesis was conducted in patients within the CORDIOPREV study (Clinical Trials.gov.Identifier: NCT00924937), an ongoing prospective, randomized, opened and controlled trial in patients with coronary heart disease who had their last coronary event over 6 months before enrolling in the study. In CORDIOPREV study the participants were randomized to receive two dietary models, a Mediterranean diet and a Low Fat diet, over a period of 5 years, in addition to conventional treatment for coronary heart disease. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethics committees, following the Helsinki declaration and good clinical practice.

Paper 1: this work was conducted in 239 patients, who were divided in two groups: the first group consisting of 138 Metabolic Syndrome patients and the other group consisted of 101 subjects without Metabolic Syndrome. Half of patients in each group followed a Mediterranean diet and the other half followed a Low Fat diet. This publication was a longitudinal study; the biochemical determinations and the analysis of the intestinal microbiota were performed at baseline and after one year and two years of follow diet. The analysis of intestinal microbiota was carried out by real-time quantitative polymerase chain reaction using specific primers for 16S rRNA gene of different bacterial taxa.

Paper 2: this study was conducted in 20 obese patients (men), 10 patients followed a Mediterranean diet and 10 patients followed a Low Fat diet. This publication

Abstract

was a longitudinal study; the biochemical determinations and the analysis of the intestinal microbiota were performed at baseline and after one year of follow diet. The analysis of intestinal microbiota was carried out, by sequencing using the 454 Life Sciences (Roche) junior platform, according to standard 454 platform protocols. In additions, we have evaluated the bacterial composition and its relationship with the whole fecal and plasma metabolome.

Paper 3: this work is a transversal study, which was conducted in 75 patients, 39 men and 36 women, who were also divided into three groups, according to their body mass index: 13 men and 13women with body mass index <30; 13 men and 10 women with 30≤ body mass index ≤33; and 13 men and 13 women with body mass index >33. The biochemical determinations and the analysis of intestinal microbiota were performed at baseline. The intestinal microbiota was analyzed by sequencing of the variable region V4 of the microbial 16S rRNA gene in the MiSeq platform of Illumina.

Results:

Paper 1: we observed, at basal time, that the abundance of *Bacteroides, Eubacterium* and *Lactobacillus* genera was higher in the control group than in Metabolic Syndrome patients, while *Bacteroidesfragilis group, Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Bifidobacterium longum, Bifidobacterium adolescentis, Ruminococcus flavefaciens subgroup* and *Eubacterium rectale* are depleted in Metabolic Syndrome patients (all P values <0.05). Additionally, we found that the long-term consumption of a Mediterranean diet partially restored the population of *Parabacteroides distasonis, Bacteroides thetaiotaomicon, Faecalibacterium prausnitzii, Bifidobacterium adolescentis* and *Bifidobacterium longum* in Metabolic Syndrome patients (all P values <0.05) by real-time quantitative polymerase chain reaction.

Paper 2: our results by 454 sequencing in 20 obese patients showed that the Low Fat diet increased the genus *Prevotella* and decreased the genus *Roseburia*, while the Mediterranean diet decreased *Prevotella* and increased the *Roseburia* and *Oscillospira* genera (all P values<0.05). In addition, the relative abundance of *Parabacteroides distasonis* (P=0.025) and *Faecalibacterium prausnitzii* (P=0.020) increased after the long-term consumption of the Mediterranean diet and Low Fat diet, respectively.

Paper 3: we observed that the intestinal microbiota was influenced by gender and body mass index by the Illumina sequencing platform. We observed that the abundance of the *Bacteroides* genus was lower in men than in women (P<0.001) when body mass index was <33. In fact, the abundance of this genus decreased in men as body mass index increased (P<0.001), whereas in women it remained unchanged in the different ranges of body mass index. We also observed a higher presence of *Veillonella* and *Methanobrevibacter* genera in fecal samples from men compared to women (all P values <0.05) and the abundance of the genus *Bilophila* was lower in men than in women, regardless of body mass index (P=0.002).

Conclusions:

Metabolic Syndrome is related with a dysbiosis of the gut microbiota, which is characterized by a decrease in the relative abundance of bacterial taxa important for human health. In addition, the relative abundance of these taxa increases after long-term consumption of a healthy diet, restoring the dysbiosis in Metabolic Syndrome patients. This main conclusion is divided in three main conclusions, which corresponding to the three papers of this Thesis:

1. *Conclusion 1- Paper 1:* The consumption of the Mediterranean diet influenced the gut microbiota composition, mainly in Metabolic Syndrome patients. At baseline these patients had a dysbiosis of the intestinal microbiota compared to the non-

Abstract

Metabolic Syndrome patients, so that the consumption of Mediterranean diet restores the homeostasis of the gut microbiota, which is particularly important in conditions where the intestinal microbiota is altered, such as obesity and Metabolic Syndrome.

2. *Conclusion 2- Paper 2:* The long-term consumption of healthy diets, such as the Mediterranean diet and the Low Fat diet, exert a protective effect on the development of Type 2 Diabetes by different specific changes in the gut microbiota which increase the abundance of the *Roseburia* genus and *Faecalibacterium prausnitzii*, respectively.

3. *Conclusion 3- Paper 3:* The gut microbiota differs between men and women, and that these differences may be influenced by the grade of obesity.

Secondary conclusions:

1. Metabolic Syndrome patients have a functional dysbiosis characterized by a reduction of the relative abundance of bacterial taxa with an important anti-inflammatory capability and saccharolytic activity. In addition, the long-term consumption of the Mediterranean diet is more effective at increasing these bacterial taxa found with lower relative abundance in Metabolic Syndrome patients.

2. The changes in the gut microbiota after one year's consumption of both diets, Mediterranean diet and Low Fat diet, are linked to amino acid digestibility.

3. The gut microbiota has a significant contribution to the individual variance seen in Body Mass Index, Triglycerides, HDL-cholesterol, LDL-cholesterol and Total Cholesterol.

I. RESUMEN

Introducción:

La comunidad microbiana albergada en el intestino humano, conocida comúnmente como microbiota intestinal es considerada como un órgano completamente integrado en el huesped y juega un importante papel en el metabolismo, la fisiología, la nutrición y en el sistema inmune. La microbiota intestinal ha co-evolucionado con nosotros y los cambios en su composición y/o estructura pueden tener consecuencias importantes para la salud y la enfermedad humana. En la actualidad, se sabe que el desequilibrio microbiano o disbiosis de la microbiota intestinal es asociado con trastornos metabólicos, tales como Síndrome Metabólico, Obesidad, Diabetes tipo 2 y Enfermedad Cardiovascular. De hecho, algunos estudios han sugerido que los cambios en la microbiota intestinal pueden desencadenar los mecanismos patogénicos que promueven inflamación, resistencia a insulina y el desarrollo de Síndrome Metabólico. En este contexto, la relación entre la composición de la microbiota intestinal y la salud humana ha llevado al diseño de estrategias para promover la prevalencia de bacterias beneficiosas que mejoran el estado de salud. Evidencias recientes han demostrado que las alteraciones significativas en la microbiota intestinal se han asociado con alteraciones en la dieta, influenciadas principalmente por el consumo de fibra dietética de frutas y verduras. En este sentido, se necesitan estudios para investigar la posibilidad de que las alteraciones inducidas por la dieta en la microbiota intestinal contribuyen a la mejora de los trastornos intestinales y de las enfermedades relacionadas.

Hipótesis:

Nuestra hipótesis es que el consumo de dos dietas saludables, dieta Mediterránea y dieta Baja en Grasa, corrige la disbiosis de la microbiota intestinal en pacientes con Síndrome Metabólico, después de una intervención prolongada de al menos un año. Por otro lado, la hipótesis nula es que el consumo a largo plazo de dos dietas saludables

dieta Mediterránea y dieta Baja en Grasa, no corrige la disbiosis de la microbiota intestinal presente en pacientes con Síndrome Metabólico.

Objetivos:

Determinar si el Síndrome Metabólico está asociado con una disbiosis de la microbiota intestinal y si esta disbiosis puede ser modulada por el consumo a largo plazo de dos dietas saludables, una dieta Mediterránea y una dieta Baja en Grasa. Este objetivo principal se divide en tres objetivos principales, los cuales corresponden con los tres artículos de esta Tesis:

1. *Objetivo 1- Artículo 1:* Evaluar los cambios en la composición de la microbiota intestinal en pacientes con Síndrome Metabólico en comparación con un grupo de personas sin Síndrome Metabólico y examinar el efecto del consumo a largo plazo de dos dietas saludables: una dieta Mediterránea y una dieta Baja en Grasa, en la restauración de la composición de la microbiota intestinal, analizando la composición de la microbiota intestinal mediante reacción en cadena de la polimerasa cuantitativa a tiempo real.

2. *Objetivo 2- Artículo 2:* Analizar los cambios en la composición de la microbiota intestinal en una población obesa, después del consumo a largo plazo de una dieta Mediterránea y una dieta Baja en Grasa, mediante secuenciación de nueva generación.

3. *Objetivo 3- Artículo 3:* Identificar las características diferenciales de la microbiota intestinal en la obesidad, en función del género y los cambios en el índice de masa corporal, mediante secuenciación de nueva generación.

Objetivos secundarios:

1. Caracterizar la disbiosis de la microbiota intestinal asociada con el Síndrome Metabólico analizando la capacidad functional a nivel de especie, y evaluar el efecto del consume a largo plazo de una dieta Mediterránea y dieta Baja en Grasa.

2. Estudiar el efecto del consumo durante un año de una dieta saludable, dieta Mediterránea y dieta Baja en Grasa, en la relación entre bacterias específicas y el metaboloma de heces y plasma.

3. Evaluar la contribución de la composición de la microbiota intestinal en la varianza individual en el Índice de Masa Corporal y los niveles plasmáticos de lípidos.

Sujetos, diseño y metodología:

La presente Tesis se llevó a cabo en pacientes del estudio CORDIOPREV (Trials.gov.Identifier clínica: NCT00924937), un estudio prospectivo, aleatorizado, abierto y controlado en pacientes con enfermedad coronaria, los cuales tuvieron su último episodio coronario como mínimo 6 meses antes de inscribirse en el estudio. En el estudio CORDIOPREV los participantes fueron asignados al azar para recibir dos modelos dietéticos diferentes, una dieta Mediterrámea y una dieta Baja en Grasa, durante un período de 5 años, además del tratamiento convencional para la enfermedad coronaria. Todos los participantes dieron su consentimiento informado por escrito para participar en el estudio. El protocolo del ensayo y todas sus modificaciones fueron aprobados por el comité de ética local, siguiendo la declaración de Helsinki y las buenas prácticas clínicas.

Artículo 1: este trabajo se llevó a cabo en 239 pacientes, quienes fueron divididos en dos grupos: el primer grupo formado por 138 pacientes con Síndrome Metabólico y el otro grupo formado por 101 pacientes sin Síndrome Metabólico. La mitad de los pacientes de cada grupo siguieron una dieta Mediterránea y la otra mitad una dieta Baja en Grasa. Esta publicación fue un estudio longitudinal; las determinaciones bioquímicas y el análisis de la microbiota intestinal se realizaron al inicio del estudio y después de un año y dos años de seguimiento de la dieta. El análisis de la microbiota intestinal se llevó a cabo mediante reacción en cadena de la polimerasa

cuantitativa a tiempo real usando cebadores específicos para el gen 16S del ARN ribosómico de diferentes taxones bacterianos.

Artículo 2: este estudio se llevó a cabo en 20 pacientes obesos (varones), 10 pacientes siguieron una dieta Mediterránea y 10 pacientes siguieron una dieta Baja en Grasa. Este trabajo es un estudio longitudinal; las determinaciones bioquímicas y el análisis de la microbiota intestinal se realizaron al inicio del estudio y después de un año de seguimiento de la dieta. El análisis de la microbiota intestinal se llevó a cabo por secuenciación usando la plataforma 454 Life Sciences (Roche), de acuerdo con los protocolos estándares para dicha plataforma. Además, hemos evaluado la composición bacteriana y su relación con todo el metaboloma fecal y plasmático.

Artículo 3: este trabajo es un estudio transversal, el cual se llevó a cabo en 75 pacientes, 39 hombres y 36 mujeres, quienes fueron divididos a su vez en tres grupos, en función de su índice de masa corporal: 13 hombres y 13 mujeres con un índice de masa corporal <30; 13 hombres y 10 mujeres con un índice de masa corporal entre 30 y 33; y 13 hombres y 13 mujeres con un índice de masa corporal >33. Las determinaciones bioquímicas y el análisis de la microbiota intestinal se realizaron al inicio del estudio. La microbiota intestinal fue analizada por secuenciación de la región variable V4 del gen microbiano 16S del ARN ribosómico en la plataforma MiSeq de Illumina.

Resultados:

Artículo 1: hemos observado, al inicio del estudio, que la abundancia de los géneros *Bacteroides, Eubacterium* y *Lactobacillus* es mayor en el grupo control que en pacientes con Síndrome Metabólico, mientras que las bacterias del *grupo Bacteroides fragilis, Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Bifidobacterium longum, Bifidobacterium adolescentis, el subgrupo Ruminococcus flavefaciens* y *Eubacterium rectale* están

reducidas en pacientes con Síndrome Metabólico (todos los valores de P <0,05). Además, se encontró que el consumo a largo plazo de la dieta Mediterranea restaura parcialmente la población de *Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Bifidobacterium adolescentis* y *Bifidobacterium longum* en pacientes con Síndrome Metabólico (todos los valores de P <0,05) mediante reacción en cadena de la polimerasa cuantitativa a tiempo real.

Artículo 2: nuestros resultados por secuenciación 454 en 20 pacientes obesos mostraron que la dieta Baja en Grasa incrementó el género *Prevotella* y disminuyó el género *Roseburia* mientras que la dieta Mediterránea disminuyó el género *Prevotella* e incrementó los géneros *Roseburia y Oscillospira* (todos los valores de P<0,05). Además, la abundancia relativa de *Parabacteroides distasonis* (P=0,025) y *Faecalibacterium prausnitzii* (P=0,020) aumentaron tras el consumo a largo plazo de la dieta Mediterránea y la dieta Baja en Grasa, respectivamente.

Artículo 3: hemos observado que la microbiota intestinal está influenciada por el género y el índice de masa corporal, mediante la plataforma de secuenciación de Illumina. Observamos que la abundancia del género *Bacteroides* fue menor en los hombres que en las mujeres (P<0,001) cuando el índice de masa corporal era <33. De hecho, la abundancia de este género se redujo en los hombres con el aumento del índice de masa corporal (P<0,001), mientras que en las mujeres se mantuvo sin cambios en los diferentes rangos del índice de masa corporal. También se observó una mayor presencia de los géneros *Veillonella* y *Methanobrevibacter* en las muestras fecales de los hombres en comparación con las de las mujeres (todos los valores de p <0,05) y la abundancia del género *Bilophila* fue menor en los hombres que en las mujeres, independientemente del índice de masa corporal (P=0,002).

Resumen

Conclusiones:

El Síndrome Metabólico está asociado con una disbiosis de la microbiota intestinal, la cual se caracteriza por un descenso de la abundancia relativa de taxones bacterianos importantes para la salud humana. Además, la abundancia relativa de dichos taxones incrementa tras el consumo a largo plazo de una dieta saludable, restaurando la disbiosis en pacientes con Síndrome Metabólico. Esta conclusión general se divide en tres conclusiones, las cuales se corresponden con los tres artículos de esta Tesis:

1. *Conclusión 1- Artículo 1:* El consumo de la dieta Mediterránea influyó en la composición de la microbiota intestinal principalmente en los pacientes con Síndrome Metabólico. Al inicio del estudio estos pacientes presentaban una disbiosis de la microbiota intestinal en comparación con el grupo de personas sin Síndrome Metabólico, por lo que el consumo restaura la homeostasis de la microbiota intestinal, que es particularmente importante en condiciones de una alteración de la microbiota como la obesidad y Síndrome Metabólico.

2. *Conclusión 2- Artículo 2:* El consumo a largo plazo de una alimentación sana, tales como la dieta Mediterránea y la dieta Baja en Grasa, ejerce un efecto protector sobre el desarrollo de Diabetes tipo 2 por diferentes cambios específicos en la microbiota intestinal, aumentando la abundancia del género *Roseburia* y la especie *Faecalibacterium prausnitzii*, respectivamente.

3. *Conclusión 3- Artículo 3:* La microbiota intestinal difiere entre hombres y mujeres, y estas diferencias parecen estar influidos por el grado de obesidad.

Conclusiones secundarias:

1. Los pacientes con Síndrome Metabólico presenta una disbiosis functional caracterizada por una redución de la abundancia relativa de taxones bacterianos con una importante capacidad anti-inflamatoria y actividad sacarolítica. Además, el consumo a largo plazo de la dieta Mediterránea es más efectiva en el aumento de estos taxones

bacterianos encontrados con menor abundancia relativa en pacientes con Síndrome Metabólico.

2. Los cambios en la microbiota intestinal despues del consumo durante un año de ambas dietas, dieta Mediterránea y dieta Baja en Grasa, están vinculados a la digestibilidad de aminoácidos.

3. La microbiota intestinal tiene una contribución significativa a la varianza individual en el Índice de Masa Corporal, Triglicéridos, colesterol HDL, colesterol LDL y Colesterol Total.

II. INTRODUCTION

II. INTRODUCTION

1. METABOLIC SYNDROME

1.1 Definition and diagnostic criteria

Metabolic syndrome (MetS) is a cluster of physiological, biochemical, clinical, and metabolic factors that increase the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2D), which is itself a CVD risk factor [1, 2]. In addition to reducing the quality of life of patients, MetS has a significant economic impact on public health expenditure due to the morbidity generated, since the increased risk for developing T2D increases more than 30% the probability of CVD, which is currently the leading cause of mortality worldwide [3-5].

Various definitions have been given for MetS through the years. In 1988, Dr. Gerald Reaven, in his Banting Lecture, described the syndrome as a series of abnormalities including hypertension, diabetes mellitus and dyslipidemia, naming it "Syndrome X", where insulin resistance constituted the main factor or pathophysiological mechanism [6]. However, he did not include obesity or visceral obesity in the definition, and this was later added as a crucial abnormality.

From then on, several groups have attempted to develop diagnostic criteria for the diagnosis, prevention and treatment of the MetS [7]. At present, the most commonly used criteria for definition have been described by the World Health Organization (WHO) [8], the European Group for the study of Insulin Resistance (EGIR) [9], the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) [10], the American Association of Clinical Endocrinologists (AACE) [11] and the International Diabetes Federation (IDF) [12]. These are summarized in Table 1.

Introduction

Table 1 Diagnostic criteria of the metabolic syndrome considering its definition, according to World Health Organization (WHO), European Group for the study of Insulin Resistance (EGIR), National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), American Association of Clinical Endocrinologists (AACE), and International Diabetes Federation (IDF).

Clinical measures	**WHO** (1998) [8]	**EGIR** (1999) [9]	**ATP III** (2001) [10]	**AAEC** (2003) [11]	**IDF** (2005) [12]
Insulin resistance	Lowered insulin sensitivity[a] plus any 2 of the following	Plasma insulin >75th percentile plus any 2 of the following	None, but any 3 of the following 5 features	IGT or IFG plus any of the following based on the clinical judgment	None
Body weight	Waist-to-hip ratio: >0.90 in men, >0.85in women and/or BMI>30 kg/m^2	WC: ≥94 cm in men ≥80 cm in women	WC: ≥102 cm in men ≥88 cm in women	BMI ≥ 25 kg/m^2	Increased WC (population specific) plus any 2 of the following
Lipids	TG ≥150 mg/dL and/or HDL-C: <35 mg/dL in men <39 mg/dL in women	TG ≥150 mg/dL and/or HDL-C <39 mg/dL in men or women	TG ≥150 mg/dLand /or HDL-C: <40 mg/dL in men <50 mg/dL in women	TG ≥150 mg/dL and HDL-C: <40 mg/dL in men <50 mg/dL in women	TG ≥150 mg/dL or on TGs Rx. HDL-C: <40 mg/dL in men <50 mg/dL in women or on HDL-C Rx
Blood pressure	≥140/90 mm Hg	≥140/90 mm Hg or on hypertension Rx	≥130/85mm Hg	≥130/85 mm Hg	≥130 mm Hg systolic ≥85 mm Hg diastolic or on hypertension Rx
Glucose	IGT, IFG, or T2D	IGT or IFG (not diabetes)	>110 mg/dL (includes diabetes)[b]	IGT or IFG (not diabetes)	≥100 mg/dL (includes diabetes)[b]
Other	Microalbuminuria			Other features of insulin resistance[c]	

[a]Insulin sensitivity measured under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation. [b]In 2003, the American Diabetes Association (ADA) changed the criteria for IFG tolerance from >110 mg/dl to >100 mg/dl [13]. [c]Includes family history of type 2 diabetes mellitus, polycystic ovary syndrome, sedentary lifestyle, advancing age, and ethnic groups susceptible to type 2 diabetes mellitus. BMI: body mass index; HDL-C: high density lipoprotein cholesterol; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; Rx: receiving treatment; TG: triglycerides; T2D: type 2 diabetes; WC: waist circumference.

However, the fact that different definitions are used for the diagnosis, in which the components or criteria are not the same, could lead to a variation in measuring the prevalence of MetS in the same population, depending on the definition chosen [14, 15]. In an effort to provide greater consistency in both clinical care and research into patients with MetS, the IDF, the American Heart Association (AHA), the National Heart, Lung and Blood Institute (NHLBI), the World Heart Federation and the International Association for the Study of Obesity published a joint statement in 2009 that provided a "harmonized" definition of MetS. This unification was published in the article entitled "Harmonizing the Metabolic Syndrome" [15] and is summarized in Table 2. The purpose of this consensus on the diagnosis of MetS was to obtain a useful, practical tool for assessing CVD risk and diabetes, while taking into consideration the study population and geographic region.

Table 2 Criteria for Clinical Diagnosis of the Metabolic Syndrome according to the current Harmonization definition.

Diagnostic criteria	**Harmonizing the Metabolic Syndrome** [15]
Waist circumference	**Elevated Waist circumference:** Population- and country-specific definitions
High triglycerides	**>150 mg/dL** or treatment with specific lipid-lowering drugs
Reduced HDL-C	**<40 mg/dL in men or <50 mg/dL in women** or treatment effect on HDL-C
High blood pressure	**SBP ≥130 mmHg and/or DBP ≥85 mmHg** or antihypertensive treatment
High fasting glucose	**Fasting glucose ≥100 mg/dL** or treatment for high blood sugar
Diagnosis	3 of the 5 components proposed

Abbreviations: HDL-C, high-density lipoprotein cholesterol; mg/dL, milligrams per deciliter; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; mmHg, milligrams of mercury.

At present, most of the publications worldwide are based on the ATP III criteria. According to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel III [16] (NCEP-ATP III) in the European population [15], a patient is considered as having metabolic syndrome when they meet at least 3 of the following diagnostic criteria:

- **Abdominal obesity**

Abdominal obesity is defined by the increase in waist circumference, and also represents a marker of insulin resistance and dysfunctional adipose tissue itself [17]. According to ATP III criteria, men are considered as having abdominal obesity when the waist circumference is equal to or greater than 102 cm and in women when it is equal to or greater than 88 cm [15, 16].

The pathophysiological consequences of abdominal obesity are due to the fact that the adipose tissue has expanded and the adipocytes become dysfunctional, which causes changes in the secretion of adipokines and cytokines. This results in a localized inflammation in adipose tissue that propagates an overall systemic inflammation associated with the development of obesity-related comorbidities such as insulin resistance and MetS [18-20].

- **Dyslipidemia**

Dyslipidemia in the MetS is characterized by the concurrent presence of both qualitative and quantitative lipoprotein abnormalities: low levels of high density lipoprotein cholesterol (HDL-C) (<50 mg/dl in women, <40 mg/dl in men) and an increase in triglycerides (TG >150 mg/dl) [21-25]. A typical feature of obesity, the metabolic syndrome, insulin resistance, and type 2 diabetes, atherogenic dyslipidemia has emerged as an important risk factor for myocardial infarction and CVD [15, 16, 26, 27].

- **Arterial hypertension**

According to criteria ATPIII for the diagnosis of MetS, a patient is considered as having hypertension when their systolic and diastolic blood pressure is greater than 130/85 mmHg respectively. Hypertension is a serious condition as it leads to a higher risk for CVD. If left untreated, hypertension can lead to atherosclerosis and increase the probability of suffering a cardiovascular event.

The walls of arteries have a layer of muscle and elastic tissue that makes them flexible and able to dilate and constrict as blood flows through them. High blood pressure can make the artery walls thicken and harden (atherosclerosis). When the artery walls thicken, the inside of the blood vessel narrows. Cholesterol and fats are more likely to build up on the walls of damaged arteries, making them even narrower. Blood clots can also get trapped in narrowed arteries, blocking the flow of blood.

Arteries narrowed by arteriosclerosis may not deliver enough blood to organs and other tissues, and reduced or blocked blood flow to the heart can cause a heart attack [28].

Most studies agree that subjects with hypertension have an abnormal metabolism and/or dislipemia more often than healthy subjects [28]. Classical studies have shown that hypertensive patients have abnormal curves of glucose overload and hyperinsulinemia. Even Reaven argues that hypertension itself is a manifestation of insulin resistance [28]. For this reason, patients with hypertension have a high prevalence of MetS [29, 30].

- **Insulin resistance**

A fasting glucose measurement of ≥100 mg/dL is usually an indicator of insulin resistance, and is frequently accompanied by other MetS components. In addition, for a percentage of those with altered fasting glucose, it is a risk factor or predictive for type 2 diabetes development [16]. Although the ATP III panel did not consider the

determination of insulin resistance a criterion for the diagnosis of MetS, they are usually accepted as a feature of this syndrome. Moreover, clinical trials show that modification of the three characteristic components of MetS, namely atherogenic dyslipidemia, hypertension and prothrombotic state, reduces the risk of cardiovascular disease [31].

1.2. Epidemiology

MetS is a public health problem closely linked to the Western lifestyle. The main risk factors of mortality in the world coincide with the components of the MetS [31]. The metabolic alterations occur simultaneously more frequently than would be expected by chance and the concurrence of several factors increases cardiovascular risk over and above the risk associated with the individual factors alone [32]. The risk increases with the number of MetS components present in an individual [33].

The prevalence of MetS depends on the definitions used as well as the population being studied [34]. Multiple studies have examined the prevalence of MetS using different criteria, study design, the age, gender, ethnicity and environment of the population being studied and obesity prevalence of the background population, so it is difficult to work from unified values [7]. In general, the IDF estimates that one-quarter of the world's adult population has MetS [12]. Higher socioeconomic status, a sedentary lifestyle and high BMI were significantly associated with MetS and, for this reason, the prevalence of MetS is high and is on the rise in many western societies [35]. Cameron et al. have concluded that the differences in genetic background, diet, levels of physical activity, smoking, family history of diabetes and education all influence the prevalence of the MetS and its components [36]. The prevalence of MetS increases with age (10% in individuals aged 20–29, 20% in individuals aged 40–49, and 45% in individuals aged 60–69) [34, 36, 37]. In addition, urban populations have higher rates of MetS than rural populations [14, 38] and recent studies demonstrate rising rates of MetS in many developing countries [39, 40].

In Spain, the existing data show similar results found in international studies. Sholze et al. report that in Spain, 22% of the hypertensive population has sufficient criteria for the diagnosis of MetS and this percentage is likely to rise steadily [41]. Furthermore, it was concluded that all the components of the MetS are significantly

more prevalent in men, except the low values of HDL-c, which is more prevalent in women, and that the prevalence of MetS increases in parallel with age; below the age of 60 it is more prevalent in males, whereas no difference is observed above this age [42].

Further studies are needed to determine the prevalence of MetS in the Spanish population, as it is highly relevant epidemiological data, directly associating the increased prevalence of MetS with increased CVD [43, 44].

1.3. Pathophysiology

MetS is a consequence of the complex interplay between genetic and environmental factors [43, 44]. Although the pathophysiology is largely unknown, obesity (especially abdominal or visceral obesity) and insulin resistance seem to be the main etiological factors of this syndrome, although other factors, such as dyslipidemia, hypertension, genetic susceptibility, oxidative stress and lifestyle (such as diet and physical activity) are all important elements in the pathophysiology of MetS.

1.3.1. Abdominal obesity

Obesity plays a central and causal role in metabolic syndrome [45-47]. In fact, the increased prevalence of obesity is related to an increased prevalence of MetS [48-49]. Adipose tissue (AT) is composed of adipocytes, stromal preadipocytes, immune cells and vascular endothelium. This tissue plays a major role in the energy homeostasis of the whole body, since it is able to respond rapidly and dynamically to the excess of nutrients through hypertrophy and hyperplasia of the adipocites to store more fat, a phenomenon which is known as expansion of adipose tissue [50].

However, this capacity for expansion is limited and is conditioned by genetic and environmental factors of the individual. When the adipose tissue cannot expand more, it is unable to store more fat. In this situation, the fat is deposited in an ectopic way in other organs, such as, liver, muscle and visceral adipose tissue. This ectopic fat causes the harmful metabolic consequences which are features of obesity [50], amongst which is insulin resistance. However, not all AT expansion is necessarily associated with pathological changes.

The concept of the “metabolically healthy obese” state [51] suggests that some individuals can preserve systemic insulin sensitivity on the basis of “healthy” AT

expansion, bypassing all of the pathological consequences associated with obesity [52, 53].

"Healthy" AT expansion consists of an enlargement of AT through effective recruitment of adipogenic precursor cells that are differentiated into small adipocytes, along with the recruitment of other stromal cell types with appropriate ratios. This is all linked to an adequate angiogenic response, minimal induction of extracellular matrix and minimal inflammation [53]. This process is illustrated in Figure 1.

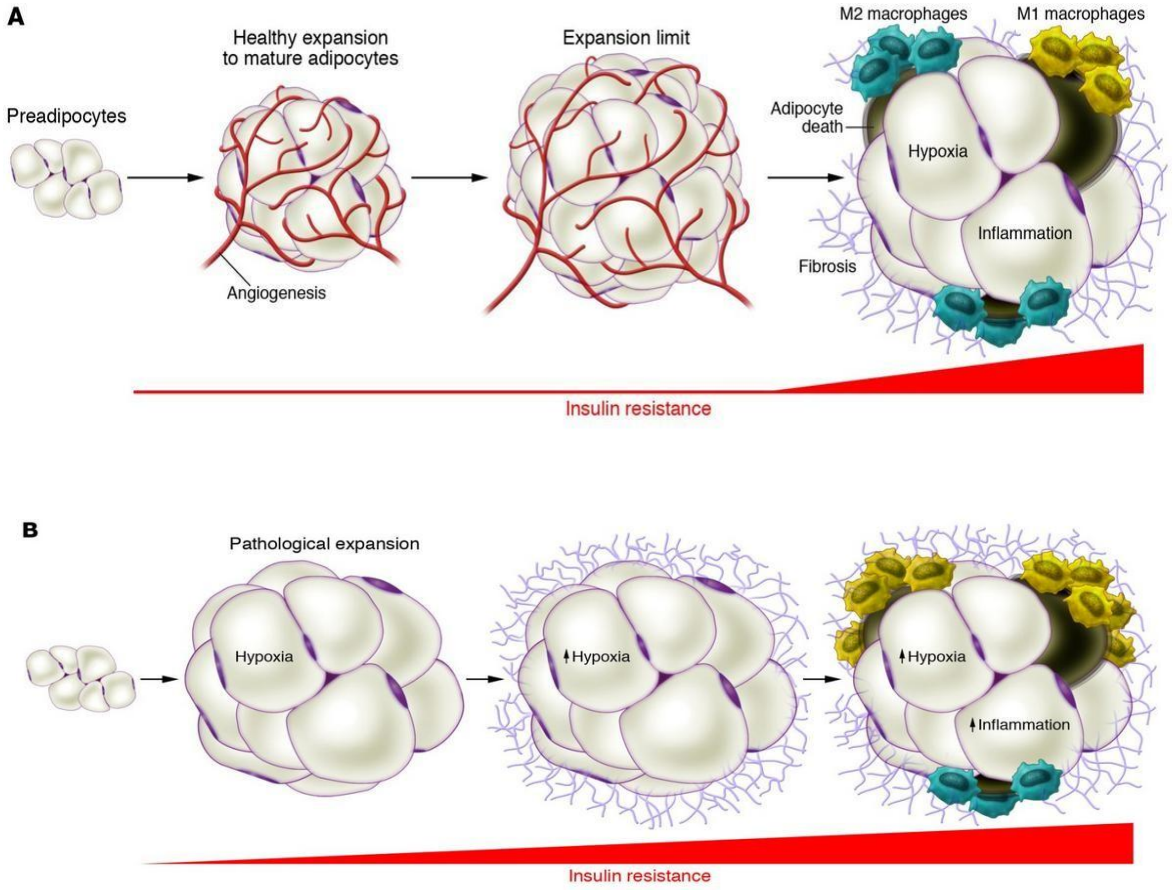

Figure 1 Healthy and unhealthy adipose tissue expansion [53].

In a pathological expansion of AT in obesity, a massive enlargement of the existing adipocytes is produced and the blood supply to the adipocytes may be reduced, with consequent hypoxia [54]. Hypoxia has been suggested as a trigger of necrosis and

the infiltration of macrophages into adipose tissue. Several studies have highlighted the infiltration of macrophages into expanding AT as an important physiological phenomenon [55], while firmly establishing the phenomenon of a macrophage-orchestrated inflammatory response leading to an inflammatory phenotype that is strongly associated with systemic insulin resistance [53]. This process is illustrated in Figure 1.

In this condition, there is an overproduction of biologically active metabolites known as adipocytokines, which include glycerol, free fatty acids (FFA), pro-inflammatory mediators (tumor necrosis factor alpha (TNFα),interleukin-6 (IL-6)), plasminogen activator inhibitor-1 (PAI-1) and C-reactive protein (CRP) [56]. This results in a localized inflammation in adipose tissue that propagates systemic inflammation associated with the development of obesity-related comorbidities [57]. Adipocytokines integrate the endocrine, autocrine and paracrine signals to mediate the multiple processes including insulin sensitivity [58]. It has been demonstrated that individuals with MetS have an abnormal adipokine profile that affects insulin sensitivity [19]. However, this infiltration capacity of adipose tissue is reversible, as has been observed in weight loss regimes that resulted in a reduction in inflammatory markers [59, 60].

1.3.2. Insulin resistance

Decreased insulin sensitivity is also a key factor in the development of MetS [61]. Some researchers give more priority to insulin resistance (IR) than to obesity in the pathogenesis of MetS; however, it is very difficult to separate these disorders and identify a unique role of IR [61, 62]. IR is defined as a pathophysiological condition in which a normal insulin concentration does not produce enough normal insulin response in the peripheral target tissues such as adipose tissue, muscle, and liver. The decline in

the tissue response to the action of insulin produces an increase in plasma insulin levels, a condition which is called hyperinsulinemia [62]. Although hyperinsulinemia may compensate for insulin resistance to some biological actions of insulin, e.g. maintenance of normoglycemia, it may, however, cause an over-expression of insulin activity in some normally sensitive tissues. This accentuation of some of the effects of insulin, coupled with a resistance to others, results in the clinical manifestations of MetS [62] as a alteration in the glucose metabolism, leading finally to hyperglycemia, due to the inability of the pancreatic beta cells to produce sufficient insulin to maintain normal glucose levels (normoglycemia)[63]. Furthermore, this insulin resistance is associated with low-grade inflammatory status and increased oxidative stress, both of which are characteristics of MetS [64].

1.3.3. Atherogenic dyslipidemia

Atherogenic dyslipidemiais characterized by increased levels of low density lipoproteins (LDL), decreased small dense high density lipoprotein (HDL) and increased blood TG levels [25, 65].

The normal lipid metabolism includes FFAs, which are released from adipocytes to the circulating blood in the liver and muscle. In the liver, some of these FFAs are oxidized and the rest are reesterified to TG. There is a continuous flow of FFAs between adipose tissue and liver; however, if the process of esterification is saturated, TG accumulation can cause fatty liver.

Insulin resistance leads to abnormal lipid profiles [66] and atherogenic dyslipidemia in several ways. First, insulin normally suppresses lipolysis in adipocytes, so an impaired insulin signalling increases lipolysis, resulting in increased FFA levels. In the liver, FFAs serve as a substrate for the synthesis of TG. FFAs also stabilize the production of apoB, the main lipoprotein in very low density lipoprotein (VLDL)

particles, resulting in greater VLDL production [67]. Second, insulin normally degrades apolipoprotein B (ApoB) through PI3K-dependent pathways, so insulin resistance directly increases VLDL production. Third, insulin regulates the activity of lipoprotein lipase, the main rate-limiting mediator of VLDL clearance. Thus, hypertriglyceridemia in insulin resistance is the result of both an increase in VLDL production and a decrease in VLDL clearance. VLDL is metabolized to remnant lipoproteins and small dense LDL, both of which can cause the formation of atheroma. The TGs in VLDL are transferred to HDL by cholesteryl ester transport protein (CETP) in exchange for cholesteryl esters, resulting in the TG-enriched HDL and cholesteryl ester-enriched VLDL particles [68]. Further, the TG-enriched HDL is a better substrate for hepatic lipase, so it is cleared rapidly from the circulation, leaving fewer HDL particles to participate in reverse cholesterol transport from the vasculature.

Thus, in the liver of insulin-resistant patients, FFA flux is high, TG synthesis and storage are increased and excess TG is secreted as VLDL [68]. Taken together, the dyslipidemic profile characterizing MetS, which includes high TG and low HDL levels, is related to insulin resistance and CVD.

1.3.4. Hypertension

Hypertension, like other components of MetS, is closely related to insulin resistance, since insulin resistance and/or hyperinsulinemia are present in the majority of hypertensive patients [69, 70]. Several studies suggest that both hyperglycemia and hyperinsulinemia activate the Renin Angiotensin System (RAS). This may contribute to the development of hypertension in patients with insulin resistance [71].

Furthermore, Insulin stimulates the sympathetic nervous system, causes renal retention of Na + and directly modifies vascular mechanisms (contracting and relaxing); in addition, insulin may influence the increase or decrease of blood pressure. When

renal sodium excretion is reduced, this results in expansion of extracellular volume and blood volume, which are factors responsible for the heart increasing cardiac output, and the arteries respond with vasoconstriction, resulting in hypertension [72-74].

1.3.5. Genetic susceptibility

Some people are genetically predisposed to developing metabolic disorders, and in these people, the factors acquired by the environment, such as physical inactivity and excess body fat, increase the probability of developing IR and MetS [16]. It is likely that each metabolic risk factor is genetically regulated independently and its own genetic regulation is influenced by environmental factors and conditions. For example, a variety of polymorphisms in genes affecting lipoprotein metabolism are associated with worsening dyslipidemia among obese people [75]. Similarly, a genetic predisposition to defective insulin secretion when combined with insulin resistance can raise plasma glucose to abnormal levels [76].

The thrifty genotype hypothesis proposed by Neel in 1962 [77], explains the mechanism by which a rapid, massive release of insulin after an abundant meal is able to reduce hyperglycemia and glucosuria, thereby allowing greater energy deposits. Those individuals capable of storing more energy were therefore better prepared to survive in times of scarcity. In this way, individuals carrying these "saver" genes had selective advantages for adaptation, which were in turn transmitted to their offspring. Currently, however, since genes do not change rapidly, these "saver" genes represent a disadvantage and can lead to chronic metabolic diseases such as T2D, abdominal obesity and cardiovascular diseases, amongst others, especially since, nowadays, there is an excessive, continuous availability of all types of food [64, 78].

Another thrifty phenotype hypothesis was introduced by Hales and Barker in 1992 [79]. This hypothesis proposes that if a fetus grows in conditions of malnutrition, it

develops adaptations that produce structural, physiological and metabolic changes to maximize opportunities for postnatal survival in conditions of food shortages. If this individual receives a normal or excessive feed in the postnatal period, such adjustments are detrimental to the individual's health throughout their life, predisposing them to changes in glucose/insulin metabolism and later developing type 2 diabetes and Metabolic syndrome in adulthood [80].

1.3.6. Oxidative stress

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and their disposal defense mechanisms, known as antioxidant [81] systems. The consequences of oxidative stress include cell damage, which leads to damage to DNA, lipids, proteins, carbohydrates, and so on. Oxidative damage, particularly in DNA, may trigger cell death by apoptosis or necrosis.

In addition, oxidative stress is associated with numerous pathological mechanisms, such as atherosclerosis, T2D, MetS, inflammatory diseases, cancer, as well as with the aging process [81, 82]. In these pathophysiological processes, the levels of ROS increase [83, 84]. In models of obese-diabetic and obese non-diabetic mice, antioxidant activity was low, possibly due to the obesity per se [83].

Several markers of oxidative damage, such as Malonaldheido, Isoprostanes F-2 and CRP, are increased in obese people and are directly correlated with BMI and body fat percentage, the oxidation of LDL and TG levels [85].

Currently, much attention is focused on excess abdominal adiposity, which is one of the most important components of the MetS [16]. Indeed, Furukawa et al., pointed to accumulated fat as a major pathogenic mechanism of obesity associated with MetS, whose scheme is explained in Figure 2 [83]. In this hypothesis, Furukawa proposes that increased oxidative stress generated in the accumulated fat is a consequence of obesity

per se; due to the increase of Nicotinamide Adenine Dinucleotide Phosphate-oxidase (NADPH oxidase) and decreasing antioxidants, this consequently causes altered adipokine production, which leads to systemic oxidative stress that damages other organs such as the liver, skeletal muscle and aorta, triggering the development of MetS [83].

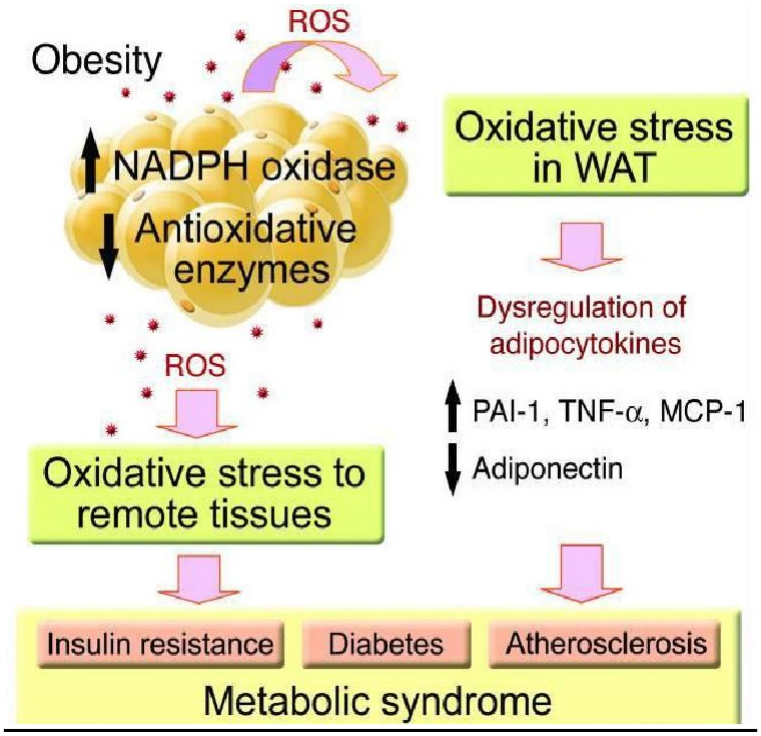

Figure 2 Furukawa's hypothesis about the role of ROS in the MetS [83].

1.3.7. Lifestyle

In the current era, changes that have occurred in our working habits, transportation, leisure, food and economic development promote a sedentary lifestyle, overweight, obesity and MetS. It is vitally important to develop health strategies to prevent the emerging global epidemic of MetS. Furthermore, the risk of cardiovascular disease and diabetes as a result of MetS should be reduced with changes in lifestyle, such as increased physical activity or weight loss [86, 87].

Among the factors contributing to the development of MetS, dietary habits play a major role. There are several dietary components that have been linked to the risk of

MetS, in particular the amount and type of dietary fat [60]. In 2006, the AHA published their "Recommendations on Diet and Lifestyle" as a guide to reduce the risk of MetS [88], which is based on the consumption of a diet low in fat and high in complex carbohydrates; these recommendations differ slightly to the Med diet, with respect to the recommended amount of fat, but they are similar in other features. The main contribution of fat in the Mediterranean diet comes from olive oil, which is the source of monounsaturated fatty acids and phenolic compounds par excellence [89, 90]. The consumption of olive oil improves the characteristic parameters of MetS, as well as reducing triglyceride levels and increasing HDL-c concentrations, thus attributing to olive oil cardioprotective effects [89-92].

The consumption of saturated fatty acids is a risk factor for the development of MetS [93, 94]. Furthermore, evidence shows that the consumption of diets rich in monounsaturated fat and carbohydrates improves insulin sensitivity, while the consumption of saturated fatty acids induces a deterioration [95, 96].

Replacing saturated fat by a diet rich in carbohydrates has been considered, but this depends on the type of carbohydrates and their glycemic index. In particular, refined carbohydrates excessively increase atherogenic dyslipidemia in parallel with insulin resistance and obesity [94]. Moreover, a diet rich in complex carbohydrates, such as rice, with its high, resistant starch content, is associated with improvement in the endothelial function, reduced postprandial glucose and oxidative stress in patients with impaired fasting glucose, impaired glucose tolerance or newly diagnosed T2D [97]. Therefore, changing dietary patterns would be an effective strategy for the prevention and treatment of metabolic syndrome.

In the treatment of MetS, physical activity plays a pivotal role. Several studies have described that cardiorespiratory exercise is able to modulate the relationship between MetS and cardiovascular events, providing a strong protective effect against

cardiovascular mortality [98, 99]. The amount and intensity of physical exercise regulate the circulating levels of lipids and other metabolic abnormalities responsible for cardiovascular risk [100]. In addition, when physical activity is combined with diet, the healthy effect is greater compared with studies where either approach is practiced alone [101, 102]. To sum up, lifestyle modification programs are necessary to maintain metabolic changes over a long period of time, and such programs should always address both nutritional treatment and physical activity [103, 104].

2. INTESTINAL MICROBIOTA

The gastrointestinal tract (GT) is the natural habitat of a large, diverse and dynamic population of microorganisms, which mainly consists of bacteria adapted to life on mucosal surfaces or in the intestinal lumen [105-107]. The term "microbiota" refers to the community of living microorganisms gathered in a particular ecological niche. The adult human gut comprises between 10 to 100 trillion microorganisms, which is equivalent to ten times the number of our total somatic and germ cells [108] and represents around 1 - 2 kg of our body weight [109]. The human gut microbiota is constituted mainly of bacteria, but there are also fungi, viruses and eukarya (or protozoa). The microbial ecosystem of the gastrointestinal tract includes native species which permanently colonize the gastrointestinal tract and a number of microorganisms that temporarily pass through [105]. Native bacteria are acquired at birth and during the first year of life, while bacteria in transit are continuously ingested through food, beverages, and so on.

Furthermore, the collective genomes of gut microbiota (microbiome) contain 100 to 150 times more genes than our own genome [110], so the intestinal microbiota is currently considered as a metabolically adaptable, flexible and rapidly renewable organ which is fully integrated in the host metabolism. This ecosystem plays an important role in obtaining energy from diet and, being fully integrated in the metabolism of the host, plays a key role in human health [111].

2.1. Composition and distribution of the intestinal microbiota.

The diversity of microorganisms varies along the GT [112] and depends on the morphological and physiological characteristics of each part of the digestive system. As we move through the GT, the quantity and diversity of microorganisms increases. Between the distal esophagus and the rectum, there is a marked difference in the diversity and number of bacteria, ranging from 10 per gram of contents in the esophagus

and stomach to 10^{12} per gram of contents in the colon and distal gut In the esophagus, the commonest genera are *Prevotella (Phylum Bacteroidetes), Streptococcus* and *Veillonela (Phylum Firmicutes)* [114]. In the stomach, despite its acidic pH, a diverse community was found through analysis of 16S rDNA in 2006, consisting of 128 phylotypes mainly belonging to the following phyla: *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Fusobacteria* [115] and the following genus*: Lactobacillus, Veillonella* (phylum *Firmicutes) and Helicobacter* (phylum *Proteobacteria)* [113].

On the other hand, the distribution of microorganisms in the small intestine varies along its length, increasing from 10^4 colony-forming units (CFU) per gram of intestinal content in the proximal duodenum to 10^7 CFU/g of intestinal content in the terminal ileum [116]. The first studies that analyzed the duodenal microbiota found a widely dispersed population in which Gram-positive microorganisms were predominant, particularly species of *Lactobacillus* and *Streptococcus* genera belonging to the phylum *Firmicutes* [117-119]. However, species of the genera *Bacteroides* (phylum *Bacteroidetes), Bifidobacterium* (phylum *Actinobacteria), Veillonella* and *Staphylococcus* (phylum *Firmicutes)* [120] have also been isolated in lesser quantities.

The number of bacteria along the jejunum and ileum also increases progressively with a predominance of aerobic Gram-negative microorganisms and certain obligate anaerobes [121]. Jejunal biopsies determined the frequent presence of the phylum *Proteobacteria* and *Bacillus-Lactobacillus-Streptococcus* group (mostly the last) [122]. Biopsies from the ileum revealed the presence of *Bacteroidetes* and *Clostridium* (XIVa and clusters of XI) as the dominant species [123].

The large intestine is where the highest concentration of bacteria in the GT is located, with about 10^{11} CFU/g of intestinal content. The large intestine accounts for over 70% of all the microbes found in the body, which is due to the fact that the transit

time in this region is much slower, which favors the fermentation of available substrates from the diet or endogenous secretions, thus allowing more microorganisms to grow [121-124]. In addition, most bacteria in the colon are strict anaerobes, due to the reducing environment in this part of the intestine. In a healthy colonic microbiota, the most predominant phyla are *Firmicutes* and *Bacteroidetes*, whereas the phylum *Proteobacteria* is less abundant. At the genera level, *Bacteroides*, *Prevotella* (phylum *Bacteroidetes)* and *Ruminococcus* (phylum *Firmicutes)* are the most abundant [125], followed by the genera *Bifidobacterium* (phylum *Actinobacteria), Eubacterium* and *Peptostreptococcus* (phylum *Firmicutes)* [126]. In addition, the genus *Clostridium* is well represented, and in lesser proportions, there are populations of facultative anaerobic or aerotolerant bacteria such as *Enterococcus*, *Lactobacillus* and *Streptococcus*, which are essential for microbial homeostasis in the large intestine, all belonging to the phylum *Firmicutes* [120, 127, 128].

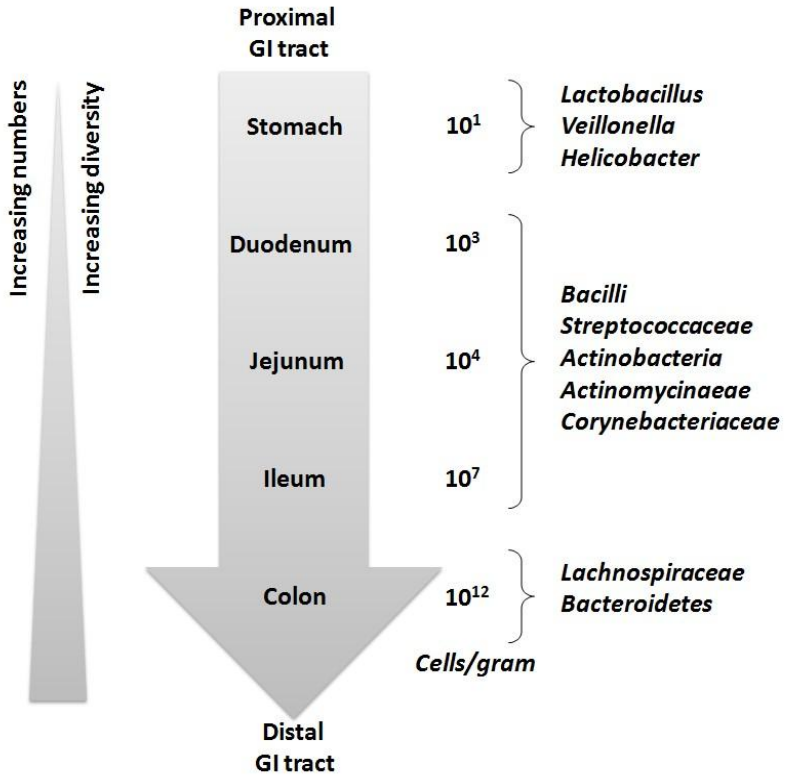

Figure 3 Composition and distribution of the gut microbiota over the whole length of the gastrointestinal tract. Adapted from Sekirov, I., et al. 2010 [113].

Not only there are differences in diversity of the GT, but there are also from the lumen to the mucosal surface of the intestine [113]. While *Bacteroides* (phylum *Bacteroidetes),* *Bifidobacterium* (phylum *Actinobacteria),* *Streptococcus*, *Enterobacteriacae*, *Enterococcus*, *Clostridium*, *Lactobacillus* and *Ruminococcus* (phylum *Firmicutes)* are the predominant luminal microbial genera, the only predominant mucosa and mucus associated genera are *Clostridium*, *Lactobacillus*, *Enterococcus* (phylum *Firmicutes)* and *Akkermansia* (phylum *Verrucomicrobia)* [129].

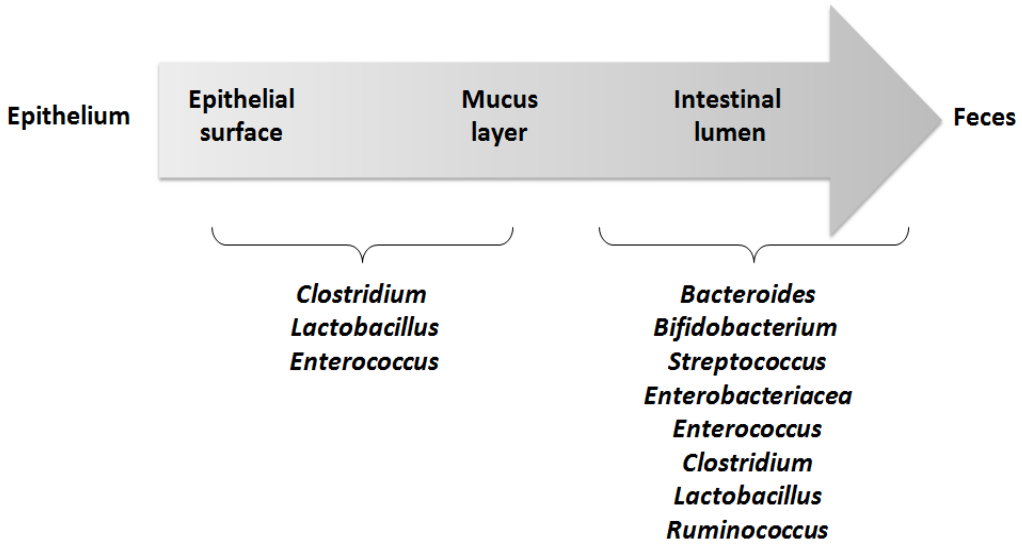

Figure 4 Longitudinal variations in the composition of the gut microbiota in the intestine. Adapted from Sekirov, I., et al. 2010 [113].

2.2. Functions of the intestinal microbiota

The gut microbiota maintains a symbiotic relationship with the gut mucosa and in a healthy individual it has mainly metabolic, trophic, protective and immune functions [130].

2.2.1. Metabolic functions

The metabolic function of the intestinal microbiota is essential for the host, since the microbial metabolism is involved in converting many substances into nutrients that

can be absorbed and utilized by the body, so the gut microbiota play an important role in energy regulation[131].

In the colon, the microbiota ferments carbohydrates of vegetable origin which cannot be digested by the digestive enzymes of mammals and escape proximal digestion. This fermentation is carried out by bacteria that mainly belong to the following genera: *Bacteroides, Roseburia, Bifidobacterium Facalibacterium* and *Enterobacteria.* The result is the production of short-chain fatty acids (SCFA), such as butyrate, propionate and acetate [132], which represent the main source of energy for colonocytes [133].

Butyric acid is, for the most part, metabolized in the intestinal epithelium, constituting the main energy source for the colonic epithelium [134]. In addition, several studies have also demonstrated that butyrate can act at the systemic level to exert anti-obesity and anti-inflammatory effects [135]. Moreover, acetic and propionic acids access the portal circulation and are involved in the metabolism of cholesterol and lipids [132]. Propionic acid decreases the expression of lipogenic enzymes in the liver, which is involved in the *de novo* synthesis of triglycerides and fatty acids, as well as reduced cholesterol levels [136]. In contrast, acetic acid reduces the serum levels of fatty acids, but increases cholesterol levels [137].

Short-chain fatty acids also control gene expression through the inhibition of histone deacetylase enzymes by butyric acid and metabolic regulation through the G protein coupled receptors GPR43 and GPR41 (also known as FFA2 and FFA3, respectively). Acetate and propionate are the most potent activators of GPR43, while GPR41 is activated in the following order of affinity: propionate> butyrate> acetate. The activation of GPR43 by SCFAs in neutrophils leads to the suppression of inflammation, while its activation in the L cells of the small intestine and colon stimulates the secretion of glucagon-like peptide 1 (GLP-1). In addition, the gut

microbiota also activates GPR41 through the production of SCFA. When this activation occurs in L cells of the intestine, it induces the secretion of peptide YY. Both GLP-1 and peptide YY decrease appetite and energy intake. Additionally, butyrate and propionate induce the expression of leptin in the adipose tissue, and so also regulate appetite [138]. Short chain fatty acids also promote the absorption of minerals (such as calcium, magnesium and iron) which is clinically relevant in the treatment and prevention of certain diseases like osteoporosis and anemia [137].

The intestinal microbiota also plays a very important role in the metabolism of bile acids. The primary bile acids (cholic and chenodeoxycholic) are synthesized in the liver and are conjugated with glycine. These conjugated bile acids are captured in the distal ileum and are used by bacteria which have the capacity to deconjugate the primary bile acids and convert them into secondary bile acids (deoxycholic and lithocholic), which allows them to escape from the intestinal uptake [139]. The secondary bile acids bind to the G protein-coupled receptor, the TGR5 receptor, in intestinal L-cells and induces the secretion of glucagon-like peptide 1 that increases insulin. Meanwhile, TGR5 receptor activation in muscle and adipose tissue increases energy expenditure [138].

The metabolic functions of the intestinal microbiota also include the production of vitamins (vitamin K, B12, biotin, folate and pantothenic) and amino acid synthesis from ammonia or urea. The anaerobic metabolism of peptides and proteins (a process known as putrefaction) occurs in more distal segments of the colon, and is also a source of SCFAs, but generates a series of potentially toxic substances including ammonia, amines, phenols thiols and indoles [140-142].

2.2.2. Trophic functions

The intestinal microbiota seems to play a highly important role in maintaining the proliferation and differentiation of intestinal cells, which are required to preserve the

structure of the intestine [141, 143, 144]. The short chain fatty acids produced by the intestinal microbiota have a trophic effect on the intestinal epithelium. Butyrate, propionate and acetate can stimulate the proliferation and differentiation of the intestinal epithelium, but butyrate plays the most important part in this function [141, 145]. In addition, the production of certain metabolites and the regulation of electrolytes in the feces by commensal bacteria play a key role in the content of water and volume of feces, in the secretion of neuropeptides intestinal motility regulators and in Xenobiotic and drug metabolism [146].

2.2.3. Protective functions

The intestinal microbiota is a very important element in the defense barrier of the intestinal mucosa, thus avoiding the colonization of other pathogenic microorganisms. Moreover the intestinal microbiota provides the right pH conditions and produces bacteriocins, among other effects, which makes the colonization by foreign elements difficult [147, 148]. To sum up, the commensal microbiota of the intestine constitutes the first defense barrier against infections.

2.2.4. Immunological functions

The gut microbiota has been proposed as a regulator of many aspects of innate and acquired immunity, based on studies in germ-free animal models, which have immature gut-associated lymphoid tissue (GALT) and a reduced number of plasmatic cells, lacking Peyer's patches, anomalies of spleen and lymph nodes and immature villi. The importance of the gut microbiota lies in the fact that these deficiencies can be repaired through colonization by species of the *Bacteroides* genus [149, 150].

The recognition of bacterial components through pattern-recognition receptors (PRRs), such as the toll-like receptors (TLRs) of innate immune cells, is considered to be the starting point of immunity, informing the immunocompetent cells to respond

correctly to each environmental stimulus (e.g. pathogens or harmless microbes) [133]. TLR-4 recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, while TLR-2 recognizes lipopeptides and lipoproteins from various pathogens, and peptidoglycan and lipoteichoic acid from Gram-positive bacteria [151].

It has been shown that both the LPS of Gram-negative bacteria and the saturated fatty acids in the diet can activate TLR4, which causes the induction of the synthesis of cytokines TNF-α IL-6, interleukin 1 beta (IL-1B), and pro-inflammatory chemokines, related with the onset of insulin resistance and increased adiposity [164]. Nevertheless, it is not known yet whether inflammation is the cause or the consequence of alterations such as insulin resistance [153].

Recently, metabolic endotoxemia, characterized by an increase in serum LPS levels, has been demonstrated to be an inflammatory factor, which causes body weight gain, insulin resistance and diabetes in animal models fed on high-fat diets [154, 155]. In contrast, the inhibition of the gut microbiota by administering antibiotics in two different mouse models of insulin resistance resulted in reduced serum LPS levels, low-grade inflammation, obesity and type-2 diabetes, demonstrating the link between the gut microbiota and certain metabolic disorders [156]. LPS stimulation also produces a cytokine-mediated increase in plasma lipid levels by increasing the synthesis of VLDL lipoproteins in the liver and inhibiting lipoprotein lipase. Therefore, common responses can be induced by “pathogenic lipid nutrients” and microorganisms mainly related to TLR-4-signaling and proinflammatory cytokine and gene transcription activation pathways. In this scenario, one can hypothesize that the shifts in gut microbiota composition caused by a diet high in saturated fatty acids [154], together with dietary lipids, would constitute synergic TLR signals, thus contributing to increasing the inflammation occurring in obesity.

2.3. Factors modifying the intestinal microbiota

Although the composition of gut microbial communities was thought to be generally stable in the adult, many factors have been described that modulate and determine this composition over the lifetime of the individual [157]. The factors that can modify the composition of the intestinal microbiota can be divided into three categories: microbial factors, intrinsic factors of the host and extrinsic factor to the host.

2.3.1. Microbial factors

Microbial factors can include the metabolic activity of the commensal microbiota itself, which can alter the conditions of the ecosystem in terms of pH, oxygen, and so on, as well as the generation of metabolites and other substances by the gut microbiota, which may promote or prevent the growth of other species (nutrients, growth factors, etc.) [124, 158].

2.3.2. Intrinsic factors

The intrinsic factors of the individual that affect the composition of the intestinal microbiota include the production of stomach acid, pancreatic secretions and bile salts, which will ultimately determine the pH level of the mucose, the production of hydrolytic enzymes such as lysozyme and trypsin, the production of antimicrobial substances such as defensins, intestinal motility and mucus production, which will determine the conditions of the habitat to be colonized. Another important intrinsic factor is the age of the individual, which in turn determines many of the metabolic activities and characteristics of the factors identified above [159-161].

The balance of the intestinal microbiota is inevitably affected by the physiological changes of the GT induced by the ageing process itself, as well as other age-related events, i.e. modification in diet and lifestyle, and reduction of functionality of the

immune system. Therefore, changes in the composition and structure of the intestinal microbiota could be related to distinctive conditions of the elderly, such as frailty, metabolic syndrome, diabetes and sarcopenia [161].

Mariat et al. [162] reported that the *Firmicutes/Bacteroidetes* ratio was lower in elderly people (between 70 and 90 years old) than in young adults, since the elderly have a different digestive physiology characterized by a reduction in transit and of digestive secretions as compared with young adults. This was confirmed by Claesson et al. [163] in their recent application of pyrosequencing performed on the faecal microbiota of Irish young and elderly adults. On the contrary, Biagi et al. [164] showed a significantly compromised gut microbiota in centenarians but not in elderly subjects aged approximately 70 years compared with a group of younger adults. The authors therefore suggested that a healthy gut microbiota community might be affected by aging-related physiological and behavioral changes that occur after 70 years of age, which was considered the threshold age for defining an individual as elderly.

At genus level, the available studies are in general agreement reporting an age-related increase in facultative anaerobes, including *Streptococcus, Staphylococcus, Enterococcus* and *Enterobacteria* [162, 165]. The enterobacteria group comprehends potentially pathogenic species (pathobionts), and could be the cause of infections when the host resistance mechanisms fail as a result of the ageing process. Antibiotic treatment and hospitalisation are known to favour the increase of enterobacteria in the microbiota of elderly people [166, 167].

As for the health-promoting bacteria, the decrease of bifidobacteria in the gut microbiota, has been a commonly accepted ageing effect in the past years magnified by the use of antibiotic treatment, or the hospitalisation [166, 167]. However, several recent studies based on molecular techniques do not seem to confirm this: Rajilic-Stojanovic et al. [165] did not find any difference between the bifidobacteria abundance in healthy

elderly and young adults, nor did Biagi et al. [164], who reported a significant decrease in the bifidobacterial population only in extremely old people. The discrepancies in the *Bifidobacterium* behaviour with respect to the ageing process may be explained, considering the remarkable temporal instability of the *Actinobacteria* (the phylum which includes the *Bifidobacterium* genus) population in the faecal microbiota of the elderly [163, 165].

However, several recent studies based on molecular techniques do not seem to confirm this: Rajilic-Stojanovic et al. [165] did not find any difference between the bifidobacteria abundance in healthy elderly and young adults, nor did Biagi et al. [164], who reported a significant decrease in the bifidobacterial population only in extremely old people.

Changes in microbial composition occur with a high degree of variability at the two extremes of life, childhood and old age, whereas they are marked by relative stability in adulthood [160, 168]. At birth the GT is sterile and during the first year of life, the gut ecosystem is prevalently colonized by opportunistic microorganisms to which a baby is exposed in its environment. The earliest colonizers are often aerobes such as *Staphylococcus, Streptococcus* and *Enterobacteria*, followed by anaerobic later colonizers such as *Eubacteria* and *Clostridia*. The microbiota of breast-fed infants is largely dominated by *Bifidobacterium*. With weaning and together the introduction of a solid diet, drive to the transition to an adult-like profile, characterised by a remarkable microbial biodiversity. The ageing of the gut microbiota starts after ‘threshold age’ and symmetrically to what happens in the early stage of our life, the aged-type microbiota shows a low microbial biodiversity, and it is characterised by an increase in opportunistic environmental facultative aerobes *Staphylococcus, Streptococcus, Enterobacteriaceae,* as well as a reduction in anaerobes, such as *Clostridium clusters IV*

and *XIVa* and *Bacteroidetes*. However, differently from the infant-type microbiota, the aged type is characterised by a low abundance of *Bifidobacterium* [161].

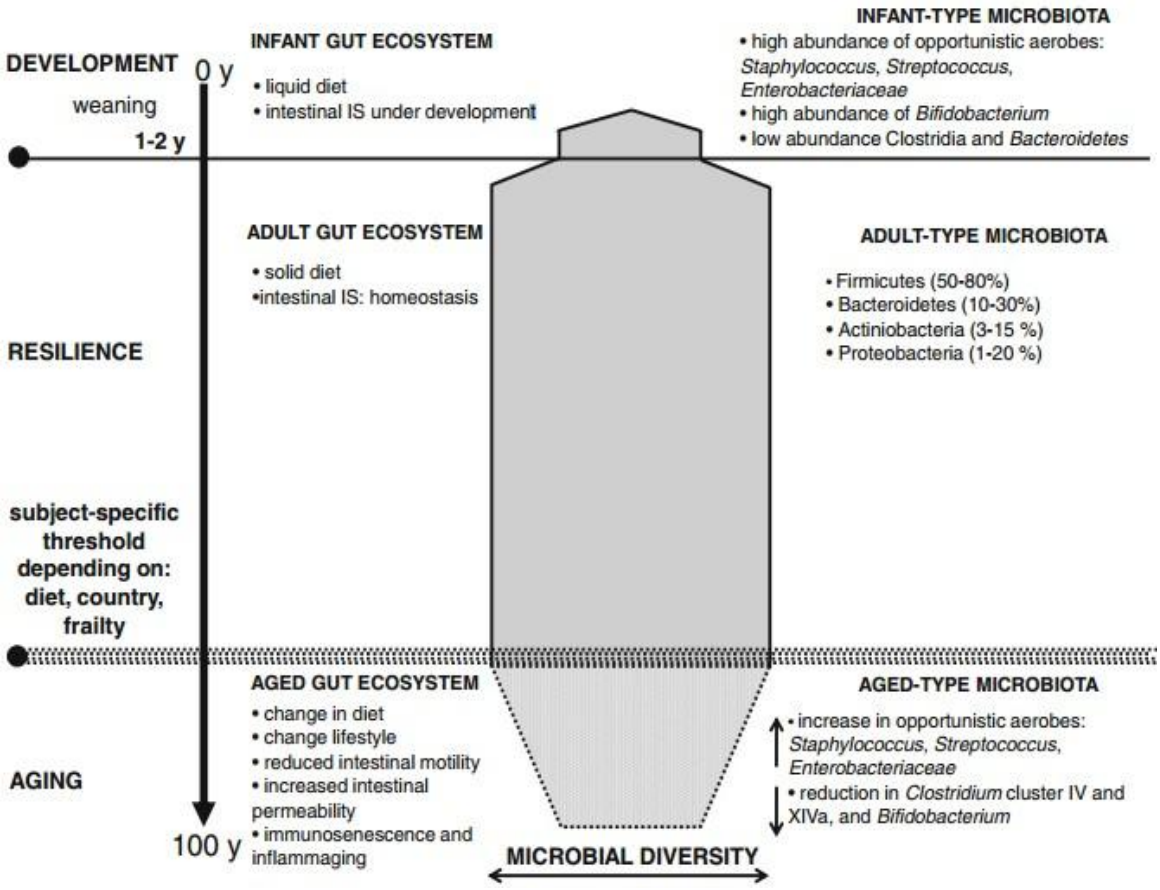

Figure 5 Changes in the intestinal microbiota composition related with age [161].

2.3.3. Extrinsic factors

Extrinsic factors include the individual's clinical history and diet, among others. The clinical history of the individual includes, especially, the use of antibiotics, neoplastic or autoimmune diseases that require the use of chemotherapy, radiation treatments, the chronic use of immunosuppressants or intestinal diseases that have required surgery or have involved a major change in bowel habits. All these factors undoubtedly have a significant effect on the commensal microbiota [158, 169-171].

Antibiotics disrupt the equilibrium among commensal populations, and lead to a decreased or altered communication between the human intestinal flora and the underlying mucosa. The possible consequences could be long-lasting and can have multiple effects on the host, including increased susceptibility to infections, the potential to develop allergies, a predisposition to develop metabolic syndrome, decreased efficacy outcomes of pharmacologic therapies, as well as the induction and spread of antibiotic resistance [172].

On the other hand some drugs, such as acetaminophen, chloramphenicol, digoxin, and sulfasalazine have also been shown to be affected by gut microbial metabolism [173]. Once these compounds are orally administered, they are transformed to bioactive, bioinactive, or toxic metabolites by intestinal microbiota before their absorption into the blood [174].

Another example is Lovastatin, which is a statin, a class of drugs used as cholesterol-lowering agents to reduce cardiovascular disease risk. Lovastatin is a lactone prodrug that is readily hydrolyzed in vivo to yield the active β-hydroxy acid metabolite, a strong inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase. In this sense, a study demonstrated the involvement of gut microbiota in the metabolism of lovastatin to its bioactive metabolite. Furthermore, the researchers found that drug-drug interaction between lovastatin and antibiotics were likely due to antibiotic-mediated inhibition of intestinal bacteria. This is the first report of gut microbe-mediated lovastatin metabolism and the consequent pharmacokinetic interactions. These findings indicated that administration of antibiotics to patients taking lovastatin may lead to decreased systemic exposure of the lovastatin active metabolite, reducing its pharmacological effects. Similar antibiotic-induced pharmacokinetic effects may occur with other drugs metabolized by gut microbial enzymes [175].

However, diet is one of the most influential extrinsic factors in the composition of the intestinal microbiota. In general, it has been shown that diets rich in fats and proteins increase the abundance of species of the genus *Bacteroides*, while diets high in carbohydrates increase the abundance of the genus *Prevotella*. Furthermore, it has been observed that the consumption of diets rich in vegetables, fruits and fiber increases the microbial richness [176, 177]. In this thesis, it has included two specific sections to address this issue in depth (*Section 4: Modulation of the intestinal microbiota by prebiotics and probiotics and Section 5: Modulation of the intestinal microbiota by diet*).

3. INTESTINAL MICROBIOTA AND METABOLIC SYNDROME.

The gut microbiota community is commonly referred to as our hidden metabolic 'organ', due to its immense impact on human wellbeing, including host metabolism, physiology, nutrition and the immune function. Our gut microbiota coevolves with us [178] and the changes in this population can have major consequences, both beneficial and harmful, for human health. At present, the imbalance or dysbiosis of the gut microbiota is associated with the pathogenesis of both intestinal and extra-intestinal disorders [179, 180]. Gut microbiota exerts a significant role in the obesity, T2D, plasma lipids metabolism and hypertension, therefore in the pathogenesis of the metabolic syndrome.

3.1. Obesity

Alterations in the composition of the gut microbiota and its relationship with obesity have been observed in many studies. The first evidence for the role of gut microbiota in the development of obesity came from studies conducted by Bäckhed et al. on germ-free mice compared to conventionally-raised mice [181]. The latter have 47% more adipose tissue than germ-free mice and these results were independent of food intake. In addition, the germ-free mice were colonized with the intestinal microbiota from conventionally-raised mice, which led to a significant increase in the body weight of these mice, development of insulin resistance and increased leptin levels and circulating glucose. The researchers concluded that the gut microbiota represents an environmental factor which affects the host's predisposition to develop obesity and increases adiposity [181].

Other studies have observed in genetically obese mice (ob/ob mice), compared to their lean counterparts, a 50% reduction in the abundance of the phylum *Bacteroidetes* and a proportional increase in the phylum *Firmicutes* [182]. These specific changes in

ob/ob mice suggest that these mice present an intestinal microbiota with a greater ability to extract energy from dietary nutrients. This fact was confirmed by transplanting stool from ob/ob mice to normal weight mice: the latter developed obesity in two weeks [182].

Studies in obese human subjects also have confirmed specific changes in gut microbiota composition, such as a reduction in the phylum *Bacteroidetes* and an increase in *Firmicutes* [183-184]. In contrast, studies in humans have yielded conflicting results, which may be explained by the inter-individual heterogeneity to which the gut microbiota is exposed, which originates mainly from different environmental factors such as diet, host metabolism, and hormonal factors [186]. In fact, the composition of gut microbiota seems to be influenced more by environmental and dietary factors than by genetic ones [187, 188]. Other research studies conducted in obese and lean twins have observed a reduction of *Bacteroidetes* and *Actinobacteria* phyla and a reduced microbial diversity, but have not detected changes in the proportion *of Firmicutes* in obese subjects [189].

In 2013, a study determined that reduced microbial diversity is correlated with adiposity, insulin resistance and inflammation. Moreover, this study showed that obese subjects were characterized by an increase in *Proteobacteria* and *Bacteroidetes* phyla and a decrease in *Akkermansia muciniphila*, an important anti-inflammatory bacterium since it has a key role in the production of butyrate [190]. Due to this capability *Akkermansia muciniphila* exerts multiple effects at the intestinal level, such as the prevention and inhibition of colonic carcinogenesis, the improvement of inflammation, oxidative status and the epithelial defense barrier, and the modulation of visceral sensitivity and intestinal motility [191].

Moreover, in overweight adolescents, the gut microbial composition seems to influence weight loss after dietary restriction and increased energy expenditure by

physical activity, independently of total food intake. This indicated that the interactions between the intestinal microbiota and body weight may be sensitive to lifestyle intervention and gut microbiota could potentially influence the efficacy of dietary interventions [192].

3.1.1. Mechanisms to explain the influence of the intestinal microbiota on obesity.

Several mechanisms for the influence of the gut microbiota on obesity have been proposed. When an imbalance occurs in the intestinal microbiota, the bacteria become more effective at extracting energy [193]. One mechanism through which intestinal microbiota contributes to increased energy absorption seems to be the production of SCFAs, resulting from the fermentation of dietary fiber. Humans lack the enzymes to degrade most dietary fiber. Therefore, these non-digestible carbohydrates pass unaffected through the upper gastrointestinal tract and are fermented in the cecum and the large intestine by the anaerobic cecal and colonic microbiota. Fermentation results in multiple groups of metabolites [194], of which SCFAs are the major group [195]. SCFAs, such as propionate, butyrate and acetate, could be absorbed and used as a source of energy, as well as possibly acting as signaling molecules that exert influence on the host's appetite [196, 197], intestinal transit time [196], energy absorption [198] to increase fat storage and energy retention, via the GPR41 and GPR43 receptors [196, 199].

The second mechanism that could explain the influence of the gut microbiota on obesity is that the intestinal microbiota promotes monosaccharide absorption and suppresses the fasting-induced adipocyte factor (FIAF) in intestinal tissue [200]. FIAF is an inhibitor of lipoprotein lipase and a dysbiosis causes a decrease in the expression of FIAF, resulting in an increase in lipoprotein lipase activity, a catalyst which captures

and stores the fatty acids in adipose and muscle tissue, as well as causing an increase in lipid storage [181, 200].

Another mechanism involved may be the increased permeability of the intestinal wall. A high-fat diet can produce changes in the normal composition of the intestinal microbiota and this alteration may lead to an increased permeability of the gut barrier by an abnormal distribution of some tight junction proteins, such as zonolin -1 (ZO-1) and ocludina, which contribute to an increase in certain molecules in plasma such as LPS, which could ultimately cause metabolic endotoxemia [156, 201-204]. LPS generates a signaling cascade by binding to its receptors TLR4 / CD14 and stimulates the production of proinflammatory cytokines, particularly TNF-α and IL-6, which are involved in the development of obesity, atherosclerosis and insulin resistance [205]. Moreover, the change in the composition of intestinal microbiota activates the endocannabinoid system through cannabinoid receptor 1 (CB1R). This activation is initially responsible for increasing the permeability of the intestinal barrier [206], which allows LPS to pass through it, causing endotoxemia. When the activation of the peripheral endocannabinoid system is increased, adipogenesis is stimulated through two routes: through the LPS molecules and by inhibition of the peroxisome proliferator-activated receptor (PPAR) [206, 207].

Finally, the intestinal microbiota may also increase adiposity by decreasing the activity of adenosine monophosphate-activated protein kinase (AMPK) in muscles and the liver, since this causes a reduction in fatty acid oxidation and thus an increase in adipogenesis [200].

In conclusion, evidence emerging from studies conducted on animal models and human subjects has confirmed the pathogenic role played by gut microbiota in the development of obesity. But given the background above, it is clear that there is not just

one single mechanism that explains how the gut microbiota increases its adiposity, although other important species that could play a role have not yet been identified.

3.2. Type 2 diabetes

Type 2 diabetes (T2D) is characterized by insulin resistance and sometimes by reduced insulin production, resulting in poor cellular uptake of glucose and high levels of blood glucose. Obesity is a major risk factor for T2D, and the two are closely linked. Recent studies have shown that the gut microbiota plays a critical role in the regulation of development of T2D.

In 2010, Larsen and colleagues [208] analyzed the intestinal microbiota of 18 subjects with T2D and 18 non-diabetic controls, all males between 31 and 73 years old with body mass indices ranging from 23 to 48. In this research, it was demonstrated that T2D is associated with compositional changes in the intestinal microbiota mostly apparent at phylum and class levels. The relative abundance of *Firmicutes* was significantly lower, while the proportion of *Bacteroidetes* and *Proteobacteria* was somewhat higher in diabetic subjects compared to their non-diabetic counterparts. Accordingly, the ratios of *Bacteroidetes* to *Firmicutes* correlated significantly and positively with reduced glucose tolerance [208]. In addition, the intestinal microbiota among subjects with T2D was relatively enriched with Gram-negative bacteria, belonging to the phyla *Bacteroidetes* and *Proteobacteria.* The main compounds of the outer membranes in gram-negative bacteria are LPS, known as potent stimulators of inflammation, which can exhibit endotoxaemia [209]. Consequently, LPS will continue to be produced within the gut, which might trigger an inflammatory response and play a role in the development of diabetes [208].

Another study analyzed 121 subjects who were divided into 3 groups based on their glucose intolerance status: 44 subjects with normal glucose tolerance, 64 pre-

diabetic patients and 13 newly diagnosed T2D patients [210]. The researchers found that a total of 28 operational taxonomic units were related to T2D status, most of which were enriched in the T2D group. Butyrate-producing bacteria (*Akkermansia muciniphila* and *Faecalibacterium prausnitzii*) had a higher abundance in the normal glucose tolerance group than in the pre-diabetes group. At genus level, the abundance of *Bacteroides* in the T2D group was only half that of the normal glucose tolerance and pre-diabetes groups. Moreover, it was observed that *Verrucomicrobiae* may be a potential marker of T2D as it had a significantly lower abundance in both the pre-diabetes and T2D groups [210]. These results indicate not only that the gut microbiota of patients with T2D differ from healthy control subjects, but also that the changes in gut microbiota are associated with the progression of glucose intolerance.

A metagenome-wide association study (MGWAS) based on deep shotgun sequencing of the gut microbial DNA was carried out in a large cohort of 345 Chinese patients with T2D and healthy control subjects [211]. The MGWAS analysis showed that patients with T2D were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulphate reduction and oxidative stress resistance [211].

However, the results obtained in the cohort of 345 Chinese subjects differ from the results of another study in 145 European women with normal, impaired or diabetic glucose control [212]. In this study, shotgun sequencing was used to characterize the fecal metagenome and Karlsson and colleagues developed a mathematical model that identified T2D with a high degree of accuracy [212]. Therefore, metagenomic predictive tools for T2D should be specific for the age, gender and geographical location of the populations studied.

In conclusion, human studies confirmed the pathogenic role of metabolic endotoxemia in the development of insulin resistance and T2D. The progressive development of glucose intolerance and diabetes is accompanied by a corresponding decrease in anti-inflammatory and butyrate-producing bacteria, as well as an increase in pathogens. Indeed, the experimental enrichment of butyrate-producing bacteria is associated with an improvement in insulin sensitivity.

3.3. Plasma lipids

Currently is known that aberrant levels of plasma lipids (particularly HDL-c and TG) are associated with MetS and a high risk of CVD. For this reason, studying the effect of the gut microbiome on blood lipid levels help us insight into the role of the microbiome in the development of MetS and CVD. Dysbiosis in the gut has been shown to induce increased permeability of the intestine, leading to increased systemic levels of bacterial products causing low-grade chronic inflammation [213]. This inflammation may directly affect atherogenesis and has also been hypothesized to lead to the development of insulin resistance with concomitant effects on plasma lipids [214].

A mechanism by which the microbiota involved in lipid metabolism is by LPS. An increase in plasma concentrations of LPS is related with increased body weight, increased adipose tissue, increased fasting glucose, insulin resistance and inflammatory state chronic: high levels (TNF-α, IL-1 and IL-6 and PAI-1 [215, 138]. On the other hand, TNF-α released the bloodstream, active the JNK signaling pathway producing a phosphorylation of a serine of the insulin receptor IRS-1. This inhibits the binding of the insulin receptor that increases in the bloodstream and finally, this leads to a hyperinsulinemia and excessive lipid storage in adipose tissue and liver [216].

The SCFAs produced by the intestinal microbiota also play a key role in lipid metabolism, especially when the SCFAs bind to GPR43 receptor in adipose tissue. This

inhibits lipolysis in this tissue which leads to a reduction of free fatty acids in plasma [217]. Moreover, acetate is the principal SCFA in the colon, and after absorption it has been shown to increase cholesterol synthesis. However, propionate, a gluconeogenerator SCFA, has been shown to inhibit cholesterol synthesis [218].

In addition, the gut microbiota can influence in the lipid metabolism through to the FIAF, since a dysbiosis of the gut microbiota causes a minor expression of FIAF and this causes an increase in the lipoprotein lipase activity, therefore increases the storage of triglycerides in the adipocytes [181, 200].

Recently Fu et al. [219] showed that age, sex, genetics, and the gut microbiome collectively explained 11.3% of the variation in BMI, 17.1% in triglycerides, and 25.9% in HDL-c, with the microbiome making a significant contribution to the explained variation in BMI, triglycerides, and HDL. Furthermore, when the researches included BMI as a risk factor, the total explained variation in lipids increased to 25% in triglycerides, 37.4% in HDL-c, 22.3% in LDL-c, and 22.3% in TC. In this case the microbiome made a lesser, but still significant, contribution to triglycerides and HDL-c, suggesting that the microbiome affects blood lipids partly independently of BMI and indicating that the intestinal microbiota is a potencially player in plasma lipid metabolism. In conclusion this study estimated that intestinal microbiota composition can explain ≤6% of the variation in lipid levels and that this effect is independent of age, sex and host genetics. These results support the potencial of the gut microbiota modulation to correct lipid disbalance and thereby help prevent MetS and CVD.

3.4. Hypertension

At present, several studies have explored the possible role of the gut microbiota for improving the health conditions in metabolic disorders that increase the risk of developing cardiovascular diseases such as hypertension.

In 2015, a pilot study using fecal and blood samples obtained from 7 hypertensive and 13 normotensive patients, observed marked decreases in microbial richness and diversity in the hypertensive patients. In addition, this group also showed a trend towards a decrease in evenness in species from certain bacterial taxa such as *Bacteriodetes* [220].

On the other hand a study in two different rat models of hypertension and a small cohort of hypertensive patients [221] has demonstrated a decrease in the microbial richness and a marked increase in the *Firmicutes / Bacteroidetes* ratio in the animal models of hypertension, implicating the existence of gut dysbiosis in hypertension. Furthermore, this dysbiosis was associated with a decrease in acetate- and butyrate-producing bacteria and an increase in the lactate-producing bacterial population. Most interesting of this study is that has confirmed a dysbiosis of the gut microbiota in a small cohort of human hypertensive patients. Yang T. et al. [221] observed a reduction in bacterial Chao richness and Shannon diversity in the patients with high systolic blood pressure when compared with normal systolic blood pressure. These findings clearly implicate the role of gut microbiota in the pathophysiology of both animal and human hypertension.

All these evidences indirectly suggest that gut microbiota may play a key role in the control of blood pressure homeostasis and that any change in microbiota composition or imbalance may potentially result in hypertension. Nevertheless, despite these evidences little is known about the role of microbiota in hypertension. Further investigations are required to evaluate the direct and indirect effects of the intestinal microbiota in the regulation of the blood pressure. Furthermore, this area of research needs to be examined thoroughly in more clinical studies to postulate the effect of probiotics in the regulation of hypertension.

4. MODULATION OF THE INTESTINAL MICROBIOTA BY PROBIOTICS AND PREBIOTICS

Due to the current knowledge between the desesquilibrios of the intestinal microbiota and its relation with the disease, treatments involving the modulation of the gut microbiota may be used in attempts to treat these illnesses. One possible treatment has been the use of prebiotics and probiotics.

The definition of a probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance [222]. Probiotics have been investigated as a potential dietary supplement that can positively contribute to an individual's health by improving survival and implantation of live microbial dietary supplements in the gastrointestinal flora, by selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's microbial balance [223]. Unlike probiotics, prebiotics are not live preparations, but instead are food ingredients that may be fermented but not digested. The fermentation of prebiotics can benefit the host by stimulating growth and activity in intestinal microbial species. Prebiotics are not absorbed by the small intestine, and their fermentation allows endogenous bacteria to produce energy and metabolic substrates. So far, the main prebiotics include fructooligosaccharides, galactooligosaccharides, lactulose, and non- digestible carbohydrates. The non-digestible carbohydrates include large polysaccharides (inulin, resistant starches, cellulose, hemicellulose, pectins, and gums), some oligosaccharides that escape digestion, and unabsorbed sugars and alcohols. Most prebiotics, including fructooligosaccharides and inulin, are digested by Bifidobacteria and stimulate the growth of their colonies. These bacteria influence homeostasis of intestinal cells and inhibit the growth of pathogenic bacteria reduce intestinal endotoxin concentration and improving glucose tolerance and inflammation [224, 225].

Although both prebiotics and probiotics have been shown to increase numbers of selected bacteria at the species and genus level, typically *Bifidobacterium* and *Lactobacillus,* changes in the overall composition of the gut microbiota are often relatively small, and generally persist only for as long as the period of the intervention.

In the last decades several studies in humans have evaluated the effects on the gut microbiota composition by probiotics and prebiotics [226, 227]. On the contrary, others study have demonstrated changes on the gut microbiota composition in healthy aduts human, for example, a study in 30 people showed an increase of the abundance of *Bifidobacterium* genus and especially *Bifidobacterium adolescentis* after the consumption of the prebiotic lactulose [228]. Others studies have also observed significant change in *Bifidobacterium* communities after of the use of the probiotic fermented oat drink containing *Bifidobacterium longum* 46 and *Bifidobacterium longum* 2C [229, 230].

A recently study [231] investigated the effect of consuming probiotic fermented milk on the microbial community structure in the human intestinal tract by using highthroughput barcoded pyrosequencing in six healthy adults ingested 2 servings of probiotic fermented milk daily for 3 wk, and their fecal microbiota were analyzed before and after 3 wk of ingestion period and for another 3 wk following the termination of ingestion. All subjects showed a similar pattern of microbiota at the phylum level, where the relative abundance of *Bacteriodetes* species increased during the probiotic fermented milk ingestion period and decreased during the noningestion period. The increase in *Bacteroidetes* was found to be due to an increase in members of the families *Bacteroidaceae* or *Prevotellaceae.* In contrast to probiotic fermented milk-induced adaptation at the phylum level, the taxonomic composition at the genus level showed a considerable alteration in fecal microbiota induced by probiotic fermented milk ingestion. The genera *Prevotella, Bacteroides,* and *Faecalibacterium* were the

predominant members in the gut microbiota. Moreover, members of *Prevotella* and *Bacteroides* were found to be major contributors producing changes in the corresponding families [231].

Despite these studies, the fact that probiotics and prebiotics are able to influence the relative abundance of specific intestinal microorganisms is questions that are currently under study.

On the other hand, besides exert effects on the composition of the intestinal microbiota; several studies have revealed that probiotics and prebiotics might maintain the potential to improve lipid profiles, including the reduction of LDL-cholesterol, serum/plasma total cholesterol, and TG or increment of HDL-cholesterol in the context of treating CVD and MetS.

Probiotics have also been studied for their cholesterol-lowering effects [232]. Anderson *et al.* [233] evaluated the effect of fermented milk containing *Lactobacillus acidophilus* L1 on serum cholesterol in forty-eight hypercholesterolemic humans in a study randomized, double-blind, placebo-controlled and crossover 10-week study. The result was that daily consumption of 200g of yogurt containing *Lactobacillus acidophilus* L1 after each dinner contributed to a significant reduction in serum cholesterol concentration (−2.4%) compared to the placebo group. In another study, Xiao et al. [234] demonstrated a significant decrease in serum total cholesterol, LDL-cholesterol and triglycerides after of the consumption of a low-fat yogurt containing 108 CFU/g of *Bifidobacterium longum* BL1 on lipid profiles of thirty-two subjects. In addition the authors also observed a 14.5% increase in HDL-cholesterol when comparing to the control (yoghurt without *Bifidobacterium longum* BL1).

Meanwhile prebiotics are also utilized by the intestinal microbial population to produce short-chain fatty acids which may lead to improvement of lipid profiles [235]. Causey et al. [236] assessed the effects of inulin from chicory root on blood cholesterol

level in twelve hypercholesterolemic men that were randomly assigned in two groups, a control group that consumed one pint of vanilla ice-cream without inulin and an inulin group consumed one pint of vanilla ice-cream containing 20 g of inulin. The 3-week study found that daily intake of 20g of inulin significantly reduced serum triglycerides. Similarly, another study involving eight healthy volunteers with a daily consumption of 10g of inulin for three weeks has also reached the same conclusion [237].

In contrast, many studies have also shown that probiotics and prebiotics had insignificant effects on lipid profiles. A study by Hatakka et al. [238] reported that the administration of two capsules daily containing *Lactobacillus rhamnosus* LC705 1010CFU/g per capsule did not influence blood lipid profiles in thirty-eight men after a 4-week treatment period. On the other hand several randomized, placebo-controlled dlouble-blind and crossover studies with prebiotics, using inulin [239] and frutooligo-sachharides [240] not showed significant improvement in lipid profiles.

However, although there are many studies with probiotics and prebiotics that have shown their influence lipid metabolism [241] to date, the association between lipids and gut microbiota is not very clear.

5. MODULATION OF THE INTESTINAL MICROBIOTA BY DIET

Many recent studies in humans have investigated the diet as the lifestyle factor that most influences the composition of the intestinal microbiota and therefore the health and diseases of the host. Having an understanding of how diet influences microbial communities will be of critical importance with respect to employing food to beneficially alter the gut microbiota. Indeed, a number of recent studies have highlighted the links between diet and distinct microbial profiles and, in turn, overall gut health [177, 242-246]. Several studies has shown that significant alterations in the intestinal microbiota have been associated with alterations in diet, mainly influenced by the consumption of dietary fiber from fruit and vegetables. In general, a dietary intake rich in fruit, vegetables and fiber is associated with a greater richness and diversity of the gut microbiota [247].

Duncan et al. [246] analyzed 19 healthy obese who followed in succession three different diets: maintenance for 3 days (399 g carbohydrate/day) and then high protein/medium (164 g/day) carbohydrate and high protein/low (24 g/day) carbohydrate each for 4 weeks. The results were that *Roseburia* spp. and *Eubacterium rectale* subgroup of cluster XIVa and *Bifidobacteria* decreased as carbohydrate intake decreased. *Roseburia spp* and *Eubacterium rectale* are taxa belong to the phylum *Firmicutes* and both are butyrate-producing bacterial that respond to dietary carbohydrate intake.

In a controlled dietary study in 14 overweight men, variations in the consumption of resistant starch or non-starch polysaccharide causes significant changes in certain bacterial taxa. Volunteers were provided successively with a control diet, diets high in resistant starch or non-starch polysaccharides and a reduced carbohydrate weight loss diet, over 10 weeks. The results showed an increase in the relative abundance of *Ruminococcus bromii, Roseburia* and *Eubacterium rectale* in most volunteers on the

resistant starch diet [247]. These taxa belong to the phylum *Firmicutes* and have demonstrated the capability to metabolize specific insoluble carbohydrate substrates in a selective way [248]. The increase of the intake of resistant starch, and important non-digestible in the human diet, can substantially alter the species composition of the colonic microbiota. It follows that the colonic microbial community must typically be in a state of continuous change over time, driven by short-term changes in dietary intake. Thus, only the most successful and versatile organisms will be found commonly among the dominant microbiota at different sampling times and in different individuals.

In addition, the abundance of these bacterial taxa decreased with a short-term animal-based diet [245]. The animal-based diet increased the abundance of bile-tolerant microorganisms and decreased the levels of *Firmicutes* that metabolize dietary plant polysaccharides. The bile-tolerant microorganisms *Alistipes, Bacteroides* belonging to phylum *Bacteroidetes* and *Bilophila*, belonging to phylum *Proteobacteria* and *Firmicutes* included *Roseburia, Eubacterium rectale*, and *Ruminococcus bromii*. These findings show that even short-term diet alters the human intestinal microbiota rapidly [245] and there are differences in the intestinal microbiota of carnivorous and vegetarian mammals [249], thus reflecting a balance between carbohydrate and protein fermentation. In this context, other studies have also showed changes in the gut microbiota composition in vegetarians people [250-252]. Matijašić BB et al. [252] have demonstrated in a Slovenian population comprising 31 vegetarian participants (11 lacto-vegetarians and 20 vegans) and 29 omnivore participants that vegetarian diet was associated with higher percentage of *Bacteroides-Prevotella, Bacteroides thetaiotaomicron (Bacteroidetes), Clostridium clostridioforme* and *Faecalibacterium prausnitzii (Firmicutes)*, but with lower percentage of *Clostridium cluster XIVa (Firmicutes)*. In addition, the authors showed that members of the *Clostridium clusters IV* and *XIVa* have been found to be enriched in the faeces of omnivores compared to

vegetarians and lactovegetarians, who generally consume higher proportions of carbohydrates as part of their diet [250–252]. These clusters of bacteria are noted for their ability to convert dietary fibre to SCFAs.

The composition of the human intestinal microbiota also may vary, based on dietary changes related to geographical and seasonal variations.

In 2010, De Fillipo C. et al. [253] discovered differences in the composition of the intestinal microbiota between 14 healthy children living in Burkina Faso (in a rural African village) and 15 healthy children in the urban area of Florence, Europe. The diet of Burkina Faso children is low in fat and animal protein and rich in starch, fiber, and plant polysaccharides, and predominantly vegetarian. In contrast, the diet of Europe children is a typical western diet high in animal protein, sugar, starch, and fat and low in fiber. In this study, the analysis of the intestinal microbiota indicated that the abundance of *Firmicutes* and *Bacteroidetes* was significantly different between Burkina Faso and Europe children.

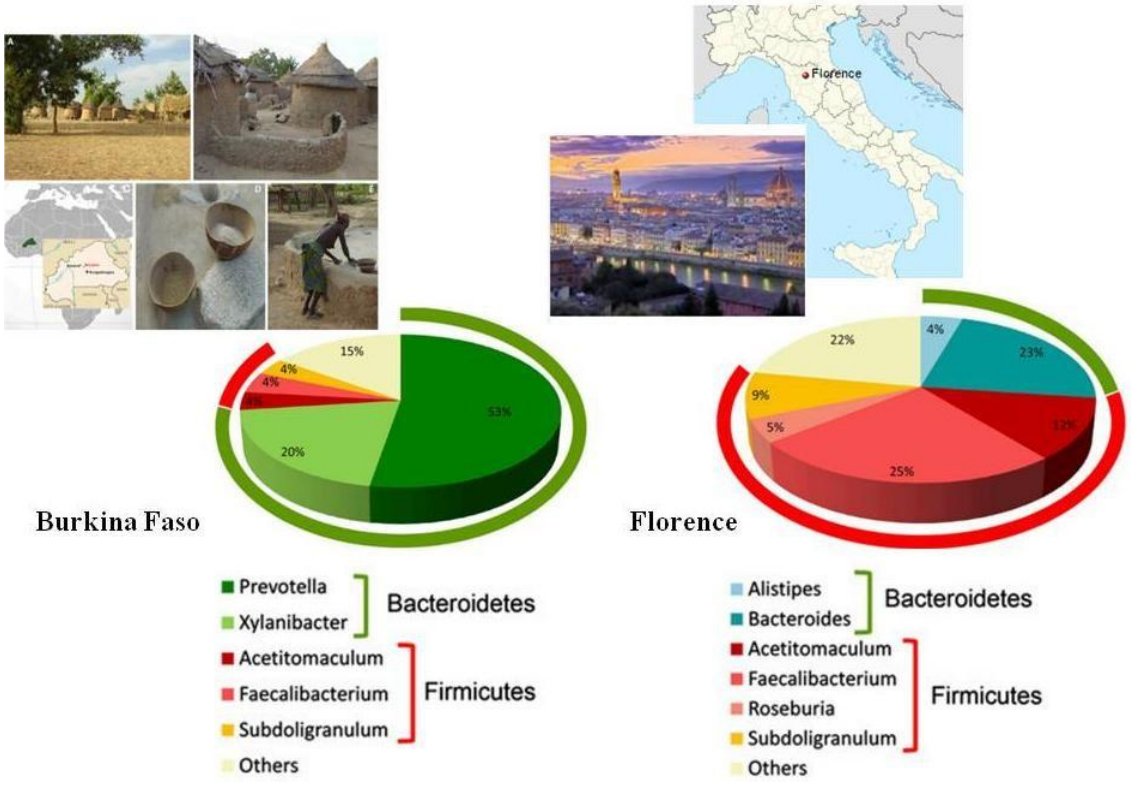

Figure 5 Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Adapted from De Fillipo C. et al, 2010 [253].

Firmicutes are twice as abundant in the Europe children suggesting a dramatically different bacterial colonization of the human gut in the two populations. Interestingly, *Prevotella, Xylanibacter* and *Treponema* are present exclusively in Burkina Faso children microbiota. These differences in the gut microbiota composition of the Burkina Faso children could be explained as a way of maximize the metabolic energy extraction from ingested plant polysaccharides.

On the other hand, within the phylum *Bacteroidetes*, Burkina Faso children had a higher abundance of *Prevotella*, while children from Europe had higher proportions of *Bacteroides.* The higher abundance of *Prevotella* results from the rural diet, rich in carbohydrate, fiber and non-animal protein that was consumed by the African children. In contrast, the children from Europe consumed a Western diet, rich in animal protein, sugar and starch, and poor in fiber, which is marked by a higher abundance of *Bacteroides* [253].

The overall dietary patterns in the De Filippo [253] study above are similar to a study in mice where conventionalised mice were switched from a low-fat diet rich in complex plant polysaccharides to an obesity-inducing high-fat/simple carbohydrate ‘‘Western’’ diet [242].

Furthermore, a study conducted in a Hutterite population demonstrated seasonal variation in the human gut microbiome composition, since the relative abundance of the phylum *Actinobacteria* was higher in this population during the winter season. The cause could be the higher intake of meat in winter as compared with the carbohydrate and fiber-rich diet more typical of the summer [254]. This study demonstrates the plastic nature of the human gut microbiome in response to variation in diet.

In 2011, the research team of the MetaHIT project determined that the human intestinal microbiota can be classified into three "enterotypes" [255]. This study

indicated the existence of a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet. Each of the three enterotypes is identifiable by variation in the levels of one of three main genera: in the enterotype 1, predominate bacteria of *Bacteroides* genus *(Bacteroidetes),* the enterotype 2, includes bacteria of the *Prevotella* genus *(Bacteroidetes*) and finally the enterotype 3 is dominated by bacteria of the *Ruminococcus* genus *(Firmicutes).* In this study, peculiarly, the enterotypes do not correlate with host characteristics such as nationality, age, body mass index and gender. However, there are phylogenetic and functional differences between the three enterotypes.

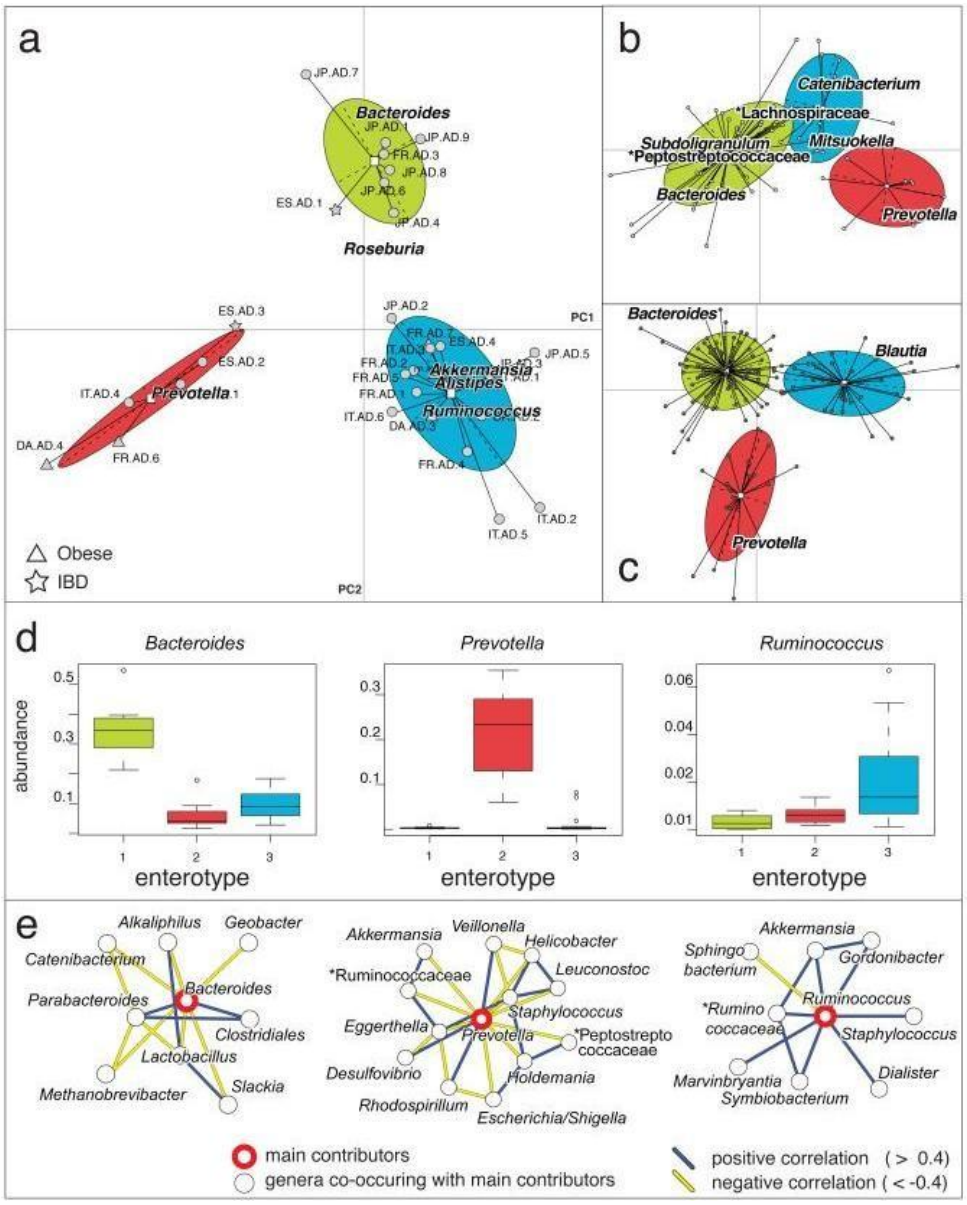

Figure 6 Phylogenetic differences between enterotypes [255].

Bacteria of enterotype 1, characterized by the presence of *Bacteroides*, get their energy mainly from the fermentation of carbohydrates and proteins, especially from polysaccharides of plant origin. This enterotype is also more effective in the synthesis of biotin, riboflavin, pantothenic acid and ascorbic acid (vitamin B8, B2, B5 and C respectively). Enterotype 2 is especially rich in *Prevotella* but also in *Desulfovibrio (Proteobacteria),* and is particularly adept in the synthesis of folic acid and thiamine (vitamin B1 and B9 respectively) and the degradation of the mucins and glycoproteins which constitute the mucosal layer surrounding the wall of the tract digestive. Finally, enterotype 3, composed mainly of *Ruminococcus* and *Akkermansia*, degrades the mucin in the same way as enterotype 2, but is also able to degrade the cellulose present in plant tissues. It is also rich in bacteria with membrane transporters, mainly sugars, indicating optimum use of their glycolytic activity [255].

However, subsequent studies have been unable to provide clear support for this concept of "enterotype" as was initially proposed [177, 256]. More recent findings support *Prevotella* or *Bacteroides* as predominant genera of intestinal microbiota [257].

Wu et al. [177] have concluded that fecal communities clustered into enterotypes distinguished primarily by levels of *Bacteroides* and *Prevotella*, both genera belonging to the phylum *Bacteroidetes.* These enterotypes were strongly associated with long-term diets, particularly protein and animal fat (*Bacteroides*) versus carbohydrates (*Prevotella*). Moreover, this study demonstrated that microbiome composition changed detectably within 24 hours of initiating a high-fat/low-fiber or low-fat/high-fiber diet in a controlled-feeding study of 10 subjects, but that enterotype identity remained stable during the 10-day study. Thus, alternative enterotype states are associated with long-term diet. The results of Wu are in consistent with the study of De Filippo et al. [253] comparing European children, who eat a typical Western diet high in animal protein and fat, to children in Burkina Faso, who eat high-carbohydrate diets low in animal protein.

Furthermore, the inverse relationship between *Prevotella* and *Bacteroides* has been reproduced in studies comparing the microbiota of subjects from agrarian societies with those from industrialized societies [258, 259]. In general, several studies support the theory that Agrarian diets high in fruit/legume fibre are associated with greater microbial diversity and a predominance of *Prevotella* over *Bacteroides*.

In contrast, 'Western'-style diets, high in fat/sugar and low in fiber, decrease the beneficial Firmicutes that metabolise dietary plant-derived polysaccharides to SCFAs. The composition of the intestinal microbiota of people on Western diets is associated with the increasing incidence of diseases such as obesity, coronary vascular disease, metabolic syndrome and certain malignancies.

Another study investigated the temporal relationship between food intake, gut microbiota and metabolic and inflammatory phenotypes in 38 obese people, and 11 overweight individuals who followed a diet-induced weight-loss and a weight-stabilization intervention. This study reported that individuals with reduced microbial gene richness present more pronounced dys-metabolism and low-grade inflammation and dietary intervention (6 week energy-restricted high-protein diet) increased gene richness significantly in individuals that originally had a low gene count. This increased gene richness remained after the subjects were switched to a 6 week weight-maintenance diet suggesting that dietary intervention as the potential to, at least partially, correct a loss of richness in the microbiota [260].

Given all this evidence in literature, one can infer that the Mediterranean Diet, common in the Western Mediterranean culture and rich in complex carbohydrates, fibres, vitamins and poor in animal proteins and fats, is able to favour the beneficial change to a saccharolytic profile, acting as a selector of "healthy" microbes that, in turn, favour and promote a general healthy status of the host.

A study assessed the gut microbiota and metabolome in a cohort of Italian individuals in relation to their habitual diets to test the effect of the consumption of Mediterranean diet [261]. The researchers obtained daily dietary information and assessed intestinal microbiota and metabolome in 153 individuals habitually following omnivore, vegetarian or vegan diets. The majority of vegan and vegetarian subjects and 30% of omnivore subjects had a high adherence to the Mediterranean diet. In this study De Filippis et al. observed that the subjects with an extraordinary adherence to Mediterranean diet showed a clear relationship between the intake of foods rich in fiber and vegetables and the concentrations of SCFA on feces. Moreover, the same microbiota that was more associated with vegetable based diets tended to correlate with SCFA levels. *Prevotella* among *Bacteroidetes* and *Lachnospira* among the *Firmicutes* appeared as the most probable candidates for fermentation of carbohydrates, leading to a higher SCFA production. In addition, detected higher urinary trimethylamine oxide levels in individuals with lower adherence to the Mediterranean diet. This study concludes that Mediterranean diet is associated with beneficial microbiome-related metabolomic profiles [261].

Another study also investigated the effect of the comsuption of Mediterranean diet on the gut microbiota composition, but to assess specific components, such as polyphenols [262]. Polyphenols antioxidants are specifically consumed in the diet through different products such as tea, coffee, wine, fruit, vegetables and chocolate. This study was carried out in healthy humans comparing red wine intake with de-alcoholized red wine and gin intake observed that drinking red wine induced an increase in the number of *Bacteroides, Prevotella* among *Bacteoidetes* and *Enterococcus* as well as an important decrease in the *Clostridium* genera among *Firmicutes* [262].

In conclusion although the mechanisms by which a diet may modulate intestinal microbiota remain largely unknown, the intestinal microbiota might be a useful

biomarker of long-term consumption of healthy or unhealthy diets. More intriguing is the possibility that diet-induced alterations in the intestinal microbiota may contribute to the improvement of intestinal disorders and related diseases.

III. HYPOTHESIS

III. HYPOTHESIS

Currently, the shaping of the gut microbiome is considered a therapeutic target, since specific changes in the gut microbial community might counteract the development of MetS and obesity. Although previous studies have pointed out that the microbiota is individual-specific and shows high stability and resistance to perturbations over time [263, 264], recent research in humans indicates that changes in eating habits could explain up to 57% of the variability in the composition of the intestinal microbiota, while the genetic polymorphisms of the host account for no more than 12% of the predisposition to suffer MetS [265].

Based on these findings, our hypothesis is that the long-term consumption of two healthy diets, Mediterranea diet (Med diet) and Low Fat diet, corrects the dysbiosis of the intestinal microbiota present in patients with MetS, after a prolonged dietary intervention of at least one year. On the other hand, the null hypothesis is that that the long-term consumption of two healthy diets, Med diet and Low Fat diet, not corrects the dysbiosis of the intestinal microbiota present in patients with MetS.

IV. OBJECTIVES

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Main objective

To determine whether the MetS is associated with a dysbiosis of the intestinal microbiota and if this dysbiosis can be modulated by the long-term consumption of a healthy diet. This general objective is divided in three objectives, which corresponding to the three papers of this thesis:

1. To evaluate the changes in the composition of the intestinal microbiota in MetS patients compared with a non- MetS people group and to test the effect of the long-term consumption of two healthy diets: a Med diet and a Low Fat diet, in restoring the gut microbiota composition analyzing the intestinal microbiota composition by real-time quantitative PCR. *This objective is addressed in paper 1 "The gut microbial community in metabolic syndrome patients is modified by diet".*

2. To analyze the changes in the intestinal microbiota composition in an obese population after of the long-term consumption of a Med diet and a Low Fat diet by NGS. *This objective is addressed in paper 2 "Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population".*

3. To identify differential signatures of the gut microbiota for obesity according to gender and changes in BMI by NGS. *This objective is addressed in paper 3 "Intestinal microbiota is influenced by gender and body mass index".*

Secondary objectives

1. To characterize the dysbiosis of the intestinal microbiota associated with MetS analyzing the functional capability to specie level, and to test the effect of the long-term consumption of a Med diet and a Low Fat diet.

Objectives

2. To study the effect of the one year consumption of a healthy diet, Med diet and Low Fat diet, in the relationship between specific bacteria and the metabolome of the feces and plasma.

3. To evaluate the contribution of the intestinal microbiota composition to the individual variance in BMI and plasma lipid levels.

V. PAPERS

1. *The gut microbial community in metabolic syndrome patients is modified by diet.*

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The gut microbial community in metabolic syndrome patients is modified by diet

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Abstract

Intestinal microbiota changes may be involved in the development of metabolic syndrome (MetS), which is a multicomponent disorder frequently associated with obesity. The aim of this study was to test the effect of consuming two healthy diets: a Mediterranean diet and a low-fat high-carbohydrate diet, for 2 years in the gut microbiota of MetS patients and those in the control group. We analyzed the differences in the bacterial community structure between the groups after 2 years of dietary intervention (Mediterranean or low-fat diet) through quantitative polymerase chain reaction using primers, targeting specific bacterial taxa. We observed, at basal time, that the abundance of *Bacteroides, Eubacterium* and *Lactobacillus* genera is lower in the control group than in MetS patients, while *Bacteroides fragilis* group, *Parabacteroides distasonis, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Fusobacterium nucleatum, Bifidobacterium longum, Bifidobacterium adolescentis, Ruminococcus flavefaciens* subgroup and *Eubacterium rectale* are depleted in MetS patients (all P values <.05). Additionally, we found that long-term consumption of Mediterranean diet partially restores the population of *P. distasonis, B. thetaiotaomicron, F. prausnitzii, B. adolescentis* and *B. longum* in MetS patients (all P values <.05). Our results suggest that the Mediterranean diet could be a useful tool to restore potentially beneficial members of the gut microbiota, although the stability of these changes over time still remains to be assessed.

Keywords: Microbiota; Obesity; Metabolic syndrome; Mediterranean diet; Cardiovascular disease

1. Introduction

The microbial communities harbored in the human intestine are involved in innate and adaptative immunity, as well as in controlling energy balance; they act collectively as an organ that is fully integrated in the host metabolism [1]. Despite mounting evidence in animal models for the role of the gut microbiota in body weight and obesity [2–4], studies in humans are scarce and causality is yet to be established. While a balanced microbiota confers benefits to the host, microbial imbalances have been associated with metabolic disorders such as dyslipidemia, insulin resistance and type 2 diabetes [5,6]. In fact, some studies have suggested that changes in the intestinal microbiota may trigger pathogenic mechanisms that promote inflammation, insulin resistance and the development of metabolic syndrome (MetS) [7,8]. Moreover, the loss of immunological tolerance associated with changes in the Firmicutes/Bacteroidetes ratio seems to play a significant role in the development of obesity and eventually the initiation of MetS [7].

The shaping of the gut microbiome is currently considered as a therapeutic target, since specific changes in the gut microbial community might counteract the development of obesity and MetS [9]. Although the adult human gut microbiota community is relatively stable over long periods of time [10], dietary interventions can influence its composition and could potentially be used as therapeutic tools to alleviate and treat conditions triggered by microbial imbalances [11]. In fact, it has already been shown that the consumption of a high-fat high-protein diet increases levels of *Bacteroides* versus *Prevotella*, which is more abundant after high-carbohydrate diets [12]. Moreover, the inverse relationship between *Prevotella* and *Bacteroides* has been reproduced in studies comparing the microbiota of subjects from agrarian societies with those from industrialized societies [13,14]. In addition, the consumption of diets higher in fruit, vegetables and fiber is linked to increased microbial richness, at either the taxonomic level or the gene level [15]. The gut

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microbiome can even respond to short-term modifications of macronutrient content in the diet, although it quickly returns to baseline composition after the intervention ceases [16]. It is therefore hypothesized that only long-term dietary interventions can substantially impact the microbiota [11].

In this study, our aim was to evaluate the differences in the bacterial community structure of the intestinal microbiota between MetS patients and a group of individuals without MetS and to test the effect of the long-term consumption of two healthy diets: a Mediterranean diet and a low-fat high-carbohydrate diet, in restoring the gut microbiota composition.

2. Materials and methods

2.1. Study subjects

The current work was conducted in a subgroup of 239 patients within the CORDIOPREV study (Clinical Trials.gov.Identifier: NCT00924937), an ongoing prospective, randomized, opened and controlled trial in patients with coronary heart disease (CHD), who had their last coronary event over 6 months before enrolling in two different dietary models (Mediterranean and low-fat) over a period of 5 years, in addition to conventional treatment for CHD [17]. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethics committees, following the Helsinki declaration and good clinical practice.

The 239 patients were divided into two groups: the first group consisting of 138 MetS patients was selected according to the National Cholesterol Education Program's Adult Treatment Panel III criteria for MetS [18] with increased abdominal fat waist circumference (>102 cm for males and >88 cm for females), high triglycerides (TG; ≥150 mg/dl), low high-density lipoprotein cholesterol (HDL-C; <40 mg/dl for males and <50 mg/dl for females), high fasting glucose (≥100 mg/dl), systolic arterial blood pressure of ≥130 mmHg and/or diastolic arterial blood pressure of ≥85 mmHg. The other group consisted of 101 subjects without MetS. The baseline characteristics of the subjects in the study are shown in Supplemental Table 1.

2.2. Study design

The study design has been previously described [19]. Briefly, participants of each of the two groups were randomized to receive two diets: a Mediterranean diet and a low-fat diet. The composition was as follows: (a) low-fat high-carbohydrate diet: 28% fat (12% monounsaturated, 8% polyunsaturated and 8% saturated) and (b) Mediterranean diet: 35% fat (22% monounsaturated, 6% polyunsaturated and 7% saturated). Furthermore, to ensure that the main fat source of the Mediterranean diet (olive oil) was identical for all patients in this group, the olive oil was given to the participants by the research team. Food packs, including low-fat foods (cereals, biscuits, pasta, etc.) of similar cost, were provided for the patients who were randomized to the low-fat group. Diet assessment was performed using a validated 14-item questionnaire to assess adherence to the Mediterranean diet [20] and a similar 9-point score to assess adherence to low-fat diet at baseline before the start of the dietary intervention and yearly follow-up visits.

2.3. Clinical plasma parameters

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA at baseline before the start of the dietary intervention and yearly follow-up visits. The plasma was separated from the red cells by centrifugation at 1500*g* for 15 min at 4°C. Analytes determined in frozen samples were analyzed centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, who were unaware of the interventions. Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche) using specific reagents (Boehringer Mannheim). Plasma TG and cholesterol concentrations were assayed by enzymatic procedures [21,22]. HDL-C was measured by the precipitation of a plasma aliquot with dextran sulfate-Mg^{2+}, as described by Warnick *et al.* [23]. Low-density lipoprotein cholesterol was calculated using the following formula: plasma cholesterol−(HDL-C+large TRL-C+small TRL-C). Therefore, glucose determination was performed by the hexokinase method.

2.4. DNA extraction from fecal samples

To collect the fecal samples, we gave the patients a box with carbonic snow and a sterile plastic bottle with a screw cap to keep the frozen sample. Once delivered to the laboratory staff, the sample was stored at −80°C until microbial DNA was extracted. Hence, this was performed using the QIAamp DNA Kit Stool Mini Kit Handbook (Qiagen, Hilden, Germany) following the manufacturer's instructions. This protocol was optimized for a 180- to 220-mg sample. Consequently, bacterial DNA was quantified using with a Nanodrop ND-1000 v3.5.2 spectrophotometer (Nanodrop Technology, Cambridge, UK); the samples were stored at −20°C.

2.5. Quantification of the bacterial composition by real-time quantitative polymerase chain reaction analysis

Specific primers for 16S rRNA gene in different bacterial species (Supplemental Table 2) were used to characterize the fecal microbiota using real-time quantitative polymerase chain reaction (PCR). We selected the bacterial species on the basis of finding specific primers and for being species with known functions. Each PCR reaction contained 5 ng of fecal DNA and 2 μl of each primer at a concentration of 5 pmol/μl using the iQ SYBR Green Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), in an iQ5 real-time PCR detection system thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The reaction was incubated at 95°C for 8 min, followed by 40 cycles of 1 min at 95°C, 30 s at 60°C and 20 s at 72°C.

In order to assess the specificity of the amplifications, PCR products were run on an agarose gel at 1.5% in a TBE buffer and the DNA bands were excised from the agarose gel for subsequent sequencing, which was performed at the Central Service for Research Support of the University of Cordoba. In addition, the nucleotide sequences were compared with known sequences in the GenBank database using the BLAST algorithm. Moreover, the specificity of PCR amplifications was checked in each PCR reaction by a melting curve program (60–95°C with a heating rate of 0.5°C/s and a continuous fluorescence measurement). The relative abundance of each bacterial species was calculated using the total bacterial abundance as a reference: the first two pairs of universal bacteria primer were used and then both were combined by the BestKeeper method to obtain an accurate reference value [relative abundance=$2^{-(Ct,\ target\ specie - Ct,\ reference)}$] [24].

2.6. Statistical analysis

We used PASW Statistics, Version 18 (Chicago, IL, USA) to perform the statistical analysis. The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. When variables followed a normal distribution (metabolic variables), one-factor analysis of variance was used to compare the baseline metabolic variables between the MetS and the non-MetS groups. When variables did not follow a normal distribution, we used nonparametric methods. The Mann–Whitney *U* test analysis was used to compare the statistically significant differences in the relative abundance of the bacterial species between MetS patients and the group without MetS. The statistically significant microbiota changes by diet were assessed by the Wilcoxon signed-rank test. A study of the relationship among parameters was also carried out using Pearson's linear correlation coefficient. All data presented are expressed as mean±S.E.M. A *P* value <0.05 was considered significant.

3. Results

3.1. Baseline characteristic of the study participants

No significant differences in age were observed among the groups. As expected, the MetS group had higher waist circumference, TG, glucose and blood pressure and lower HDL-C plasma levels in metabolic variables than the non-MetS group. No significant differences were observed between the patients assigned to Mediterranean or low-fat diets for either MetS or non-MetS groups (Supplemental Table 1).

3.2. MetS and gut microbiota

Relative abundance of *Bacteroides*, *Eubacterium* and *Lactobacillus* genera at basal time was higher in the MetS patients than in the non-MetS group ($P<.05$). We also analyzed the differences in the relative abundance of 18 bacterial species belonging to the most abundant phyla and genera, known to be present in human gut intestinal microbiota in both groups (Fig. 1). We observed that the relative abundance of *Bacteroides fragilis* group, *Parabacteroides distasonis*, *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, *Fusobacterium nucleatum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Ruminococcus flavefaciens* subgroup and *Eubacterium rectale*, at basal time, was lower in MetS patients than in the control subjects ($P<.05$).

3.3. Relationship between MetS-related variables and the gut microbiota

We observed a negative relationship between the waist circumference and the relative abundance of *B. thetaiotaomicron*, *P. distasonis*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup and *B. adolescentis* ($R=-0.162$, $P=.022$; $R=-0.213$, $P=.002$; $R=-0.294$, $P<.001$; $R=-0.297$, $P<.001$; $R=-0.176$, $P=.013$; $R=-0.211$, $P=.003$). We

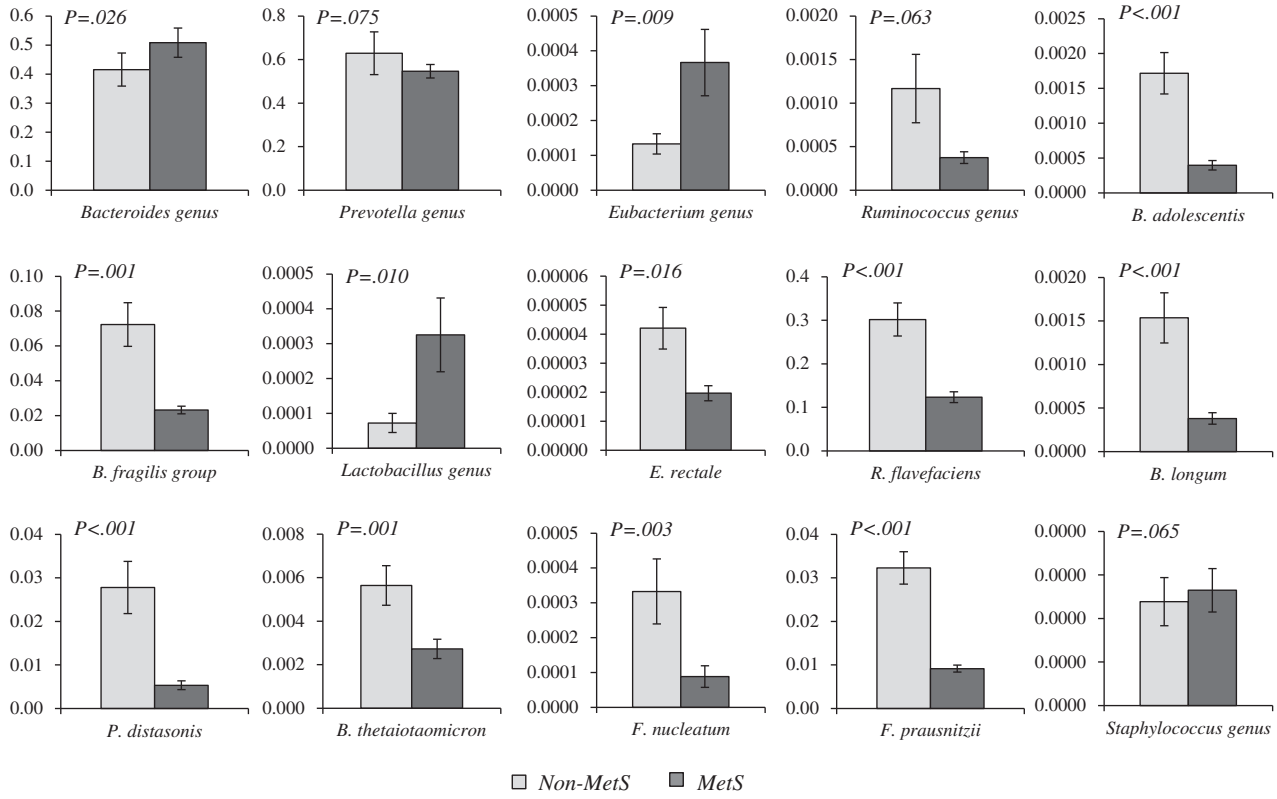

Fig. 1. Differences in the gut microbiota composition of MetS patients. Values are the mean±S.E.M. The statistically significant differences between each group were tested by the Mann–Whitney test.

also observed a positive relationship between the c-HDL plasma levels and the relative abundance of *B. fragilis* group, *B. thetaiotaomicron*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup, *B. adolescentis* and *F. nucleatum* (R=0.146, P=.039; R=0.265, P<.001; R=0.296, P<.001; R=0.190, P=.007; R=0.163, P=.021; R=0.141, P=.046; R=0.140, P=.048). Furthermore, there was a negative relationship between the TG plasma levels and the relative abundance of *B. fragilis* group, *B. thetaiotaomicron*, *P. distasonis*, *F. prausnitzii*, *B. longum*, *R. flavefaciens* subgroup and *B. adolescentis* (R=−0.175, P=.013; R=−0.211, P=.003; R=−0.200, P=.005; R=−0.279, P<.001; R=−0.145, P=.040; R=−0.275, P<.001; R=−0.174, P=.014). In addition a negative relationship exists between the glucose plasma levels and the relative abundance of *P. distasonis* and *B. longum* (R=−0.161, P=.022; R=−0.145, P=.040). Finally, there was also a negative relationship between the systolic blood pressure and the relative abundance of *B. longum* (R=−0.167, P=.018) (Supplemental Fig. 1).

3.4. Mediterranean diet affects microbiota composition

In order to assess whether diet significantly impacts the microbiota profile of MetS patients, we analyzed bacterial composition after 2 years of consumption of a Mediterranean or a low-fat diet (Table 1). We observed that Mediterranean diet induced a statistically significant increase in the abundance of *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *B. adolescentis* and *B. longum* (P<.05) in the MetS, but not in the non-MetS group. By contrast, we observed a statistically significant increase in the abundance of *E. rectale* (P<.05) in the non-MetS, but not in the MetS group. Additionally, the consumption of the low-fat diet for 2 years decreased the abundance of *P. distasonis* (P<.05) in the non-MetS group, which remained unchanged in the MetS patients group.

We also observed a weak but significant relationship between the Mediterranean diet score after 2 years of dietary intervention and the abundance of *F. prausnitzii* (R=0.158, P=.028), as well as the changes in the abundance of *B. adolescentis* (R=0.147, P=.040).

4. Discussion

Our data show that the *Bacteroides*, *Eubacterium* and *Lactobacillus* genera were significantly increased, while *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *F. nucleatum*, *B. longum*, *B. adolescentis*, *R. flavefaciens* subgroup and *E. rectale* were decreased significantly in MetS patients compared with the non-MetS group. More interestingly, our results suggest that long-term consumption of Mediterranean diet increases the abundance of *P. distasonis*,

Table 1
Diet-induced relative abundance fold change in microbiota composition in the panel of bacterial species.

Experimental group	Non-MetS group		MetS group	
Bacterial species/diet	Low fat	Mediterranean	Low fat	Mediterranean
P. distasonis	**0.87±0.29** *	1.70±0.23	1.79±0.22	**1.75±0.22** *
B. thetaiotaomicron	1.38±0.25	1.37±0.20	1.29±0.19	**1.58±0.19** *
F. prausnitzii	1.47±0.28	1.63±0.23	1.29±0.21	**1.78±0.22** *
B. adolescentis	1.11±0.34	1.50±0.28	1.40±0.26	**2.26±0.27** *
B. longum	1.54±0.33	1.16±0.27	1.58±0.25	**2.01±0.25** *
E. rectale	1.08±0.25	**1.90±0.21** *	1.39±0.19	1.23±0.20
B. fragilis group	1.51±0.26	1.65±0.21	1.17±0.20	1.37±0.20
R. flavefaciens subgroup	1.32±0.29	1.64±0.24	1.10±0.22	1.63±0.22
F. nucleatum	1.32±0.27	1.50±0.22	1.10±0.21	1.04±0.21

Fold change normalized versus relative abundance values at baseline. The statistically significant microbiota changes by diet were assessed by the Wilcoxon signed-rank test.
* P<.05.

B. thetaiotaomicron, *F. prausnitzii*, *B. adolescentis* and *B. longum* in the MetS patients, although MetS persists.

Several studies have shown evidence that alterations in gut microbiota may lead to obesity and MetS, directly or as a consequence of the disturbances in the gut microbiota that causes the "low-grade" inflammation that may promote the development of MetS [25,26]. In this regard, we note that our results show a negative correlation between the abundance of *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *F. nucleatum*, *B. longum*, *B. adolescentis*, *R. flavefaciens* subgroup and *E. rectale* with plasma levels of glucose and TG and a positive correlation with plasma levels of HDL. These results further support the idea that gut microbiota acts collectively as a fully integrated organ in the host metabolism [1]. However, it also modulates host energy and lipid metabolism [4]. Thus, changes in the intestinal microbiota may trigger pathogenic mechanisms once obesity is established; this promotes insulin resistance and the development of MetS [7–9].

Moreover, the observed reduction in MetS patients in the abundance of several bacterial species within the *Bacteroides* and *Ruminococcus* genera with important saccharolytic activity, such as *B. fragilis* group, *P. distasonis*, *B. thetaiotaomicron* and the *R. flavefaciens* subgroup [27–29], suggests a reduction in carbohydrate degradation capacity in MetS patients, which may also cause a reduction in propionate and acetate production [30,31]. The latter point is particularly relevant in this context, as a reduction of acetate levels in the gut may also reduce the abundance of beneficial bacteria (as observed in our study) such as *F. prausnitzii* and *E. rectale*. Hence, this bacteria consume acetate and produce butyrate [32,33], in addition to the decrease in *E. rectale*, *F. nucleatum* and *F. prausnitzii*, which directly degrade carbohydrate to produce butyrate.

Although previous studies have described an individual-specific microbiota with high stability over time [10] and resistance to perturbations [34,35], recent research indicates that changes in the gut microbiota composition may occur after dietary interventions [12,16,36,37] and that long-term periods following a specific diet can affect the microbiota in a substantial way [11]. Our results further strengthen this hypothesis, as the consumption of a Mediterranean diet over 2 years resulted in a significant modification of the gut microbiota composition of MetS patients.

Previous studies have shown that specific foods consumed in the traditional Mediterranean diet have an influence on the gut microbiota composition [38,39]. Antioxidant phenolic compounds are consumed in the Mediterranean diet through different products such as fresh fruit, vegetables, red wine and olive oil. In fact, it has been shown that red wine consumption increases the growth of *Enterococcus*, *Prevotella*, *Bacteroides* and *Bifidobacterium* genera abundance in healthy humans [37]. In addition, a study performed using culture fermentation systems reflective of the distal region of the human large intestine showed that a pomegranate product significantly enhances the growth of *Bifidobacteria* and *Lactobacilli* [40], suggesting that the fruit, another source of antioxidants in Mediterranean diet, may also influence gut microbiota composition.

In line with this, our study showed that the consumption of a Mediterranean diet, containing phenolic-compound-rich foods such as fresh fruit, vegetables, red wine and olive oil, is more effective in increasing the levels of bacterial species found to be lower in MetS patients, such as *P. distasonis*, *B. thetaiotaomicron*, *F. prausnitzii*, *B. adolescentis* and *B. longum*. Consequently, the consumption of a low-fat diet that is more abundant in whole grains significantly lowers in sources of phenolic compounds and lowers in fiber intake than the Mediterranean diet, which did not result in a similar increase in the abundance of these bacteria. Hence, this was evidenced by the nutritional assessment of the diet compliance by surveys. Moreover, the positive correlation between Mediterranean diet score and the abundance of *F. prausnitzii* and *B. adolescentis* further supports the hypothesis that Mediterranean diet induces significant changes in gut microbiota composition.

Additionally, in terms of fat percentage, our study supports the idea that the consumption of diet with a high percentage of fat as Mediterranean diet in comparison with the low-fat diet administered increases the abundance of bile resistance taxa such as *Bacteroides* [16]. However, this is because the intake of fat increases the secretion of bile acids [41].

Moreover, it is particularly important due to the fact that the consumption of Mediterranean diet increased the abundance of the *Bacteroides* genus member *B. thetaiotaomicron* and *F. prausnitzii*, which suggest that the consumption of this diet may increase or maintain a microbiota with antiinflammatory capability [42]. Thus, this is in agreement with the antiinflammatory effects associated with the consumption of Mediterranean diet consumption [43]. Overall, our study showed that the consumption of Mediterranean diet influenced the gut microbiota composition mainly in the MetS patients. This was presumed by the dysbiosis observed in this population compared to the non-MetS, suggesting that its consumption may help in maintaining the gut microbiota homeostasis, which is particularly important in conditions of an alteration of microbiota such as obesity and MetS. Hence, this could contribute to explaining the low rates of cardiovascular mortality found in Southern European Mediterranean countries, in comparison with other Western populations [44].

However, our study has the limitation that the relationship between cardiovascular risk factors and microbiota species, although significant, was low. Moreover, the long-term consumption of a Med diet partially restores the alteration in the gut microbiota composition observed in MetS patients, without the disappearing of the syndrome, which suggests that longer periods of Med diet consumption may be needed.

In conclusion, our results suggest that Mediterranean diet could be a useful tool in manipulating the gut microbiota. Thus, further studies will be required to fully understand the effect of the Mediterranean diet in shaping gut microbiota and its effect on human health.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2015.08.011.

Conflict of Interest

None of the authors has any conflict of interest that could affect the performance of the work or the interpretation of the data.

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2. *Two healthy diets modulate gut microbial community improving insulin sensitivity in a human obese population.*

Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population

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Context: Gut microbiota, which acts collectively as a fully integrated organ in the host metabolism, can be shaped by long-term dietary interventions after a specific diet.

Objective: The aim was to study the changes in microbiota after 1 year's consumption of a Mediterranean diet (Med diet) or a low-fat, high-complex carbohydrate diet (LFHCC diet) in an obese population.

Design: Participants were randomized to receive the Med diet (35% fat, 22% monounsaturated) and the LFHCC diet (28% fat, 12% monounsaturated).

Setting and Participants: The study was conducted in 20 obese patients (men) within the Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention (CORDIOPREV) study, an ongoing prospective, randomized, opened, controlled trial in patients with coronary heart disease.

Main Outcome Measure: We evaluated the bacterial composition and its relationship with the whole fecal and plasma metabolome.

Results: The LFHCC diet increased the *Prevotella* and decreased the *Roseburia* genera, whereas the Med diet decreased the *Prevotella* and increased the *Roseburia* and *Oscillospira* genera ($P = .028$, .002, and .016, respectively). The abundance of *Parabacteroides distasonis* ($P = .025$) and *Faecalibacterium prausnitzii* ($P = .020$) increased after long-term consumption of the Med diet and the LFHCC diet, respectively. The changes in the abundance of 7 of 572 metabolites found in feces, including mainly amino acid, peptide, and sphingolipid metabolism, could be linked to the changes in the gut microbiota.

Conclusions: Our results suggest that long-term consumption of the Med and LFHCC diets exerts a protective effect on the development of type 2 diabetes by different specific changes in the gut microbiota, increasing the abundance of the *Roseburia* genus and *F. prausnitzii*, respectively. **(*J Clin Endocrinol Metab* 101: 233–242, 2016)**

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Abbreviations: CHD, coronary heart disease; CORDIOPREV, Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention; LFHCC diet, low-fat, high-complex carbohydrate diet; Med diet, Mediterranean diet; OGTT, oral glucose tolerance test; OTU, operational taxonomic unit; SCFA, short-chain fatty acid; T2D, type 2 diabetes.

Obesity was formerly thought to be caused only by a positive caloric balance when caloric intake exceeds caloric expenditure and the excess of energy is stored in adipose tissue (1). However, this simple idea has been altered for different reasons, because gut microbiota was proposed as an additional contributing factor to the pathophysiology of obesity—a link between gut microbial ecology and obesity (2). In fact, several studies have suggested that changes in the gut microbiota trigger pathogenic mechanisms to promote the development of obesity, type 2 diabetes (T2D), and metabolic syndrome (3, 4).

Gut microbiota, the complex, diverse, and vast microbial community harbored in the human intestine (5, 6), acts collectively as a fully integrated organ in the host metabolism, involved in extracting energy from nutrients, regulating innate and adaptive immunity, and helping to control the energy balance (7). Animal model studies have shown that obesity is associated with an increase in the Firmicutes/Bacteroidetes ratio, also known as "obese microbiota," which is transmissible between individuals (2). Moreover, the intestinal absorption of bacterial components, such as the endotoxin lipopolysaccharide, bacterial DNA, and flagellins, activate the Toll-like receptors, inducing inflammation, which favors insulin resistance (8), although it is not well established whether insulin resistance precedes the changes in the microbiota or vice versa (9). In addition, microbiota produces bioactive metabolites, such as short-chain fatty acids (SCFAs), originated by the fermentation of carbohydrates, which are involved in energy metabolism and appetite regulation, promoting healthy body weight, and secondary bile acid, which play a key role in glucose metabolism (10, 11).

Studies in humans have shown that gut microbiota seem to have coevolved with the dietary habits of the population. For example, the microbiota of children in Burkina Faso is adapted to extract calories from the polysaccharide-rich (carbohydrates and fibers) diet consumed in that country (12). Thus, the human gut microbiota community seems to be very stable and more influenced by ambient and dietary factors than by genetic factors. However, there have been very few studies on the modification of microbiota composition by dietary intervention (13). Moreover, it has been shown that the consumption of a Western diet increases endotoxemia, which suggests a disruption of the intestinal barrier in addition to an increase in the Gram-negative bacterial content of the microbiota (14). In fact, a high-fat, high-carbohydrate meal induces comprehensive endotoxemia and inflammation, increasing the expression of Toll-like receptor-4, the specific receptor for endotoxin, and suppressor of cytokine signaling-3, a protein that interferes with insulin signal transduction (15), whereas a high-fruit, high-fiber meal or the intake of orange juice or a polyphenol preparation with resveratrol does not induce any of these effects (16, 17).

Although previous studies have pointed out that microbiota is individual specific and shows high stability and resistance to perturbations over time (18, 19), recent research indicates that changes in gut microbiota composition may occur after dietary interventions (14, 20–22). In addition, whereas short-term dietary interventions tend to induce only minor changes, long-term periods of consumption of a specific diet may affect the microbiota to a substantial degree (13). For example, it has been shown that the consumption of a high-fat, high-protein diet increases the abundance of Bacteroidetes vs *Prevotella*, which is more abundant after high-carbohydrate diets (21). In addition, we have shown that the consumption of a Mediterranean diet (Med diet) partially restores the alteration in the gut microbiota composition observed in patients with metabolic syndrome (22).

Our aim was to study the lasting changes in the microbiota after the long-term consumption (1 year) of a Med diet and a low-fat, high-complex carbohydrate diet (LFHCC diet) in an obese population with coronary heart disease (CHD), using the 16S sequencing method and analyzing the relationship between specific bacteria and the metabolomics profile found in feces and plasma.

Materials and Methods

Study subjects

The current work was conducted in a subgroup of 20 obese patients (men) within the Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention (CORDIOPREV) study (Clinical Trials.gov registration no. NCT00924937), an ongoing prospective, randomized, opened, controlled trial in patients with CHD, who had their last coronary event more than 6 months before enrollment on 2 different dietary models (Med diet and LFHCC diet) over a period of 5 years, in addition to conventional treatment for CHD. CORDIOPREV inclusion and exclusion criteria are summarized as follows: patients were eligible if they were >20 but <75 years old, had established CHD without clinical events in the last 6 months, were thought to follow a long-term dietary intervention, and did not have severe diseases or an estimated life expectancy of <5 years (23). Antibiotic usage was included as an exclusion criterion for the current study, in addition to the general exclusion criteria defined in the CORDIOPREV study. All the subjects were receiving a standardized treatment for CHD. No differences were observed between groups. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethic committees, following the Helsinki Declaration and good clinical practices. The baseline characteristics of the study subjects are shown in Table 1.

Table 1. Baseline Characteristics of the Study Population

Parameter	LFHCC Diet	Med Diet	*P* Value
Age, y	61.4 ± 2.6	65.2 ± 3.2	.362
Weight, kg	86.9 ± 1.9	88.3 ± 1.8	.606
Waist circumference, cm	105.9 ± 2.5	109.6 ± 2.2	.285
BMI, kg/m^2	31.6 ± 0.8	32.8 ± 0.5	.214
Glucose, mg/dL	94.2 ± 3.1	91.2 ± 2.7	.466
HbA_{1c}, %	6.3 ± 0.1	6.0 ± 0.1	.312
SBP, mm Hg	129.0 ± 9.4	136.0 ± 3.7	.495
DBP, mm Hg	72.7 ± 3.6	75.1 ± 3.5	.635
TG, mg/dL	102.2 ± 8.1	98.7 ± 7.6	.757
TC, mg/dL	149.8 ± 6.8	150.2 ± 7.0	.968
HDL, mg/dL	41.7 ± 2.5	42.1 ± 1.9	.900
LDL, mg/dL	83.7 ± 4.9	88.0 ± 6.4	.599

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. Values are means ± SEM. One-way ANOVA *P* values.

Study design

The study design was described previously (23). In brief, participants were randomized to receive 2 diets: a Med diet and an LFHCC diet. The compositions were as follows: the LFHCC diet contained 28% fat (12% monounsaturated; 8% polyunsaturated and 8% saturated) and the Med diet contained 35% fat (22% monounsaturated; 6% polyunsaturated and 7% saturated). To ensure that the main fat source of the Med diet (olive oil) was identical for all patients in this group, the olive oil was given to the participants by the research team. Food packs, including low-fat foods (eg, cereals, biscuits, and pasta) of similar cost, were provided for the patients randomized to the low-fat group.

Clinical plasma parameters

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. The plasma was separated from red cells by centrifugation at 1500 × *g* for 15 minutes at 4°C. Analytes determined in frozen samples were studied centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, Córdoba, Spain, who were unaware of the interventions. An oral glucose tolerance test (OGTT) was performed (75 g of dextrose monohydrate in 250 mL of water) with sampling at 0, 30, 60, and 120 minutes to establish plasma glucose and insulin levels (for details, see Supplemental Materials and Methods).

DNA extraction from fecal samples

DNA extraction was performed using the QIAamp DNA Stool Mini Kit Handbook (QIAGEN), following the manufacturer's instructions, and quantified using a NanoDrop ND-1000 v3.5.2 spectrophotometer (NanoDrop Technology).

Microbiota analysis

A total of 40 fecal samples (20 basal and 20 after a year of dietary intervention, for each participant) were used for the microbial community analysis using the 454 Life Sciences (Roche) Junior platform, according to standard 454 platform protocols (see Supplemental Materials and Methods).

Phylogenetic analysis of sequencing reads

The samples were processed and analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (version v1.8.0; http://qiime.org/) with default parameters unless otherwise noted (see Supplemental Materials and Methods).

Metabolomic analysis

Samples were sent to Metabolon and prepared using the automated MicroLab STAR system from Hamilton Company (see Supplemental Materials and Methods).

Statistical analysis

All of the data presented are expressed as means ± SEM. PASW statistical software, version 20.0 (IBM Inc) was used for the statistical analysis of individual data. We analyzed the changes in the abundance of bacterial genera and species when detected in at least 8 subjects per diet. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. The data were analyzed using ANOVA for repeated measures with time as the intrasubject factor and diet as the intersubject factor. Post hoc statistical analysis was performed by using the Bonferroni multiple comparison test. A study of the relationship among parameters was also performed using the Pearson linear correlation coefficient. A *P* value of $<.05$ was considered significant.

Results

Baseline characteristic of the study population

No significant differences ($P > .05$) were observed in the baseline characteristics of the 20 obese people participating in the dietary intervention (Table 1). In brief, the subjects (20 men) had an average age of 63.3 ± 2.0 years and an average body mass index of 32.2 ± 0.5 kg/m^2.

Effect of the dietary intervention on the main metabolic variables

No statistically significant differences ($P > .05$) were observed in the main metabolic variables of the 20 obese people after 1 year of dietary intervention (Table 2). However, we observed an increase in the insulin sensitivity index for both the LFHCC and Med diets ($P = .019$ and $P = .005$, respectively), when measured from an OGTT performed at basal time and after 1 year of dietary intervention.

Microbiota composition of the study population

Global pattern

For the bacterial community analyses of the 40 samples, after screening our data for poor quality sequences, we recovered 162 871 high-quality 16S rRNA gene sequences with an average of 4072 ± 2745 (SD) sequences per sample. From those, we obtained a total of 140 566 sequences (86% of the total), which could be classified

Table 2. Effect of the Dietary Intervention on the Main Metabolic Variables in the Population in Study

			P Value		
	LFHCC Diet	Med Diet	Diet	Time	Interaction
Glucose (mg/dL)					
Basal	94.2 ± 3.1	91.2 ± 2.8	.609	.059	.679
1 year	97.9 ± 3.6	96.8 ± 3.5			
HbA_{1c} (%)					
Basal	6.30 ± 0.17	6.06 ± 0.16	.520	.049	.115
1 year	6.06 ± 0.14	6.03 ± 0.13			
ISI					
Basal	3.07 ± 0.58	3.13 ± 0.58	.762	.001	.660
1 year	5.40 ± 1.13[a]	6.02 ± 1.13[a]			
TG (mg/dL)					
Basal	102.2 ± 7.9	98.7 ± 7.9	.350	.499	.313
1 year	104.8 ± 11.5	85.8 ± 11.5			
TC (mg/dL)					
Basal	149.8 ± 6.9	150.2 ± 6.9	.696	.254	.501
1 year	142.2 ± 5.8	148.2 ± 5.8			
HDL (mg/dL)					
Basal	40.3 ± 2.4	42.1 ± 2.4	.240	.459	.250
1 year	39.7 ± 2.0	44.8 ± 2.0			
LDL (mg/dL)					
Basal	83.7 ± 5.7	88.0 ± 5.7	.526	.410	.917
1 year	81.0 ± 5.1	85.9 ± 5.1			

Abbreviations: DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; HDL, high-density lipoprotein; ISI, insulin sensitivity index; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides. Values are means ± SEM. ANOVA for repeated-measures *P* values.

[a] $P < .05$, post hoc Bonferroni's multiple comparison tests between the values at 1 year and the values at baseline.

into operational taxonomic units (OTUs) with a mean of 3514 classifiable sequences per sample, that were used in the subsequent analyses (range, 945–13 788). Of these nonsingleton OTUs, 74 430 were found in the subjects that consumed the low-fat diet, and 68 136 were found in the subjects that consumed the Med diet.

The phylogenetic characterization of all the samples uncovered 3 main bacterial phyla in the following proportions: Bacteroidetes (57.3%), Firmicutes (39.6%), and Proteobacteria (1.8%). Less abundant bacterial phyla (<0.4%–0.001%) including Tenericutes, Actinobacteria, Fusobacteria, Verrucomicrobia, and Lentisphaerae were also present (Figure 1). Across all taxa, 116 genera and 10 480 OTUs, with an average of 768 observed OTUs per sample, were identified. The main taxa that accounted for 70.5% of the sequences were *Bacteroides* (33.0%), *Prevotella* (13.7%), unknown Lachnospiraceae (6.2%), *Faecalibacterium* (4.1%), unknown Clostridiales (3.1%), unknown Ruminococcaceae (3.0%), *Oscillospira* (2.7%), *Parabacteroides* (2.4%), and unknown Bacteroidales (2.3%). At the species level, 3 unknown *Bacteroides* (3.9%), 2 *Prevotella copri* (2.6%), *Bacteroides uniformis* (1.5%), *Bacteroides plebeius* (0.8%), and 1 unknown Lachnospiraceae (0.7%) represented the most abundant taxa (10.2%).

α- and β-diversity and taxon representation patterns between diet samples

First, we used jackknifed hierarchical clustering (unweighted pair group method with arithmetic mean), based on weighted UniFrac distances, to investigate the relationships among the bacterial communities of the different samples according to the diet, after 1 year of intake. In an initial analysis, we examined the results from 40 samples and 2 sequencing runs in which the sampling yielded sufficient depth (>1000 sequences per samples, with the exception of 1 sample). With this analysis, most samples, with the exception of those for 2 individuals, were grouped according to the subject independently of the sampling period. In this way, samples from each patient at time zero (T0) and after 1 year of dietary intervention (T1) were grouped together. To test whether the outgrouping of those 2 subjects was due to amplification or sequencing errors, we performed a third run including 6 subjects at both sampling times (12 samples). The clustering of the samples from this new run paired them with the expected sample from previous runs, which supported the idea that a major change in gut microbiota occurred for those 2 subjects in 1 year. Finally, we combined all of the sequences from the 3 runs for each patient to have only 1 dataset per patient and sampling period for downstream analysis. Unweighted UniFrac analysis based on either a

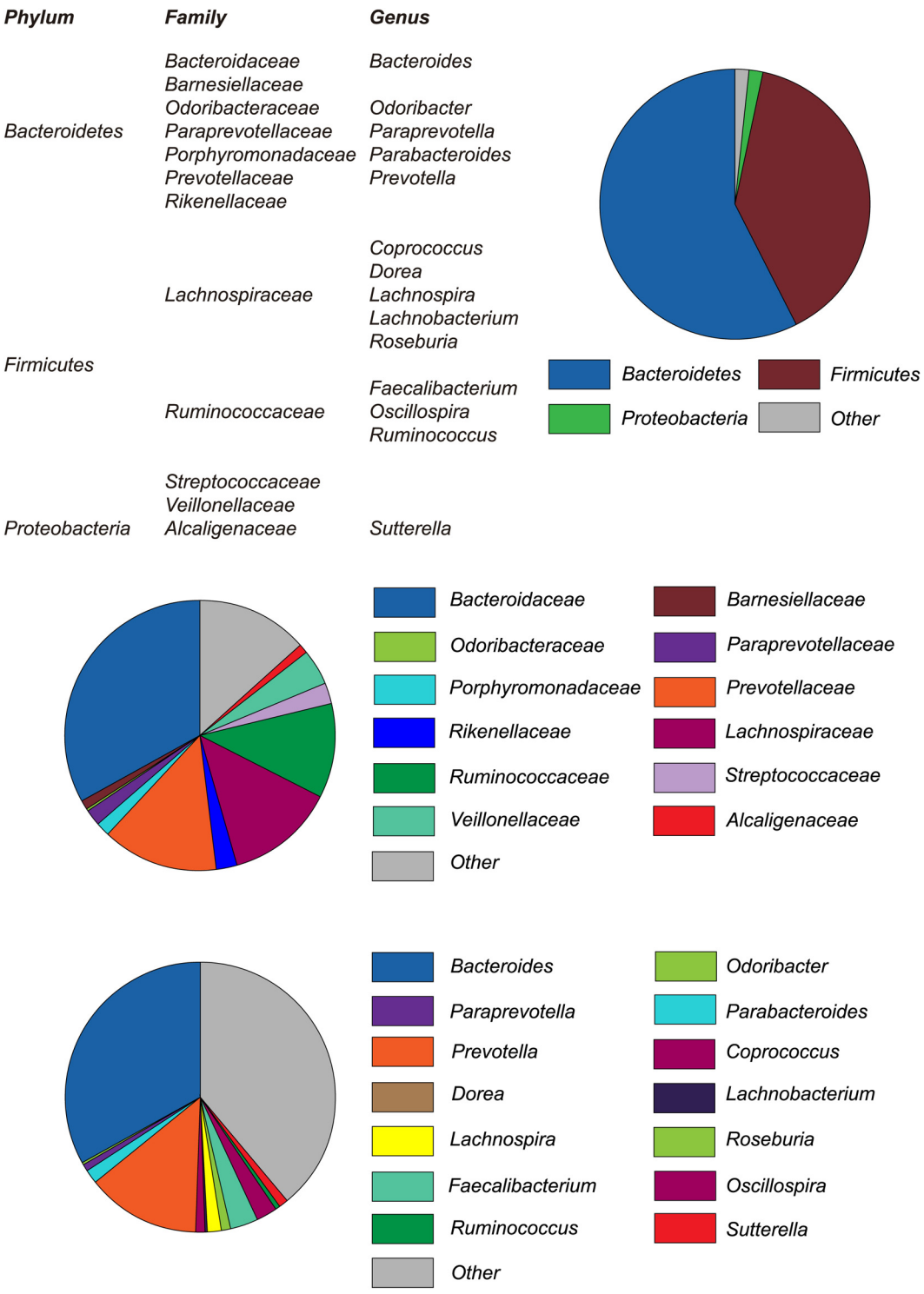

Figure 1. Microbiota composition of the study population.

hierarchical unweighted pair group method with arithmetic mean tree or principal coordinates analysis did not segregate the subject samples into different clusters according to the diet intervention or the sampling period, suggesting that the microbiota of the subjects was stable and similar in terms of proportion of the main taxonomic phyla. Thus, the global bacterial profile of feces samples from each individual cluster together and separately from those of other subjects (Supplemental Figure 1).

When α-diversity indexes (Chao1, richness and PD estimation) for the samples at the level of 800 per sample were analyzed, the samples from the different diets after 1 year of intake showed similar numbers of the 3 estimated α-diversity indexes ($P > .180$) as determined by a non-

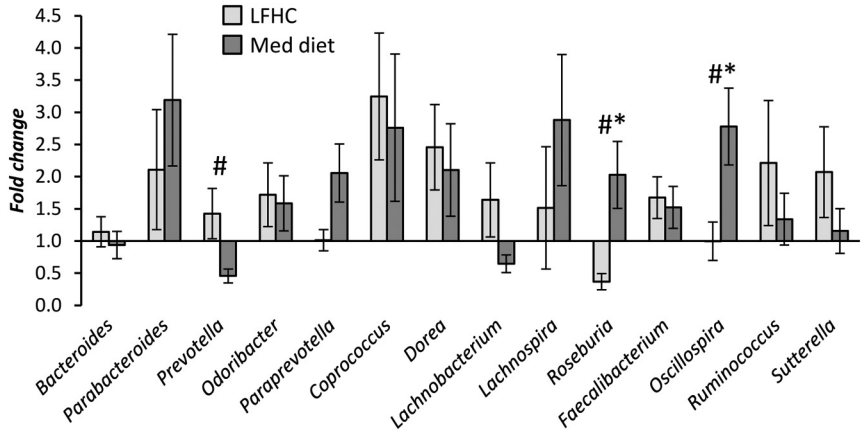

Figure 2. Microbiome changes by diet in the bacterial genus most commonly represented in our population. Values represent means ± SEM of the fold change after the consumption of LFHCC or Med diet. Fold change was calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. #, $P < .05$ between sampling time by diet interaction in the ANOVA for repeated measures. *, $P < .05$ in the post hoc Bonferroni multiple comparison tests between the bacterial abundance at 1 year and the abundance at baseline.

parametric two-sample *t* test. The rarefaction α-diversity curves level off, showing that the sequencing effort was sufficient to detect most of the OTUs for all samples (Supplemental Figure 2).

Changes in specific bacterial taxa and their relationships with the consumption of different diets

To assess whether specific changes occurred in some bacterial taxa after the intake of each diet, we compared the frequency of occurrence of each taxa between both sampling times for each diet after intrasubject normalization. We observed an increase in the relative abundance of the *Prevotella* bacterial genus with the LFHCC diet, whereas a decrease was observed after the Med diet (time × diet interaction $P = .028$). Furthermore, the Med diet increased the relative abundance of *Roseburia* and *Oscillospira* bacterial genera ($P = .017$ and $P = .001$, respectively), whereas the LFHCC diet decreased the relative abundance of *Roseburia* without changing the abundance of the *Oscillospira* bacterial genera (time × diet interaction $P = .009$ and .016, respectively) (Figure 2). On the other hand, in terms of bacterial species, long-term consumption of the Med diet increased the relative abundance of *Parabacteroides distasonis* ($P = .025$), whereas the LFHCC diet increased *Faecalibacterium prausnitzii* ($P = .020$) (Table 3).

Relationship between diet-induced changes in metabolome and changes in microbiota

A total of 572 compounds were identified in feces. Analysis by ANOVA for repeated measures identified 37 compounds exhibiting a significant interaction between time and diet. The main diet-induced effects on the fecal metabolome were changes in amino acid, peptide, and sphingolipid metabolism. The plasma analysis identified 697 compounds. Analysis by ANOVA for repeated measures identified 6 compounds exhibiting significant interaction between time and diet, principally in amino acid and peptide metabolism. To analyze the relationship between the observed changes in both microbiota composition and fecal and plasma metabolome, we performed a correlation analysis between the individual fold changes in the bacterial genera and species whose abundance significantly changed by the consumption of either of the diets (6 bacterial genera and 2 bacterial species), and the individual fold changes of the metabolites with diet-induced statistically significant changes (43 metabolites: 37 metabolites in feces and 6 metabolites in plasma). This strategy allowed us to avoid interindividual variability, and in addition, search for relationships between fold changes (relationship in a dynamic system) instead of using the values obtained at basal or at the end of the intervention (relationship in a static system), thus strengthen-

Table 3. Microbiome Changes by Diet in the Bacterial Species Most Commonly Represented in Our Population

			P Value	
Bacterial Species	**LFHCC Diet**	**Med Diet**	**Time**	**Interaction**
Bacteroides ovatus	1.37 ± 0.42	0.78 ± 0.20	.734	.236
Bacteroides plebeius	1.75 ± 0.46	1.56 ± 0.67	.147	.836
Bacteroides uniformis	0.98 ± 0.23	0.83 ± 0.21	.556	.667
Parabacteroides distasonis	1.42 ± 0.40	2.32 ± 0.62[a]	.034	.296
Coprococcus eutactus	2.28 ± 0.88	1.51 ± 0.45	.104	.469
Faecalibacterium prausnitzii	1.91 ± 0.27[a]	1.53 ± 0.33	.007	.516

Means values ± SEM of the abundance of bacterial species; fold change was calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. ANOVA for repeated-measures *P* values.

[a] $P < .05$, post hoc Bonferroni's multiple comparison tests between the abundance at 1 year and the abundance at baseline.

Table 4. Relationship Between the Observed Changes in Both Microbiota Composition and Fecal and Plasma Metabolome

	FC LFHCC Diet	FC Med Diet	*P* Value Interaction	Bacterial Genera and Species	Pearson Coefficient	*P* Value for Pearson Correlation
Metabolite in feces						
N-Acetylaspartate	0.50	1.01	.020	*Roseburia*	0.734	<.001
Glutamate	1.25	0.69	.018	*Prevotella; Oscillospira*	0.498; −0.520	.025; .019
Arginylproline	1.78	0.85	.007	*Prevotella; Roseburia*	0.490; −0.467	.028; .044
Leucylvaline	1.87	0.79	.039	*Faecalibacterium prausnitzii*	0.470	.037
Pantothenate	1.56	0.69	.027	*Oscillospira*	−0.474	.035
cis-Vaccenate (18:1n7)	0.43	1.41	.010	*Oscillospira*	0.511	.021
3,7-Dimethylurate	2.48	0.29	.044	*Prevotella*	0.499	.025
Metabolite in plasma						
XHWESASXXR	0.02	1.72	.042	*Roseburia*	0.479	.038
Valylglutamine	0.26	0.99	.045	*Oscillospira*	0.563	.010
Glycocholenate sulfate	1.14	0.84	.027	*Bacteroides uniformis*	0.450	.049

Abbreviation: FC, fold change in metabolite abundance calculated by dividing the bacterial abundance at 1 year by the abundance at baseline. *P* value interaction: ANOVA for repeated-measures *P* value for time by diet interaction.

ing our findings. Our results showed that the changes in 7 of 572 metabolites in feces and 3 of 697 metabolites in plasma were related to the changes in 3 bacterial genera and 2 bacterial species (Table 4).

Discussion

Based on the jackknifed hierarchical clustering analysis, our data suggest that interindividual differences in the microbiota are greater than the changes undergone by the diet. However, after we normalized between individuals, several diet-induced changes were observed after 1 year of dietary intervention. The principal findings were an increase in relative abundance of *Prevotella* and a decrease in *Roseburia* genera after the LFHCC diet, whereas the Med diet significantly increased the abundance of the *Roseburia* and *Oscillospira* genera. Finally, a complete fecal metabolome analysis showed changes in the feces of 10 metabolites involved in amino acid, peptide, and sphingolipid metabolism, reflecting the changes in gut microbiota.

The increase in the *Prevotella* genus with the LFHCC diet might be expected, in agreement with the findings in the Burkina Faso population. It is known that *Prevotella* (together with other genera such as *Xylanibacter*, *Butyrivibrio*, and *Treponema*) may enhance the ability to extract calories from resistant starch and oligosaccharides, as well as carbohydrates that escape digestion in the small intestine and are fermented in the gut (24). In addition, another study showed the strong association of the *Prevotella*-rich enterotype with long-term diets rich in carbohydrates (21). In addition, an interesting finding was that the LFHCC diet increased the abundance of *F. prausnitzii*, a butyrate-producing bacteria whose abundance has been found to decrease in patients with T2D (25, 26) and that is negatively associated with inflammatory markers in T2D (27). Moreover, we have previously shown that Med diet consumption increases the abundance of several butyrate-producing bacteria including *F. prausnitzii* in patients with metabolic syndrome, whereas no changes were observed in this bacterial species after the consumption of an LFHCC diet (22), which suggests that the degree of dysbiosis associated with the pathophysiological conditions may be a determinant in the response to a specific therapeutic diet-based treatment. Although *Roseburia* spp. have been shown to decrease after high-fat feeding in animal models (28), in humans this does not seem to be the case, as Med diet consumption increased its abundance, despite it being a high-fat diet compared with the LFHCC diet. This butyrate-producing genus comprises a high proportion of the human bacteria and could play an important role in the maintenance of gut health (29), taking into account the fact that the abundance of this genus, known to be anti-inflammatory, has been found to be low in T2D patients (25, 26). This finding suggests a protective effect of Med diet consumption on the development of T2D, as evidenced from epidemiological studies (30, 31). Considering that *Roseburia* produces an inhibitory substance against *Bacillus subtilis* (28), this result suggests that some of the changes in the microbiota induced by the Med diet could be mediated by the antimicrobial effect of this genera, which modifies the microbial population in the colon. In addition, the Med diet increased the relative abundance of *Oscillospira*, a genus belonging to the Ruminococcaceae family, associated with the feeding of fresh forage in ruminants such as cattle and sheep, suggesting a microbiota adaptation to a vegetable-rich diet such as the Med diet (32). In agreement with that, Med diet consumption

has been associated with the low rate of cardiovascular mortality found in Mediterranean countries (33, 34).

In summary, the abundance of 2 bacterial populations related with T2D (25–27), the *Roseburia* genus and the bacterial species *F. prausnitzii*, was modulated by diet. On the one hand, the consumption of the Med diet increased the abundance of the *Roseburia* genus (found to be low in patients with T2D), in parallel with an increase in insulin sensitivity after the consumption of this diet for 1 year. On the other hand, LFHCC consumption decreased the abundance of this genus, while increasing the abundance of another diabetes-protective bacterial species, *F. prausnitzii* (found to be low in patients with T2D). These 2 changes could have a protective influence for the prevention of T2D, as suggested by the findings of an improvement in insulin sensitivity after the consumption of the LFHCC (evaluated with the OGTT). These data suggest that the consumption of the LFHC and Med diets could be a therapeutic and preventive tool for T2D, and this could open a new hypothesis to be tested in the future in bigger populations: on whether the consumption of healthy diets reduces the risk of T2D by influencing the microbiota profile.

The metabolic activity of the gut microbiota involves the anaerobic breakdown of dietary fiber, carbohydrates, protein, and peptides (35), producing SCFAs, which play an important role in maintaining intestinal health (36), and also at a systemic level (37). In line with this, the adaptation of the microbiota to the LFHCC diet, with an increase in the *Prevotella* genus and *F. prausnitzii*, facilitates the fermentation of the carbohydrates that escape intestinal digestion and increases the production of SCFAs (24). In contrast, the Med diet decreases the abundance of this genus but increases other SCFA-producing genera such as *Roseburia* (38). These apparently opposite changes in terms of bacterial populations seem to have a neutral net effect in terms of the production of SCFAs, based on the short- or medium-chain fatty acid levels in feces and plasma (7 and 10 fatty acids from 4 to 12 atoms of carbon in feces and plasma, respectively), which did not change after the dietary intervention. Furthermore, we did not observe any changes in other microbiota-derived metabolites, such as secondary bile acid (feces, 13 secondary bile acids; plasma, 15 secondary bile acids). These results suggest that the changes in gut microbiota composition may be functionally compensated for in terms of their capacity to metabolize or produce metabolites or bioactives, thus having a neutral net effect, as shown for SCFAs and secondary bile acid.

However, the changes in the abundance of 10 metabolites (7 in feces and 3 in plasma), mainly related with amino acid, peptide, and sphingolipid metabolism, can be explained by the changes in 3 bacterial genera and 2 bacterial species. In addition, the correlation analysis results suggest that the fecal metabolome changes observed after the dietary intervention may occur through changes in microbiota.

Finally, because dietary proteins are absorbed in the gastrointestinal tract after their degradation, the relationship between protein-related metabolites and microbiota, found in our study, explains how the composition of the intestinal microbial community may influence amino acid digestibility, a term that describes the proportion of consumed amino acid that is absorbed as a result of the interaction between the food and the individual eating it (39). In fact, it is known that the quantities of amino acids absorbed intact from the large intestine are very low, as the microbiota alters the amino acid composition (39, 40).

In conclusion, our results suggest that long-term consumption of both healthy diets, the Med diet and the LFHCC diet, exerts a protective effect on the development of T2D by different specific changes in the gut microbiota, which increase the abundance of the *Roseburia* genus and *F. prausnitzii*, respectively. In addition, the changes in the gut microbiota after 1 year's consumption of both diets may be linked to amino acid digestibility. Our study provides insight into microbial functions and their role in mediating the effect of diet when used as a therapeutic tool in human health; however, further studies are required to fully understand the potential role of gut microbiota modification by diet in the prevention of metabolic diseases.

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3. Intestinal microbiota is influenced by gender and body mass index.

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RESEARCH ARTICLE

Intestinal Microbiota Is Influenced by Gender and Body Mass Index

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Abstract

Intestinal microbiota changes are associated with the development of obesity. However, studies in humans have generated conflicting results due to high inter-individual heterogeneity in terms of diet, age, and hormonal factors, and the largely unexplored influence of gender. In this work, we aimed to identify differential gut microbiota signatures associated with obesity, as a function of gender and changes in body mass index (BMI). Differences in the bacterial community structure were analyzed by 16S sequencing in 39 men and 36 post-menopausal women, who had similar dietary background, matched by age and stratified according to the BMI. We observed that the abundance of the *Bacteroides* genus was lower in men than in women ($P<0.001$, $Q = 0.002$) when BMI was > 33. In fact, the abundance of this genus decreased in men with an increase in BMI ($P<0.001$, $Q<0.001$). However, in women, it remained unchanged within the different ranges of BMI. We observed a higher presence of *Veillonella* (84.6% vs. 47.2%; X^2 test $P = 0.001$, $Q = 0.019$) and *Methanobrevibacter* genera (84.6% vs. 47.2%; X^2 test $P = 0.002$, $Q = 0.026$) in fecal samples in men compared to women. We also observed that the abundance of *Bilophila* was lower in men compared to women regardless of BMI ($P = 0.002$, $Q = 0.041$). Additionally, after correcting for age and sex, 66 bacterial taxa at the genus level were found to be associated with BMI and plasma lipids. Microbiota explained at $P = 0.001$, 31.17% variation in BMI, 29.04% in triglycerides, 33.70% in high-density lipoproteins, 46.86% in low-density lipoproteins, and 28.55% in total cholesterol. Our results suggest that gut microbiota may differ between men and women, and that these differences may be influenced by the grade of obesity. The divergence in gut microbiota observed between men and women might

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have a dominant role in the definition of gender differences in the prevalence of metabolic and intestinal inflammatory diseases.

Introduction

Gut microbiota acts collectively as an organ fully integrated in host's metabolism, and is involved in energy extraction from nutrients, regulating innate and adaptative immunity, and participating in the control of the energy balance [1]. However, several studies have proposed that changes in intestinal microbiota may trigger the pathogenic mechanisms which are involved in the development of obesity and insulin resistance [2–4], both associated with high cardiovascular risk [5]. Moreover, studies in animal models have shown that obesity is associated with an increase in the *Firmicutes/Bacteroidetes* ratio [6]. Studies with gnotobiotic mice colonized with the microbiota of lean or obese twins have also shown that this phenotype is also transmissible [7]. In contrast, studies in humans have yielded conflicting results, which may be explained by the inter-individual heterogeneity to which the gut microbiota is exposed. Fundamentally, this comes from different environmental factors such as diet, host metabolism, and hormonal factors [8]. In fact, gut microbiota composition seems to be more influenced by ambient and dietary cues than by genetic factors [9, 10].

In line with this, studies in humans have shown that gut microbiota seems to have coevolved with dietary habit [11, 12]. Recent research indicates that changes in gut microbiota composition may occur after dietary interventions [13–15]. In addition, it has been shown that microbial exposure and sex hormones exert potent effects on autoimmune diseases, which is more prevalent in women than in men [16]. Nevertheless, other studies have also linked gut microbiota to phospholipid metabolism and cardiovascular risk [17]. Overall, the incidence of metabolic diseases and their co-morbidities is sexually dimorphic and varies depending on gonadal status; e.g., increases after menopause [18]. Likewise, sex hormones are thought to play an important role in the development of cardiovascular diseases [19–21].

In addition, alteration of the intestinal microbiota has been demonstrated to be a key player in the protracted course of inflammation in inflammatory bowel diseases (IBD) [22]. This disease is more prevalent in females than in males and has the highest rates in developed Western parts of the world [23]. Therefore, the latter suggests that environmental exposures may be contributing to the pathogenesis of IBD [24].

The influence of gut microbiota in the incidence of metabolic and intestinal diseases is rather complex due to the inter-individual heterogeneity. Furthermore, the proportion of Firmicutes/Bacteroidetes in lean and obese humans has yielded contradictory results, as well as studies describing gender-related differences in the gut microbiome [25, 26]. In order to clarify this question, our objective was to identify the gut microbiota signatures associated with obesity as a function of changes in gender and BMI.

Materials and Methods

Study Subjects

This current study was conducted in a subgroup of 75 patients (39 men and 36 women) within the CORDIOPREV study (Clinical Trials.gov.Identifier: NCT00924937), an ongoing prospective, randomized, opened, and controlled trial in patients with coronary heart disease (CHD), who had their last coronary event over six months before enrolling in two different dietary

models (Mediterranean and low-fat) over a period of five years, in addition to conventional treatment for CHD [27].

We analyzed the baseline fecal samples of 75 patients (39 men and 36 women), who were also divided into three groups, according to their BMI: 13 men and 13 women with BMI < 30; 13 men and 10 women with $30 \leq$ BMI ≤ 33; and 13 men and 13 women with BMI > 33. The metabolic characteristics of the subjects in the study are shown in S1 Table.

Ethics, consent and permissions

The trial protocol and all amendments were approved by the Reina University Hospital Ethics Committee, following the Helsinki declaration and good clinical practice. However, all patients gave written informed consent to participate in the study.

Diet assessment

We performed a validated 14-item questionnaire to assess adherence to the Mediterranean Diet [28] and a similar 9-point score to assess adherence to low-fat diet at baseline before the start of the dietary intervention (and yearly follow-up visits, in the original study). Fiber intake was calculated using the Spanish food composition tables and through a validated food frequency questionnaire [29].

Clinical plasma parameters

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. The plasma was separated from the red cells by centrifugation at 1500 X g for 15 min at 4°C. Analytes determined in frozen samples were analyzed centrally by the laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, who were unaware of the interventions. Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche) using specific reagents (Boehringer-Mannheim). Plasma triglycerides (TG) and cholesterol concentrations were assayed by enzymatic procedures [30, 31]. High-Density Lipoprotein—cholesterol (HDL-c) was measured by the precipitation of a plasma aliquot with dextran sulphate-Mg2+, as described by Warnick et al. [32]. Low-Density Lipoprotein—cholesterol (LDL-c) was calculated using the following formula: plasma cholesterol–(HDL-C + large Triglyceride-Rich Lipoproteins-Cholesterol (TRL-C) + small TRL-C). Also, glucose determination was performed using the hexokinase method.

DNA extraction from fecal samples

To collect the fecal samples, we gave the patients a box with carbonic snow and a sterile plastic bottle with a screw cap to keep the frozen sample. Once it was delivered to the laboratory staff, the sample was stored at -80°C until microbial DNA was extracted. This was performed using the QIAamp DNA kit Stool Mini Kit Handbook (Qiagen, Hilden, Germany) following the manufacturer's instructions. This protocol is optimized for a 180–220 mg sample. DNA was quantified using a Nanodrop ND-1000 v3.5.2 spectrophotometer (Nanodrop Technology®, Cambridge, UK) and the samples were stored at -20°C.

Sequencing the V4 16S microbial rRNA on the Illumina MiSeq

Sample preparation was performed similarly to that described by Costello et al. [33]. Briefly, the 75 samples were amplified in triplicates by polymerase chain reaction (PCR) to generate an amplification library (modified from Sarah Owens, Argonne National Labs), with each sample being amplified in 3 replicate 25 μL PCR reactions. The PCR experimental condition for the

515–806 bp region of the 16S rRNA gene, and the sequencing procedures with the Illumina platform has been described by Caporaso et al. [34].

Upstream informatics analysis of the 16S sequences

The obtained 16S rRNA sequences were analyzed using QIIME with default parameters unless indicated otherwise [34]. Briefly, raw sequencing data was de- multiplexed and low quality reads were discarded. Reads were clustered using a closed-reference OTU picking protocol that assigned reads to reference sequences from Greengenes v13-8 [35]. Taxonomy was assigned to the OTUs against the Greengenes v13-8 preclustered at 97% identity. Differences between bacterial communities were calculated in QIIME using rarefaction curves of alpha-diversity indexes including estimates of community richness (such as the Chao1 estimator, Good´s coverage, the observed number of OTUs present in each sample and Phylogenetic diversity (PD) or the length of the phylogenetic branch observed in each sample). Due to the unequal size of our library per sample and with the purpose to retain all samples each library was sub-sampled to an even sequencing depth of exactly 2000 sequences per sample (the lowest number of reads obtained for any of the 75 samples analyzed) to mitigate biases arising from different depths of sequence across samples. Beta diversity was estimated using weighted and unweighted UniFrac distance [36]. Beta-diversity distance matrices were built after sub-sampling all the samples to an even depth of 2000 sequences per sample, which is the same with the depth provided to script alpha_rarefaction.py. Relative taxonomic abundance was measured as the proportion of reads over the total in each sample assigned to a given taxonomy.

Statistical analysis

All the data presented in this study are expressed as mean±SEM. PASW statistical software, version 20.0 (IBM Inc., Chicago, IL, USA) and R software, version 3.0.2 (R Foundation for Statistical Computing, http://www.R-project.org/) were used for statistical analyses of data. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. The statistical differences in the main metabolic variables between groups were evaluated using One-way ANOVA. In order to assess whether specific differences occurred in some bacterial taxa between genders and bacterial species, we compared the abundance of taxa present at least in the 75% of the human fecal DNA samples from men and at least in 75% of the total human fecal DNA samples from women. The specific differences in bacterial taxa between genders and bacterial species were evaluated by the Mann-Whitney *U* test. The specific differences in bacterial taxa by BMI were evaluated by the Kruskal-Wallis test. EPIDAT program v4.1 was used to evaluate power and sample size calculations (Epidat: programa para análisis epidemiológico de datos. Versión 4.1, octubre 2014. Consellería de Sanidad, Xunta de Galicia, España; Organización Panamericana de la salud (OPS-OMS); Universidad CES, Colombia.). Furthermore, we also analyzed the frequency of occurrence of taxa identified at least in 25% of the total human fecal DNA samples from men and at least in 25% of the total human fecal DNA samples from women. The X^2 test was applied to establish differences in bacterial prevalence between the studied groups. Results were adjusted by False Discovery Rate (FDR) using Benjamini and Hochberg method. FDR adjusted p-value (or q-value) of 0.05 was considered statistically significant.

A two part model for association analysis between BMI and lipids with either OTUs or taxonomic (at the genus level) units adjusting for age and gender was performed as described by Fu et al. [37]. This approach overcomes the problem of an ab non-normal distribution, which is a feature of the majority of gut bacteria OTUs or taxa. Briefly, the first part describes a binomial analysis that tests for the association of detecting a microbe (represented by an OTU or taxonomy) with a trait. The second part of the quantitative analysis tests for association between the

lipid level and the abundance of bacteria, but only for the subjects where that microbe is present. To further combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted *Z* method. Then, a final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from binary analysis, quantitative analysis, and meta-analysis [37].

Finally, the proportion of variation in BMI and lipids could be explained by the gut microbiome, based on significantly associated OTUs identified in the two-part model at a certain *P* value (ranging from 0.001 to 0.1). Also, the risk (*rm*) of the gut microbiome on BMI or lipids for each individual using an additive model was estimated according to Fu et al. [37]. The variation in BMI and lipids explained by the gut microbiome was represented as the squared correlation coefficient between the traits and *rm*, after correcting for age and gender.

Results

Baseline characteristic of the study participants

No statistical significant differences in age, BMI, glucose, TG, LDL-c, total cholesterol and systolic blood pressure were observed between men and women. However, women had higher HDL-c levels than men ($P = 0.022$) and men higher diastolic blood pressure ($P = 0.035$) (S1 Table). In addition, we did not find any differences in the diet (S2 Table) and in macronutrients intake (S3 Table) between men and women.

Gender and gut microbiota

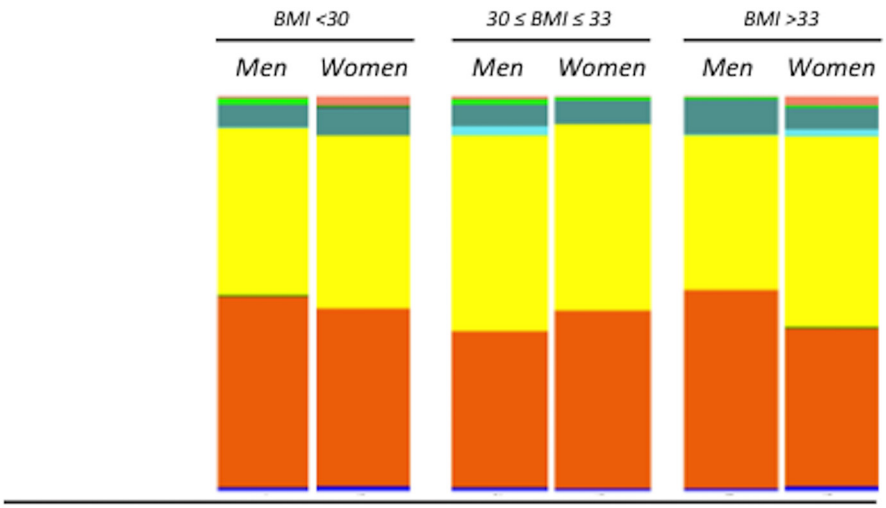
For the bacterial community analyses of the 75 samples, after screening our data for poor quality sequences, we recovered 1,296,641 high-quality 16S rRNA gene sequences with 706,186 and 590,455 sequences for men and women, respectively, with an average of 17,469 sequences per sample (Min-2015; Max-33,319). There were no significant differences in bacterial diversity between males and females with any of the alpha diversity estimators used and at a rarefaction level of 2,000 sequences per sample. With this depth we reached satisfactory coverage of the diversity for all samples since all Good's coverage values ranged between 98.43 and 99.77 (data not shown). Similarly, Principal Coordinate Analysis (PCoA) or UPGMA clustering based on unweighted and weighted UniFrac distances did not show significant differences in microbiota composition between men and women (S1 and S2 Figs).

Although diversity and overall community composition were not significantly different between males and females, we investigated whether the relative abundance of specific taxa might differ among groups. Regardless of BMI, we did not find any differences at phylum level between men and women. However, at the genera level, we observed that the abundance of the *Bilophila* genus was higher in women than in men ($P = 0.002$, $Q = 0.041$). Higher presence of *Veillonella* (33/39, 84.6% vs. 17/36, 47.2%; X^2 test $P = 0.001$, $Q = 0.019$) and *Methanobrevibacter* genera (33/39, 84.6% vs. 17/36, 47.2%; X^2 test $P = 0.002$, $Q = 0.026$) was observed in fecal samples from men compared to women.

In addition, at the bacterial species level, we observed that the abundance of *Bacteroides caccae* was higher in women than in men ($P = 0.009$, $Q = 0.035$). On the other hand, the abundance of *Bacteroides plebeius* was higher in men than in women ($P = 0.001$, $Q = 0.006$). Moreover, we observed a higher presence of *Coprococcus catus* (30/39, 76.9% vs. 13/36, 36.1%; X^2 test $P<0.001$, $Q = 0.011$) in fecal samples from men compared to women.

Gender differences in the gut microbiota are influenced by BMI

In order to assess whether specific differences in bacterial taxa between genders were influenced by BMI, we stratified both men and women into three groups each: BMI $<$ 30;



	BMI <30		30 ≤ BMI ≤ 33		BMI >33	
	Men	Women	Men	Women	Men	Women
Firmicutes / Bacteroidetes ratio	1.3	1.2	1.5	1.2	0.9	1.4
Verrucomicrobia	0.4	2.4	0.7	0.4	0.3	2.2
Tenericutes	1.7	0.2	1.3	0.7	0.4	0.5
Synergistetes	0.0	0.1	0.0	0.0	0.0	0.0
Proteobacteria	5.7	7.0	5.5	5.9	9.0	5.7
Lentisphaerae	0.1	0.0	0.1	0.0	0.0	0.0
Fusobacteria	0.2	0.0	2.3	0.0	0.3	1.7
Firmicutes	42.6	44.0	49.6	47.4	39.2	48.6
Cyanobacteria	0.1	0.1	0.1	0.1	0.1	0.1
Bacteroidetes	48.6	45.2	39.6	44.9	50.4	40.3
Actinobacteria	0.6	0.8	0.7	0.6	0.3	1.0
Euryarchaeota	0.1	0.1	0.1	0.0	0.0	0.0

Fig 1. Gender differences in the gut microbiota at the phylum level. The abundance of the bacterial phyla was obtained by analyzing the 16S rRNA sequences using QIIME. *Firmicutes/Bacteroidetes* ratio was calculated dividing the abundance of *Firmicutes* and *Bacteroidetes* for each subject. **BMI**: body mass index.

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30 ≤ BMI ≤ 33; and BMI > 33. Similar to the results on the non-stratified data, both alpha and beta diversity were not significantly different between males and females in any of the BMI groups (S1 and S2 Figs). No differences were observed at phyla level and *Firmicutes/Bacteriodetes* ratio between men and women when considered independently of the BMI. However, when we stratified men and women according to their BMI, we observed that men had higher *Firmicutes/Bacteriodetes* ratio under a BMI of 33. By contrast, men had a significantly lower *Firmicutes/Bacteriodetes* ratio than women in the BMI > 33 group ($P = 0.018$) (Fig 1).

At genera level, we observed a significantly higher abundance of the *Bacteroides* genus in women than in men ($P<0.001$, $Q = 0.002$) with a BMI > 33, whereas we did not find any difference between genders in the abundance of this genus when the BMI was < 33 (Fig 2). This was consistent with the decrease in the abundance of *Bacteroides* genus in men with the increase of the BMI ($P<0.001$, $Q<0.001$), whereas in women, it remained unchanged in the different ranges of BMI (Table 1).

In addition, we observed that the abundance of *B. plebeius* was higher in BMI > 33 group in men than in women ($P = 0.005$, $Q = 0.041$) (Table 2). Thus, this was in line with the trend to increase in the abundance of this bacterial species observed with the BMI in men ($P = 0.021$, $Q = 0.055$).

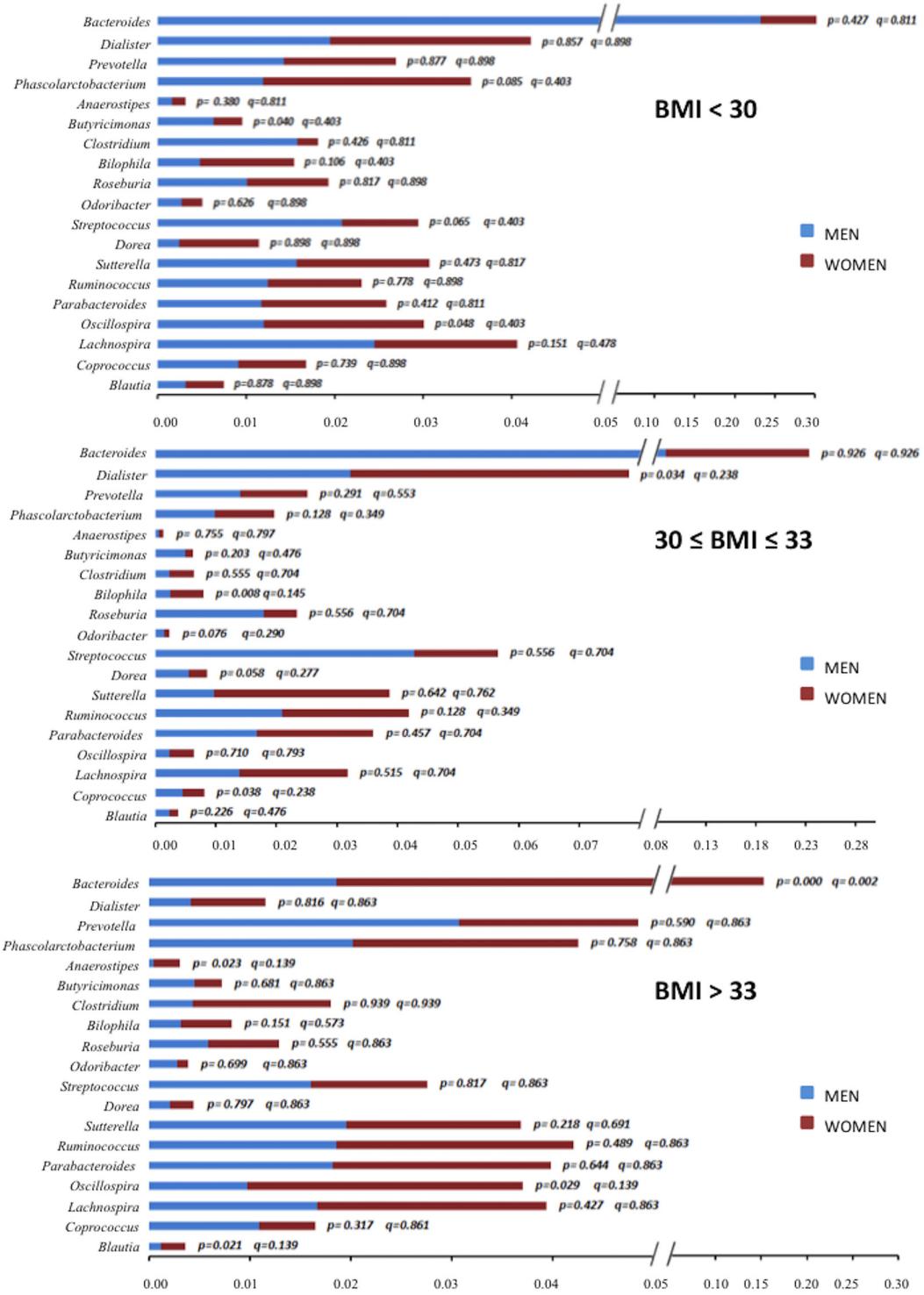

Fig 2. Gender differences in the gut microbiota at different BMI ranges at the genus level. The abundance of the bacterial phyla was obtained by analyzing the 16S rRNA sequences using QIIME. Bars show the comparison of the abundance of the different bacterial species between men and women at different BMI ranges by the Mann-Whitney *U* test (*P-value*). *Q-value*: False Discovery Rate (FDR) using Benjamini and Hochberg method. **BMI:** body mass index.

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Table 1. Gender differences in the gut microbiota by BMI at genus level.

	Men					Women				
	BMI < 30	30 ≤ BMI ≤ 33	BMI > 33	P-value	Q-value	BMI < 30	30 ≤ BMI ≤ 33	BMI > 33	P-value	Q-value
Bacteroides	0.232 ± 0.040	0.151 ± 0.025	0.019 ± 0.005	<0.001	<0.001	0.179 ± 0.039	0.165 ± 0.045	0.134 ± 0.022	0.919	0.952
Dialister	0.019 ± 0.004	0.018 ± 0.009	0.004 ± 0.002	0.008	0.053	0.023 ± 0.006	0.055 ± 0.015	0.007 ± 0.003	0.018	0.333
Prevotella	0.014 ± 0.008	0.014 ± 0.004	0.031 ± 0.012	0.712	0.798	0.013 ± 0.005	0.010 ± 0.004	0.018 ± 0.007	0.893	0.952
Phascolarctobacterium	0.012 ± 0.004	0.017 ± 0.003	0.020 ± 0.005	0.229	0.435	0.024 ± 0.005	0.011 ± 0.003	0.022 ± 0.006	0.181	0.619
Anaerostipes	0.002 ± 0.001	0.001 ± 0.000	0.000 ± 0.000	0.047	0.226	0.002 ± 0.001	0.001 ± 0.000	0.003 ± 0.001	0.854	0.952
Butyricimonas	0.006 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.171	0.406	0.003 ± 0.001	0.002 ± 0.000	0.003 ± 0.001	0.228	0.619
Clostridium	0.016 ± 0.011	0.004 ± 0.001	0.004 ± 0.001	0.960	0.960	0.002 ± 0.000	0.004 ± 0.001	0.014 ± 0.008	0.732	0.952
Bilophila	0.005 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.756	0.798	0.011 ± 0.003	0.007 ± 0.002	0.005 ± 0.001	0.586	0.952
Roseburia	0.010 ± 0.002	0.015 ± 0.005	0.006 ± 0.001	0.259	0.448	0.009 ± 0.002	0.007 ± 0.002	0.007 ± 0.002	0.797	0.952
Odoribacter	0.003 ± 0.001	0.003 ± 0.000	0.003 ± 0.001	0.145	0.393	0.002 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.115	0.619
Streptococcus	0.021 ± 0.005	0.018 ± 0.012	0.016 ± 0.006	0.117	0.369	0.009 ± 0.003	0.012 ± 0.004	0.012 ± 0.002	0.624	0.952
Dorea	0.002 ± 0.000	0.005 ± 0.002	0.002 ± 0.001	0.202	0.426	0.009 ± 0.004	0.002 ± 0.001	0.002 ± 0.001	0.228	0.619
Sutterella	0.016 ± 0.003	0.016 ± 0.003	0.020 ± 0.003	0.601	0.798	0.015 ± 0.005	0.026 ± 0.009	0.017 ± 0.004	0.744	0.952
Ruminococcus	0.012 ± 0.003	0.021 ± 0.006	0.019 ± 0.005	0.589	0.798	0.011 ± 0.002	0.014 ± 0.007	0.024 ± 0.006	0.186	0.619
Parabacteroides	0.012 ± 0.002	0.017 ± 0.005	0.018 ± 0.006	0.679	0.798	0.014 ± 0.003	0.023 ± 0.006	0.022 ± 0.006	0.641	0.952
Oscillospira	0.012 ± 0.003	0.010 ± 0.001	0.010 ± 0.002	0.732	0.798	0.018 ± 0.003	0.010 ± 0.001	0.027 ± 0.009	0.042	0.403
Lachnospira	0.025 ± 0.007	0.011 ± 0.004	0.017 ± 0.006	0.076	0.287	0.016 ± 0.006	0.017 ± 0.006	0.023 ± 0.006	0.541	0.952
Coprococcus	0.009 ± 0.003	0.009 ± 0.001	0.011 ± 0.003	0.494	0.781	0.008 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.952	0.952
Blautia	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.000	0.007	0.053	0.004 ± 0.002	0.002 ± 0.000	0.002 ± 0.000	0.726	0.952

BMI: body mass index. **Rows**: comparison of the abundance of the different bacterial genera between different BMI ranges in men and women together and in men and women separately by the Kruskal-Wallis test. **Q-value**: False Discovery Rate (FDR) using Benjamini and Hochberg method.

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We also observed a higher presence of *C. catus* (10/13, 76.9% vs. 1/13, 7.7%; X^2 test $P<0.001$, $Q = 0.011$), in fecal samples from men compared to women when the BMI was < 30. In fact, the prevalence of *C. catus* tended to increase with the BMI in women (1/13, 7.7% BMI<30; 5/10, 50.0% 30<BMI>33; 7/13, 53.9% BMI>33; X^2 test $P = 0.028$, $Q = 0.873$), whereas it remained unchanged in men at the different BMI ranges.

Moreover, we observed a higher presence of *Bifidobacterium adolescentis* (10/13, 76.9% vs. 3/13, 23.1%; X^2 test $P = 0.006$, $Q = 0.047$), *Eubacterium biforme* (10/13, 76.9% vs. 3/13, 23.1%; X^2 test $P = 0.006$, $Q = 0.047$), and *Oxalobacter formigenes* (11/13, 84.6% vs. 3/13, 23.1%; X^2 test $P = 0.002$, $Q = 0.026$) in fecal samples from men compared to women when the BMI was < 30. However, the prevalence of these bacterial species was not statistically different between men and women when the BMI was > 30.

Relationship between gut microbiota and plasma lipid levels

In addition, we study the relationship between gut microbiota, BMI, and plasma lipid levels. After adjusting for age and sex, a total of 1428 associated OTUs were detected at FDR = 0.05. Out of them, 299 OTUs were associated with BMI, 312 with TG, 335 with HDL, 276 with LDL-c, and 223 with total cholesterol (S4a to S4e Table; S3A Fig). None of the OTUs was shared by all five traits, 1 to 3 OTUS were shared by four traits (7 OTUs in total), 1 to 9 OTUs were shared by three traits (41 OTUs in total), and 13 to 62 were shared by two traits (289 OTUs in total) (S3A Fig). Across traits, 726 associations (50.24%) were detected by binary analysis (presence/absence); 521 associations (36%) were detected by the quantitative model, and 723

Table 2. Gender differences in the gut microbiota at bacterial species level.

Bacterial species	*Men (m)*	*Women (w)*	*P-value (m/w)*	*Q-value (m/w)*
B. uniformis				
All range BMI	0.0228±0.0036	0.0253±0.0040	0.722	0.747
BMI < 30	0.0282±0.0061	0.0285±0.0084	0.719	0.956
30 ≤ BMI ≤ 33	0.0252±0.0079	0.0278±0.0074	0.495	0.660
BMI > 33	0.0151±0.0035	0.0201±0.0046	0.608	0.857
P-value (BMI)	0.201	0.626		
Q-value (BMI)	0.268	0.715		
F. prausnitzii				
All range BMI	0.0209±0.0032	0.0236±0.0057	0.270	0.539
BMI < 30	0.0242±0.0056	0.0274±0.0105	0.504	0.806
30 ≤ BMI ≤ 33	0.0223±0.0072	0.0107±0.0025	0.120	0.241
BMI > 33	0.0162±0.0036	0.0297±0.0114	0.857	0.857
P-value (BMI)	0.526	0.552		
Q-value (BMI)	0.602	0.715		
B. ovatus				
All range BMI	0.0048±0.0009	0.0049±0.0012	0.440	0.586
BMI < 30	0.0058±0.0020	0.0079±0.0028	0.836	0.956
30 ≤ BMI ≤ 33	0.0065±0.0013	0.0040±0.0015	0.097	0.241
BMI > 33	0.0022±0.0005	0.0025±0.0005	0.750	0.857
P-value (BMI)	0.016	0.369		
Q-value (BMI)	0.055	0.590		
P. distasonis				
All range BMI	0.0080±0.0012	0.0115±0.0023	0.438	0.586
BMI < 30	0.0060±0.0012	0.0120±0.0033	0.268	0.630
30 ≤ BMI ≤ 33	0.0103±0.0020	0.0103±0.0039	0.382	0.611
BMI > 33	0.0076±0.0026	0.0120±0.0048	0.291	0.775
P-value (BMI)	0.155	0.783		
Q-value (BMI)	0.248	0.783		
P. copri				
All range BMI	0.0916±0.0210	0.0732±0.0137	0.264	0.539
BMI < 30	0.0336±0.0256	0.0583±0.0211	0.013	0.106
30 ≤ BMI ≤ 33	0.0809±0.0275	0.0792±0.0230	0.756	0.864
BMI > 33	0.1603±0.0458	0.0836±0.0271	0.426	0.851
P-value (BMI)	0.021	0.310		
Q-value (BMI)	0.055	0.590		
B. caccae				
All range BMI	0.0061±0.0011	0.0100±0.0016	0.009	0.035
BMI < 30	0.0081±0.0024	0.0123±0.0032	0.315	0.630
30 ≤ BMI ≤ 33	0.0072±0.002	0.0118±0.0029	0.066	0.241
BMI >33	0.0029±0.0006	0.0062±0.0017	0.063	0.252
P-value (BMI)	0.099	0.069		
Q-value (BMI)	0.199	0.275		
H. parainfluenzae				
All range BMI	0.0052±0.0013	0.0041±0.0009	0.747	0.747
BMI < 30	0.0036±0.0015	0.0034±0.0015	0.958	0.958
30 ≤ BMI ≤ 33	0.0067±0.0032	0.0036±0.0006	0.949	0.949
BMI > 33	0.0052±0.0019	0.0052±0.0020	0.733	0.857

(*Continued*)

Table 2. (*Continued*)

Bacterial species	*Men (m)*	*Women (w)*	*P-value (m/w)*	*Q-value (m/w)*
P-value (BMI)	0.885	0.367		
Q-value (BMI)	0.885	0.590		
B. plebeius				
All range BMI	0.0310±0.0041	0.0129±0.0027	0.001	0.006
BMI < 30	0.0221±0.0079	0.0085±0.0017	0.230	0.630
30 ≤ BMI ≤ 33	0.0242±0.0055	0.0081±0.0042	0.054	0.241
BMI > 33	0.0467±0.0063	0.0208±0.0062	0.005	0.041
P-value (BMI)	0.015	0.067		
Q-value (BMI)	0.055	0.275		

BMI: body mass index. **Rows**: comparison of the abundance of the different bacterial species between men and women with different BMI range or all BMI ranges together by the Mann-Whitney *U* test. **Columns**: comparison of the abundance of the different bacterial species between different BMI ranges in men and women separately by the Kruskal-Wallis test. **Q-value**: False Discovery Rate (FDR) using Benjamini and Hochberg method.

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associations (50.03%) were detected by the meta-analysis of binary and quantitative analyses (S4a to S4e Table).

At the genus taxonomy level, we identified 90 significant associations for 66 unique taxonomies at FDR = 0.05: 23 were associated with BMI, 18 with triglycerides, 17 with HDL, 14 with LDL, and 18 with cholesterol (S5a to S5e Table; S3B Fig). Also, none of the OTUs was shared by five or four traits, only 3 OTUS were shared by three traits, and 17 (1 to 4) OTUs were shared by two traits (S3B Fig).

Furthermore, we then estimated the proportion of variation in the metabolic traits that was explained by the microbiome. The OTUs identified at $P = 0.001$ level explained 31.17% variation in BMI, 29.04% in TG, 33.70% in HDL, 46.86% in LDL-c, and 28.55% in total cholesterol. As the significance level increases, the risk model included a higher number of OTUs increasing the proportion of the explained variance. Thus, the OTUs identified at $P = 0.05$ explained 64.06% variation in TG, 55.97% in HDL, 68.98% in LDL-c, and 58.97% in total cholesterol. However, this is with the exception of BMI for which the proportion of the explained variation decreased with the increase in the significance level, being estimated in 19.40% at $P = 0.05$ (S4 Fig).

Discussion

Our study shows that the gut microbiota differs in men and women at the bacterial phyla level (*Firmicutes/Bacteriodetes* ratio), at the genus level (*Bacteroides*, *Bilophila*, *Veillonella*, *and Methanobrevibacter*), and at the species level (*B. plebeius*, *B. caccae*, *C. catus*). In fact, our results suggest that in this cohort of age- and diet-matched obese subjects, microbiota composition can be affected by gender in a BMI-specific manner.

Our study reveals that the *Firmicutes*/*Bacteroidetes* ratio, which has a great importance in the development of obesity [2], changed with the BMI and between genders. Notably, previous estimations of the proportion of *Firmicutes*/*Bacteroidetes* in lean and obese humans have yielded contradictory results. As reported in mice, several studies in humans found that this ratio is increased in obesity [38, 39]. However, others did not confirm these observations [40], or even show that the relative abundance of *Firmicutes* was reduced in obese subjects [41]. Furthermore, some studies reported differences in gut microbiota composition by age group [42, 43], and that gut microbiota may also differ between sexes in animal models [16]. Moreover, previous study testing for a relatively reduced number of taxa by fluorescent in situ hybridization showed that

the abundance for *Bacteriodes-Prevotella* group (together *Bacteroidetes phylum)* was higher in men [25]. However, a recent study performed by NGS (Next Generation Sequencing) Roche 454 platform testing for the global pattern of the bacterial community showed that women was characterized by a lower abundance of *Bacteroidetes* [26]. The latter study also analyzed the gut microbiome according to BMI in women and men separately, but failed to find differences as the cohort studied had a 25.0±4.06 Kg/m^2 (mean±SD) as the mean of BMIand therefore including include lean and mostly overweight people, but not obese people. This was such that a presumably too short range of BMI was analyzed. Our approach by NGS Illumina platform also test for the global pattern of the bacterial community which confirmed the lower abundance of *Bacteroidetes* in women as compared with men when BMI is around 25 Kg/m^2 as was previously shown [26]. Consequently, this also showed differences at bacterial phyla, genus, and species levels between men and women. Thus, these differences were influenced by the grade of obesity as the BMI cohort which was included in the current work ranged from 23.44 to 41.88. Therefore, the conflicting results in term of intestinal bacterial proportions might be explained by the men/women ratio, range of age, and the grade of obesity in the different cohorts studied. In addition, in other human-associated microbial habitats such as the skin surface, various differences between genders have also been observed [44]. Additionally, diet modulates significantly the composition of the microbiota. Although the microbiota is generally highly stable over an extended period of time in the absence of significant perturbations [45], dietary interventions can induce quick changes in composition [13, 15].

In this study, we comparatively analyzed the microbiota of lean and obese men and women under a similar nutritional background, matched by age (with a mean age of 60 years) and stratified according to BMI. A higher proportion of *Firmicutes* was found in women regardless of the BMI. Interestingly, a higher proportion of *Firmicutes* was found in men under a BMI of 33, whereas a lower proportion was detected when BMI was > 33. Thus, this reflects a potential sexual dimorphism in gut microbiota composition that is variably influenced by BMI. In addition, we observed that the abundance of the *Bacteroides* genus was lower in men than in women when BMI was > 33. This is presumably a consequence of the decrease in the abundance of this genus in men with the increase of the BMI. Nevertheless, in women, it remained unchanged in the different ranges of BMI. Gender differences in fat distribution have been previously reported, and these are related to the differences in sex hormone levels [46]. Yet, little is still known about the cellular and molecular mechanisms underlying this phenomenon. The differences observed herein regarding microbiota architecture may stem from the actual differences in sex hormone levels in elder men and women. On the other hand, it might reflect the residual influence of the dramatic differences in sex steroid profiles early in life between sexes, which may have a persistent effect on gut microbiota over time.

The composition of the gut microbiota may determine how excess energy is stored in the body, and this effect might be sex dependent. Animal experiments have provided solid proof for this phenomenon, as the sexual dimorphism in total body fat content seen in rodents (males exhibiting higher fat content than females) has shown a fade away in germ free animals, thus suggesting a role for the gut microbiota [47]. Thus, it is plausible that the gender-related differences found in our study regarding bacterial composition may have an impact on how men and women differentially store excess energy.

In addition, increasing evidence suggests that the intestinal microbiota may play a role in the development of IBD [48, 49]. Moreover, the prevalence of IBD has been shown to be higher in females and with increasing age, and most common in Caucasians as compared with other ethnicities [50, 51]. In this context, it is tempting to hypothesize that the differences in gut microbiota composition reported here might contribute in determining gender differences in the prevalence of IBD. In fact, our study showed that *Bilophila* and *Blautia*, two IBD-related

genera, were more abundant in women than in men. *Bilophila wadsworthia*, a sulphite-reducing bacteria, has been associated with an increased incidence of colitis [52], and emerges under pathological conditions such as appendicitis and other intestinal inflammatory disorders [52]. Moreover, the genus *Blautia*, recently reorganized to refer to several misclassified species belonging to the *Clostridium* cluster XIVa, which according to our study is more abundant in women than in men under obesity condition, display a high incidence in patients with IBD [53, 54]. Of note, the difference in the abundance of *Bilophila* and *Blautia* between men and women was influenced by the BMI. Thus, while the differences in *Bilophila* were more evident below 33 of BMI, we observed a significant trend of the differences in *Blautia* only above 33 of BMI. These findings support the possibility that obesity, by influencing microbiota composition, might influence the development of IBD. Although admittedly, the pathogenic role of overweight in IBD remain poorly understood.

In a recent study by Fu et al. [37], it has been shown that gut microbiota composition has little effect on LDL or TC levels. However, it makes a significant contribution to the individual variance seen in BMI and to the blood levels of triglycerides and HDL. In our study, we also found that gut microbiota has a significant contribution to the individual variance seen in BMI, triglycerides, and HDL. Thus, as a difference, we found that it also contributed to the variance seen in LDL and cholesterol.

Our study shows gender-related differences analyzing the full microbiome by using a NGS method. Our experimental design included a broad BMI range, including lean, overweight, obese, and morbidly obese people which allowed us to find out gut microbiota signatures associated with obesity as a function of changes in gender and the BMI. Consequently, this could open a new hypothesis to be tested in bigger populations as one limitation of this study is the reduced sample size, although large enough to detect relative gender-related changes in gut microbiota. However, small differences in taxa with high inter-individual variability may not have been detected, or we may not have had a sufficient sample size to detect small differences in taxa between groups. Further investigations in larger populations are needed to confirm these results and extend the knowledge about the gender differences in the gut microbiota.

In conclusion, our results suggest that gut microbiota may differ between men and women, and that these differences may be influenced by the grade of obesity. Thus, these results might be relevant for the proper understanding of the basis of gender differences in the prevalence of metabolic and intestinal inflammatory diseases. Further studies will however be needed to unveil the specific mechanisms, such as sex steroid milieu, gonadal status, or genetic factors, underlying this phenomenon, and to what extent this may play a role in the sexual dimorphism in cardiovascular disease.

Supporting Information

S1 Fig. Evaluation of microbial diversity using a variety of alpha diversity metrics. Rarefaction curves were generated using phylogenetic metrics (a,d,g,j) and non-phylogenetic metrics (b,c,e,f,h,i,k,l). Horizontal and vertical axes represent rarefaction depth and alpha diversity values, respectively. Error bars correspond to standard deviation for alpha diversity values at each rarefaction depth. Rarefaction curves for gut microbiome richness estimated by gender in all subject (a,b,c), in subject with a BMI lower than 30 (d,e,f), in subject with BMI equal or greater than 30 and equal or less than 33 (g,h,i), and in subject with a BMI greater than 33 (j,k.l). (PPTX)

S2 Fig. Evaluation of microbial diversity through beta-diversity, including unweighted and weighted UniFrac measures. 3D PCoA Plots were generated using quantitative measures (unweighted unifrac) and qualitative measures (weighted unifrac). Proportion of variance

explained by each principal coordinate axis is denoted in the corresponding axis label. Beta diversity was estimated by gender in all subject (a,b), in subject with a BMI lower than 30 (c,d), in subject with BMI equal or greater than 30 and equal or less than 33 (e,f), and in subject with a BMI greater than 33 (g,h).
(PPTX)

S3 Fig. The number of OTUs (a) or taxonomies (b) associated with TG, HDL, LDL, cholesterol and BMI at FD $<$ 0.05, and their overlaps with each other. BMI indicates body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and TC, total cholesterol.
(PPTX)

S4 Fig. Contribution of the gut microbiome to body mass index and lipids. Variation explained by gut microbes at different levels of significance. BMI indicates body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and TC, total cholesterol.
(PPTX)

S1 Table. Metabolic characteristic of the participants in the study. Values correspond to the mean±SEM of the main metabolic variables. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI $<$ 30 group, 13 men and 13 women; 30 $\leq$ BMI $\leq$ 33 group, 13 men and 10 women; and BMI $>$ 33 group, 13 men and 13 women.
(DOCX)

S2 Table. Dietary assessment of the participant in the study. Values correspond to the mean ±SEM of a 14-item questionnaire to assess adherence to the Mediterranean Diet and a 9-point score to assess adherence to low-fat diet. Fiber intake was calculated using the Spanish food composition tables. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI $<$ 30 group, 13 men and 13 women; 30 $\leq$ BMI $\leq$ 33 group, 13 men and 10 women; and BMI $>$ 33 group, 13 men and 13 women.
(DOCX)

S3 Table. Macronutrients intake of the participant in the study. Values correspond to the mean±SEM. Macronutrient percentage from total energy (E) intake was calculated using the Spanish food composition tables and food frequency questionnaires. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI $<$ 30 group, 13 men and 13 women; 30 $<$ BMI $<$ 33 group, 13 men and 10 women; and BMI $>$ 33 group, 13 men and 13 women.
(DOCX)

S4 Table. OTUs associated with Body mass index (a), triglycerides (b); HDL, high-density lipoprotein (c); LDL, low-density lipoprotein (d); and TC, total cholesterol (e) at FDR $<$ 0.05 level.
(PDF)

S5 Table. Taxonomies associated with Body mass index (a), triglycerides (b); HDL, high-density lipoprotein (c); LDL, low-density lipoprotein (d); and TC, total cholesterol (e) at FDR $<$ 0.05 level.
(PDF)

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Author Contributions

Conceived and designed the experiments: BBL JCC JL-M FP-J AC. Performed the experiments: CH OAR-Z JFA-D FG-D PP-M GMQ-N JD-L JCC. Analyzed the data: PP-M GMQ-N JD-L BBL JAN-C MT-S JCC. Contributed reagents/materials/analysis tools: PP-M JD-L JCC BBL JAN-C JL-M FP-J AC. Wrote the paper: BBL JAN-C MT-S JCC JL-M FP-J AC.

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VI. CONCLUSIONS

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Main conclusion

MetS is related with a dysbiosis of the gut microbiota, which is characterized by a decrease in the relative abundance of bacterial taxa important for human health. In addition, the relative abundance increases after long-term consumption of a healthy diet, restoring the dysbiosis in MetS patients. This main conclusion is divided in three conclusions, which corresponding to the three papers of this thesis:

1. The consumption of the Med diet influenced the gut microbiota composition, mainly in MetS patients. At baseline these patients had a dysbiosis of the intestinal microbiota compared to the non-MetS patients, so that the consumption of Med diet restores the homeostasis of the gut microbiota, which is particularly important in conditions where the intestinal microbiota is altered, such as obesity and MetS. *Conclusion from paper 1*.

2. The long-term consumption of healthy diets, such as the Med diet and the Low Fat diet, exert a protective effect on the development of T2D by different specific changes in the gut microbiota which increase the abundance of the *Roseburia* genus and *Faecalibacterium prausnitzii*, respectively. *Conclusion from paper 2.*

3. The gut microbiota differs between men and women, and that these differences may be influenced by the grade of obesity. *Conclusion from paper 3*.

Secondary conclusions

1. MetS patients have a functional dysbiosis characterized by a reduction of the relative abundance of bacterial taxa with an important anti-inflammatory capability and saccharolytic activity. In addition, the long-term consumption of the Med diet is more

effective at increasing these bacterial taxa found with lower relative abundance in MetS patients.

2. The changes in the gut microbiota after one year's consumption of both diets, Med diet and Low Fat diet, are linked to amino acid digestibility.

3. The gut microbiota has a significant contribution to the individual variance seen in BMI, TG, HDL-c, LDL-c and TC.

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VIII. SUPPLEMENTAL INFORMATION

PAPER 1

1. PAPER 1

Group (men/women)	***Non-MetS (87/14)***	***MetS (111/27)***	***p-value***
Age (years)	61.41±0.94	60.16±0.74	0.290
Waist circumference (cm)	101.92±1.08	109.31±0.88	<0.001
HDL-c (mg/dL)	45.65±1.22	40.13±0.94	<0.001
TG (mg/dL)	122.52±5.81	158.66±7.39	<0.001
Glucose (mg/dL)	102.27±3.23	126.47±5.27	<0.001
Sistolic BP (mm Hg)	134.75±1.99	141.40±1.60	0.009
Diastolic BP (mm Hg)	74.49±1.04	78.33±0.95	0.007

Supplemental Table 1. Baseline characteristics. Values presented are the mean ± SEM. Abbreviations: MetS, metabolic syndrome. HDL-c, high-density lipoprotein-cholesterol; TG, triglycerides, BP, blood pressure. One-way ANOVA statistical analysis *p*-value.

Supplemental Tabla 2. Primers used for Quantification of the bacterial composition by real-time qPCR analysis.

Bacterial species	*Primer sequence*
Genus *Bacteroides* [1]	F – GAGAGGAAGGTCCCCCAC R - CGCTACTTGGCTGGTTCAG
Genus *Prevotella* [2]	F – GGTTCTGAGAGGAAGGTCCCC R - TCCTGCACGCTACTTGGCTG
Genus *Ruminococcus* (modified from [3])	F – GCTCTTTAATCGGAGCTTTCCTTC R - TTATCGGTCCCACCTTCGGCAGCT
Genus *Eubacterium* [4]	F – AGAGTTTGATCCTGGCTCAG R - GCCTTAAACCCTRCGCTT
Genus *Lactobacillus* [2]	F – GAGGCAGCAGTAGGGAATCTTC R - GGCCAGTTACTACCTCTATCCTTCTTCTTC
Genus *Staphylococcus* [5]	F – GGCCGTGTTGAACGTGGTCAAATCA R - TIACCATTTCAGTACCTTCTGGTAA
Bacteroides fragilis group [6, 7]	F - ATAGCCTTTCGAAAGRAAGAT R - CCAGTATCAACTGCAATTTTA
Bacteroides vulgatus [8]	F - GCATCATGAGTCCGCATGTTC R - TCCATACCCGACTTTATTCCTT
Parabacteroides distasonis (reclassificated :previously Bacteroides distasonis[a]) [8]	F – GTCGGACTAATACCGCATGAA R –TTACGATCCATAGAACCTTCAT
Bacteroides thetaiotaomicron [8]	F - GGCAGCATTTCAGTTTGCTTG R - GGTACATACAAAATTCCACACGT
Clostridium coccoides group [6, 7]	F – AAATGACGGTACCTGACTAA R – AAGCGTTCTTACTTTGAGTTTC
Clostridium leptum group (Clostridium cluster IV)[9]	F - GTTGACAAAACGGAGGAAGG R - GACGGGCGGTGTGTACAA
Fusobacterium nucleatum [10]	F - GGATTTATTGGGCGTAAAGC R- GGCATTCCTACAAATATCTACGAA
Faecalibacterium prausnitzii (reclassificated: previously Fusobacterium prausnitzii[b]) [8]	F - AGATGGCCTCGCGTCCGA R - CCGVAAGACCTTCTTCCTCC
Desulfovibrio desulfuricans group [11]	F - GGTACCTTCAAAGGAAGCAC R - GGGATTTCACCCCTGACTTA
Escherichia coli [8]	F - GACCTCGGTTTAGTTCACAGA R - CACACGCTGACGCTGACCA
Clostridium clostridiiforme [8]	F - CCGCATGGCAGTGTGTGAAA R - CTGCTGATAGAGCTTTACATA
Ruminococcus flavefaciens subgroup [9]	F - CAGCAGCCGCGGTAATA R - CCCACACCTAGTAATCATCGTT
Eubacterium rectale [6]	F - TAATGACGGTACCTGACTAA R - AAGCGTTCTTACTTTGAGTTTC
Lactobacillus acidophilus [8]	F - CATCCAGTGCAAACCTAAGAG R - GATCCGCTTGCCTTCGCA
Lactobacillus casei [12]	F - CTATAAGTAAGCTTTGATCCGGAGATTT R - CTTCCTGCGGGTACTGAGATGT
Bifidobacterium adolescentis [8]	F - GGAAAGATTCTATCGGTATGG R - CTCCCAGTCAAAAGCGGTT
Bifidobacterium longum [13]	F - GCCGTATCTCTACGACCGTCG R - TATCGGGGAGCAAGCGAGAG
Universal Primer pair 1 [14]	F - ACTCCTACGGGAGGCAGCAGT R - ATTACCGCGGCTGCTGGC
Universal Primer pair 2 [15]	F - ACTCCTACGGGAGGCAGCAG R - ATTACCGCGGCTGCTGG

Key to symbols: Y=C+T; R=A+G; I= nucleotide analog inosine; W= A+T

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	B. fragilis group	*B. thetaiotaomicron*	*P. distasonis*	*F. prausnitzii*	*B. longum*	*R. flavefaciens subgroup*	*B. adolescentis*	*F. nucleatum*	*E. rectale*
MetS	-0,318 0,000	-0,277 0,000	-0,330 0,000	-0,472 0,000	-0,359 0,000	-0,358 0,000	-0,370 0,000	-0,223 0,002	-0,251 0,000
Waist circunference	-0,115 0,106	-0,162 0,022	-0,213 0,002	-0,294 0,000	-0,297 0,000	-0,176 0,013	-0,211 0,003	0,006 0,929	-0,069 0,335
c-HDL	0,146 0,039	0,265 0,000	0,049 0,489	0,296 0,000	0,190 0,007	0,163 0,021	0,141 0,046	0,140 0,048	0,030 0,672
TG	-0,175 0,013	-0,211 0,003	-0,200 0,005	-0,279 0,000	-0,145 0,040	-0,275 0,000	-0,174 0,014	-0,091 0,200	-0,116 0,101
Glucose	-0,154 0,030	-0,120 0,091	-0,161 0,022	-0,124 0,081	-0,145 0,040	-0,098 0,169	-0,109 0,125	-0,137 0,054	-0,106 0,134
Systolic BP	0,064 0,366	0,032 0,656	-0,051 0,471	0,017 0,807	-0,167 0,018	-0,078 0,273	-0,004 0,951	-0,060 0,399	-0,088 0,214
Diasystolic BP	-0,019 0,794	-0,071 0,320	-0,009 0,900	0,062 0,385	-0,019 0,791	-0,074 0,296	0,003 0,966	-0,029 0,683	-0,113 0,111

Supplemental Figure 1. Relationship between the metabolic syndrome features and the gut microbiota. Within each square, the top number (R) corresponds to the Pearson correlation coefficient and the bottom number (P), the p-value. MetS: correlation between the number of metabolic syndrome criteria the patients had and the bacterial species.

PAPER 2

2. PAPER 2

Supplemental materials and methods.

Study subjects.

The current work was conducted in a subgroup of 20 obese patients (men) within the CORDIOPREV study (Clinical Trials.gov.Identifier: NCT00924937), an ongoing prospective, randomized, opened, controlled trial in patients with CHD, who had their last coronary event over six months before enrolment on two different dietary models (Med diet and LFHCC diet) over a period of five years, in addition to conventional treatment for CHD. CORDIOPREV inclusion and exclusion criteria are summarized as follows: patients were eligible if they were over 20 years old, but under 75, had established CHD without clinical events in the last 6 months, were thought to follow a long-term dietary intervention and did not have severe diseases or an estimated life expectancy of less than 5 years (1). Antibiotic usage was included as an exclusion criteria for the current study, in addition to the general exclusion criteria defined in the CORDIOPREV study. All the subjects were receiving a standardized treatment for coronary heart disease. No differences were observed between groups. All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethic committees, following the Helsinki declaration and good clinical practices. Baseline characteristics of the study subjects are shown in Table 1.

Study design.

The study design has been previously described (1). Briefly, participants were randomized to receive two diets: a Med diet and an LFHCC diet. The composition was: (a) LFHCC diet: 28% fat (12% monounsaturated; 8% polyunsaturated and 8% saturated) and (b) Med diet: 35% fat (22% monounsaturated; 6% polyunsaturated and 7% saturated). To ensure that the main fat source of the Med diet (olive oil) was identical

for all patients in this group, the olive oil was given to the participants by the research team. Food packs, including low-fat foods (cereals, biscuits, pasta, etc.), of similar cost, were provided for the patients randomized to the low-fat group.

Measurement of insulin sensitivity using the oral glucose tolerance test.

Before the test started, the patients had fasted for 12 h and were asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 7 days. They were also asked to avoid strenuous physical activity the day before the test was taken. At 8:00 a.m., the patients were admitted to the laboratory and an oral glucose tolerance test (OGTT) (75g dextrose monohydrate in 250 ml water) was performed with 0, 30, 60, and 120 min sampling to establish plasma glucose and insulin levels. The insulin sensitivity index (ISI) was calculated as previously described (2, 3).

Clinical plasma parameters.

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. The plasma was separated from red cells by centrifugation at 1500 X g for 15 min at 4º C. Analytes determined in frozen samples were analyzed centrally by laboratory investigators of the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, Córdoba, Spain, who were unaware of the interventions. Glucose was measured by the hexokinase method and insulin was determined by chemioluminiscent microparticle immunoassay (CMIA) for quantitative determination using the Architect c-16000 analyzer (Abbott®, Chicago (IL), USA). HbA1c was measured by means of high performance liquid chromatography (HPLC) with an VARIANT II Turbo Bio-Rad® analyzer (Hercules, California, USA). Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche) using specific reagents (Boehringer-Mannheim). Plasma TG and cholesterol concentrations were assayed by

enzymatic procedures (4,5). HDL-c was measured by precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}, as described by Warnick et al. (6). LDL-c was calculated using the following formula; plasma cholesterol – (HDL-C + large TRL-C+ small TRL-C). Glucose levels were measured using the hexokinase method.

DNA extraction from fecal samples.

To collect the fecal samples, we gave the patients a container of carbonic snow and a sterile plastic bottle with screw cap to maintain the frozen sample. Once delivered to the laboratory staff, the sample was stored at -80ºC until microbial DNA extraction, which was performed using the QIAamp DNA kit Stool Mini Kit Handbook (Qiagen, Hilden, Germany) following the manufacturer´s instructions. This protocol was optimized for a 180-220 mg sample. Fecal DNA was quantified using a Nanodrop ND-1000 v3.5.2 spectrophotometer (Nanodrop Technology®, Cambridge, UK) and samples were stored at -20°C.

Microbiota analysis.

A total of 40 fecal samples (20 basal and 20 after a year of dietary intervention, for each participant) were used for the microbial community analysis. The DNA was submitted to PCR-amplification of the V2-V3 hypervariable region of the bacterial 16S rRNA gene. Three independent 20-µl PCRs were performed for each sample using a two-step PCR protocol with the 16S rRNA gene primers 8F (5'-*AGTTTGATCCTGGCTCAG*-3') and 357R (5'-*CTGCTGCCTYCCGTA*-3') linked to universal M13/pUC forward (5'-GTTGTAAAACGACGGCCAGT-3') and M13/pUC reverse (5'-CACAGGAAACAGCTATGACC-3') primers (M13F-*8F* and M13R-*357R*) in an approach similar to that previously described (7). Next, second PCR reactions using a 10x dilution of the first PCR product were performed using the fusion forward primer of the Lib-L, consisting of the A-adaptor sequence 5'-

CCATCTCATCCCTGCGTGTCTCCGAC-3' followed by the 4-base calibration sequence 5'-TCAG-3', a 10-base MID oligonucleotide to differentiate each of the 20 samples and the 20-base M13F/pUC forward oligonucleotide. The reverse fusion primer consists of the Lib-L B-adaptor sequence 5'-CCTATCCCCTGTGTGCCTTGGCAGTC-3' followed by the 4-base calibration sequence, and the 20-base M13/pUC reverse oligonucleotide. HPLC-purified oligonucleotides were synthesized by TIB MOLBIOL (Berlin, Germany). All PCR reactions were carried out in a T100TM Thermal Cycler (Bio-rad, Madrid Spain) using the FastStart High Fidelity Polymerase (Roche Diagnostics GmBH, Mannheim, Germany) and under the conditions recommended by the manufacturer for pyrosequencing analyses. The PCR products were purified using the Amicon Ultra 0.5 ml 30k, (Merck Millipore, Merck KGaA, Darmstadt, Germany), further purified (twice) using the AgencourtH AMPureH XP PCR purification system (Agencourt Bioscience Co., Beverly, MA, USA) and quantified using the QuantiT dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA) and a fluorometer (BioTek Instruments, Winooski, VT, USA). Subsequently, all samples from each run were pooled in equimolar concentrations. Pools of 20 samples (including 10 patients' samples, 5 samples from each diet at baseline and after a year of dietary intervention from each patient run in parallel) were diluted to obtain a total of 1x105 copies/µl (1.5 molecules per bead), and emulsion PCR was performed with the Lib-L kit (454 Life Sciences). DNA positive beads were enriched, counted on the GS Junior Bead Counter, and loaded onto a picotiter plate for pyrosequencing on the 454 Life Sciences (Roche) Junior platform, according to standard 454 platform protocols. The 40 16S rRNA amplicon samples were sequenced on two runs (20 samples in each). Additionally, 12 samples were taken in a third run to increase the number of sequences from those samples and test the reproducibility of results.

Phylogenetic analysis of sequencing reads.

Sequencing of the PCR products generated a mean of 99150 raw sequences per run. The samples were processed and analyzed following the procedure described by Caporaso et al. (8), using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (version v1.8.0. http://qiime.sourceforge.net/) with default parameters unless otherwise noted. The sequences were first screened for quality using the following parameters: minimum quality score of 25, minimum sequence length of 150 bp, maximum length of 500 bp and no ambiguous bases in the entire sequence or mismatches in the primer sequence. Any sequences not meeting these parameters were excluded from downstream analyses. The sequences were then sorted by barcode into their respective samples and the barcode and primer sequences were removed. The sequences were denoised using the QIIME denoiser default parameters. Chimeras were removed and operational taxonomic unites (OTUs) were clustered de novo from the denoised sequences using USEARCH at 97% identity. Representative sequences for each OTU were aligned using PyNAST against Greengenes template alignment (February 2011 release), and taxonomy was assigned to the OTUs detected using Basic Local Alignment Search Tool (BLAST) against the Greengenes reference database (9) preclustered at 97% identity (http://qiime.org/home_static/dataFiles.html). A phylogenetic tree was constructed using the FastTree 2.1.3 with default parameters (10) for use in phylogenetic diversity calculations. Singleton OTUs were filtered out of the entire dataset to reduce the noise caused by PCR or sequencing error, and we also discarded those OTUs with a number of sequences < 0.005% of the total number of sequences (11).

Differences between bacterial communities were calculated in QIIME using rarefaction curves of alpha-diversity indexes including estimates of community richness (such as the Chao1 estimator and the observed number of OTUs present in each sample and

Phylogenetic diversity (PD) or the length of the phylogenetic branch observed in each sample) and beta-diversity (weighted and unweighted UniFrac distance) estimates. Rarefaction analysis was performed using rarefied OTU tables (rarefied to 800 sequences; the lowest number of reads obtained for any of the 40 DNA samples analyzed to control for differing depths of sequencing across the samples), 100 replications, and cut-offs of 97% sequence similarity, respectively. Beta-diversity distance matrices were built after sub-sampling all the samples to an even depth of 800 sequences per sample. UniFrac distances were based on the fraction of branch length shared between two communities in a phylogenetic tree. Unweighted UniFrac accounts for membership only; weighted UniFrac accounts for membership and relative abundance (12,13). UniFrac-based jackknifed hierarchical clustering was performed using the unweighted pair group method with an arithmetic mean (UPGMA) in QIIME. Principal coordinates analysis (PCoA) was also performed on the UniFrac distance matrices to visualize the differences between the sample types and those visualized using the KiNG graphics program (http://kinemage.biochem.duke.edu/software/king.php). The statistical significance of differences in alpha- and beta-diversity was performed with QIIME using a nonparametric two sample t-test with 999 Monte Carlo permutations on a number of observations, Chao1 and PD and nonparametric ANOSIM tests on unweighted and weighted UniFrac distance matrices.

Metabolomic Analysis.

Sample Preparation. Samples were sent to Metabolon and prepared using the automated MicroLab STAR® system from Hamilton Company. A recovery standard was added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with

methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into four fractions: one for analysis by UPLC-MS/MS with positive ion mode electrospray ionization, one for analysis by UPLC-MS/MS with negative ion mode electrospray ionization, one for analysis by GC-MS, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. Each sample was then frozen and dried under vacuum, then prepared for the appropriate instrument.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC- MS/MS).

The LC-MS portion of the platform was based on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a ThermoFisher Scientific Q-Exactive high resolution/accurate mass orbitrap mass spectrometer operated at a 35,000 mass resolution, which was interfaced with a heated electrospray ionization (HESI) source. The sample extract was dried then reconstituted in acidic or basic LC-compatible solvents, each of which contained 12 or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion-optimized conditions and the other using basic negative ion-optimized conditions in two independent injections using separate dedicated columns (Waters UPLC BEH C18-2.1×100 mm, 1.7 µm). Extracts reconstituted in acidic conditions were gradient eluted using water and methanol containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS/MS scans using dynamic exclusion and the scan range was from 80-1000 m/z. Raw data files are archived.

Gas Chromatography-Mass Spectroscopy (GC-MS).

The samples destined for analysis by GC-MS were dried under vacuum for a

minimum of 18 h prior to being derivatized under dried nitrogen using bistrimethyl-silyltrifluoroacetamide. Derivatized samples were separated on a 5% diphenyl / 95% dimethyl polysiloxane fused silica column (20 m x 0.18 mm ID; 0.18 um film thickness) with helium as carrier gas and a temperature ramp from 64° to 340°C in a 17.5 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization (EI) and operated at unit mass resolving power. The scan range was from 50–750 m/z. Raw data files are archived.

Quality assurance/QC.

For QA/QC purposes, additional samples were included with each day's analysis. These samples included extracts of a pool of well-characterized human plasma, extracts of a pool created from a small aliquot of the experimental samples, and process blanks. QC samples were spaced evenly among the injections and all experimental samples were randomly distributed throughout the run. A selection of QC compounds was added to every sample for chromatographic alignment, including those under test. These compounds were carefully chosen so as not to interfere with the measurement of the endogenous compounds.

Data Extraction and Compound Identification.

Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. These systems are built on a web-service platform utilizing Microsoft's .NET technologies, which run on high-performance application servers and fiber-channel storage arrays in clusters to provide active failover and load-balancing. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Metabolon maintains a library based on authenticated standards that contains the retention time/index (RI), mass to charge ratio (m/z), and chromatographic data (including MS/MS spectral data) on all molecules present in the

library. Furthermore, biochemical identifications are based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 0.005 amu, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. While there may be similarities between these molecules based on one of these factors, the use of all three data points can be utilized to distinguish and differentiate biochemicals. More than 3300 commercially available purified standard compounds have been acquired and registered into LIMS for distribution to both the LC-MS and GC-MS platforms for determination of their analytical characteristics. Additional mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral). These compounds have the potential to be identified by future acquisition of a matching purified standard or by classical structural analysis. Missing values (if any) are assumed to be below the level of detection. However, biochemicals those were detected in all samples from one or more groups but not in samples from other groups were assumed to be near the lower limit of detection in the groups in which they were not detected. In this case, the lowest detected level of these biochemicals was imputed for samples in which that biochemical was not detected.

Statistical analysis.

All the data presented are expressed as mean±SEM. PASW statistical software, version 20.0 (IBM Inc., Chicago, IL, USA) was used for statistical analysis of individual data. We analyzed the changes in the abundance of bacterial genera and species when detected in at least 8 subjects per diet. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. The data were analyzed using analysis of variance

(ANOVA) for repeated measures with time as intra-subject factor and diet as the inter-subject factor. Post hoc statistical analysis was completed by using the Bonferroni's multiple comparison tests. A study of the relationship among parameters was also carried out using Pearson's linear correlation coefficient. A P value <0.05 was considered significant.

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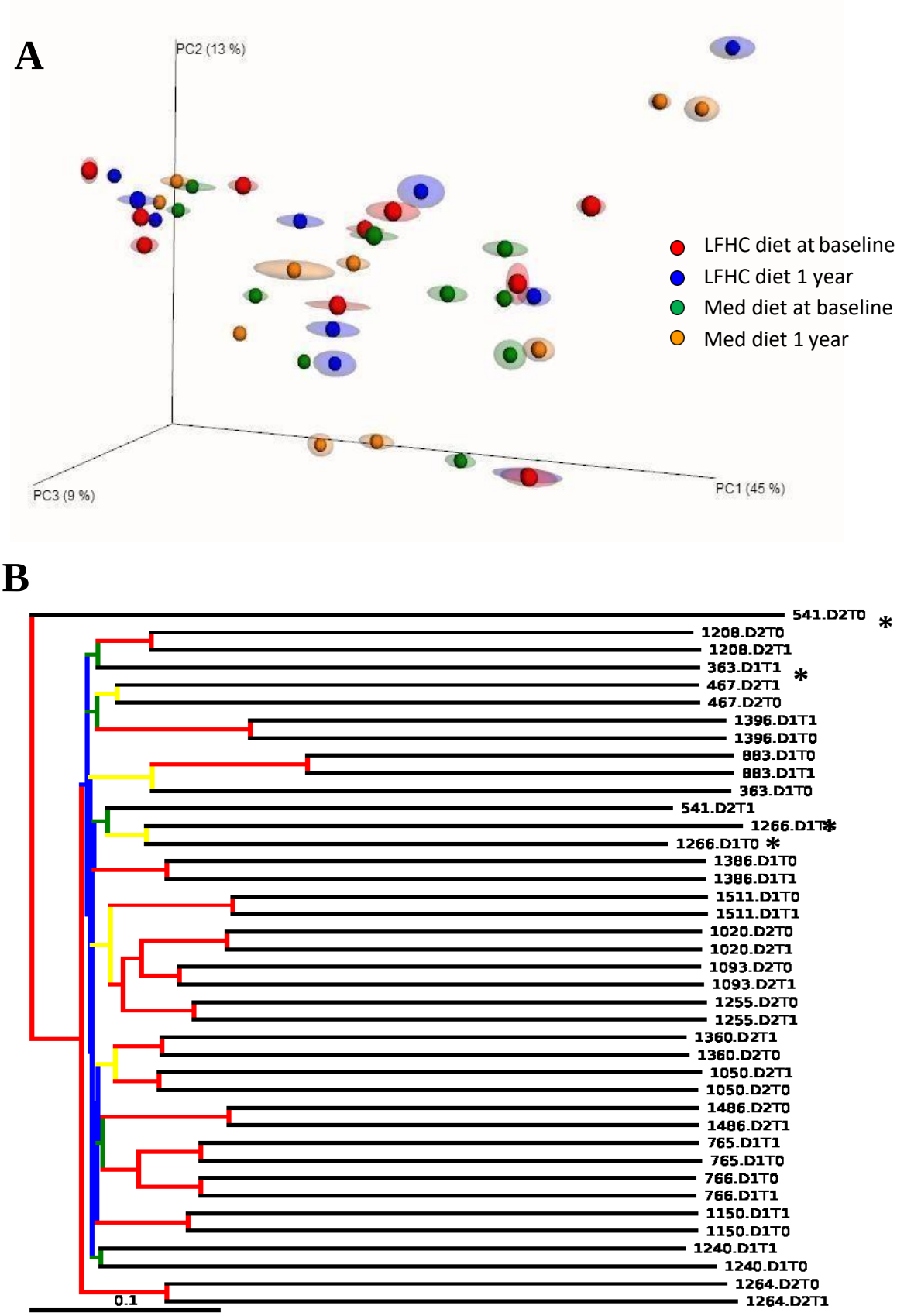
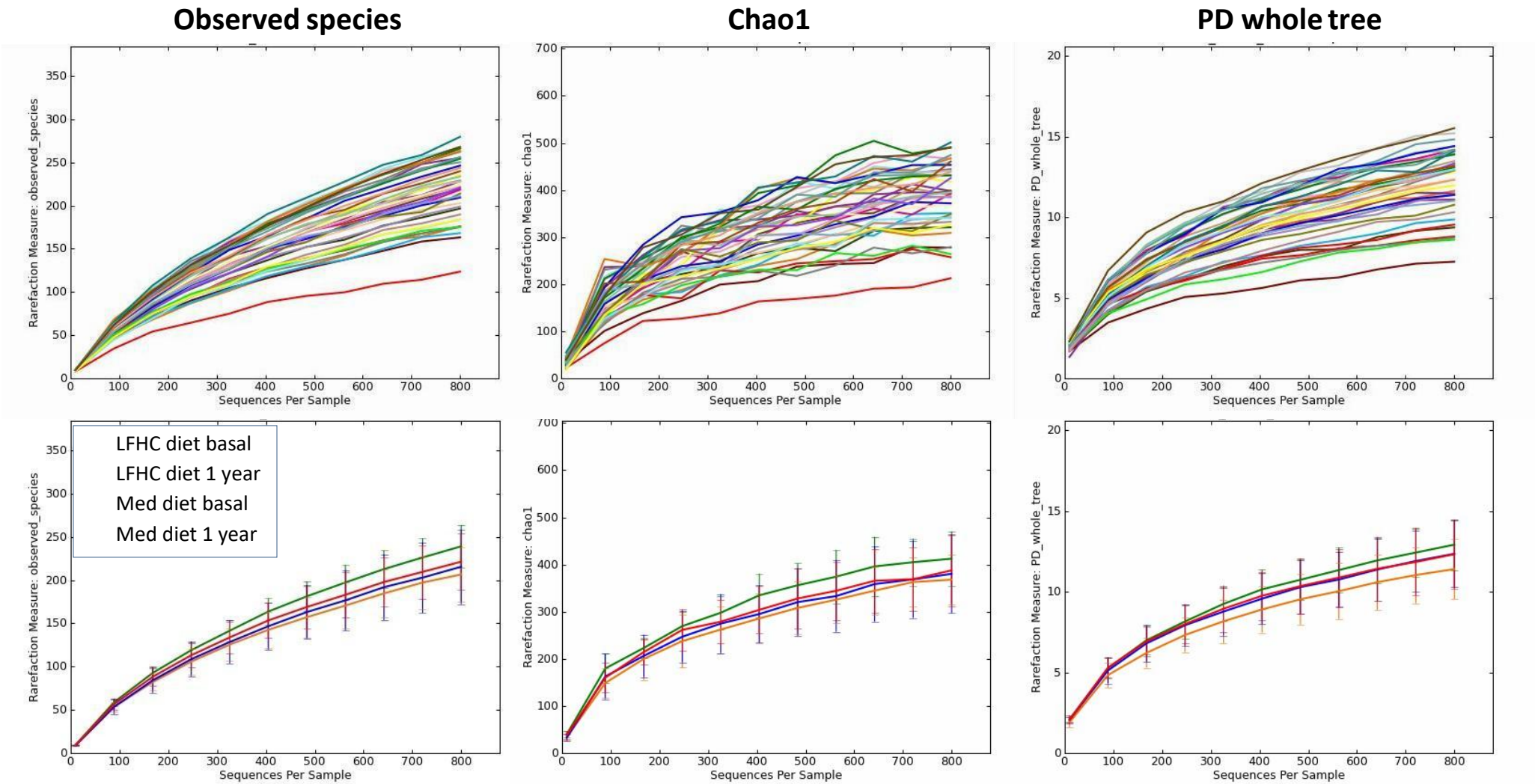
Supplemental Figure 1. 16S gene clustering of bacterial communities by treatments.



Supplemental Information

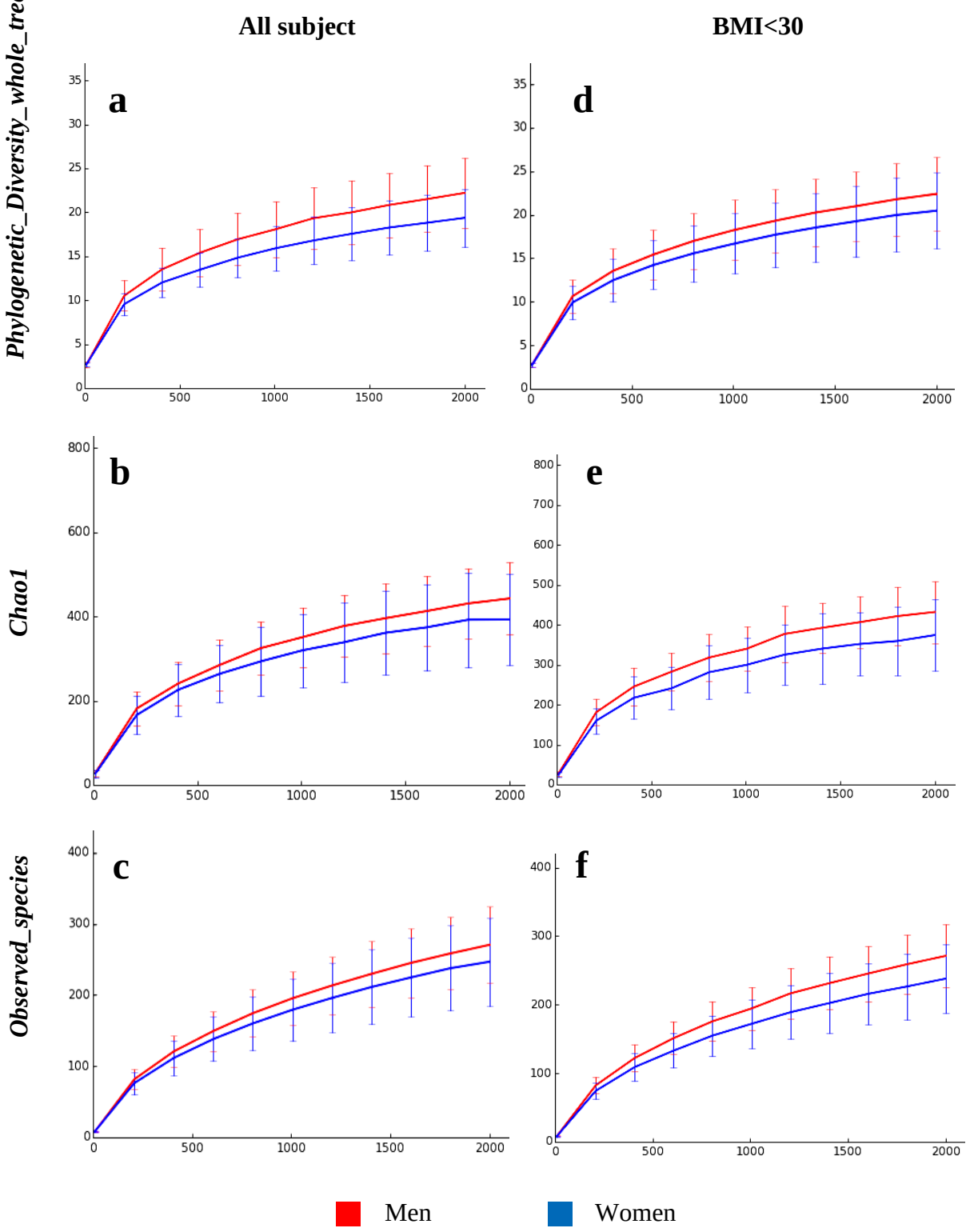
Supplemental Figure 2. Alpha-diversity curves using different indexes



PAPER 3

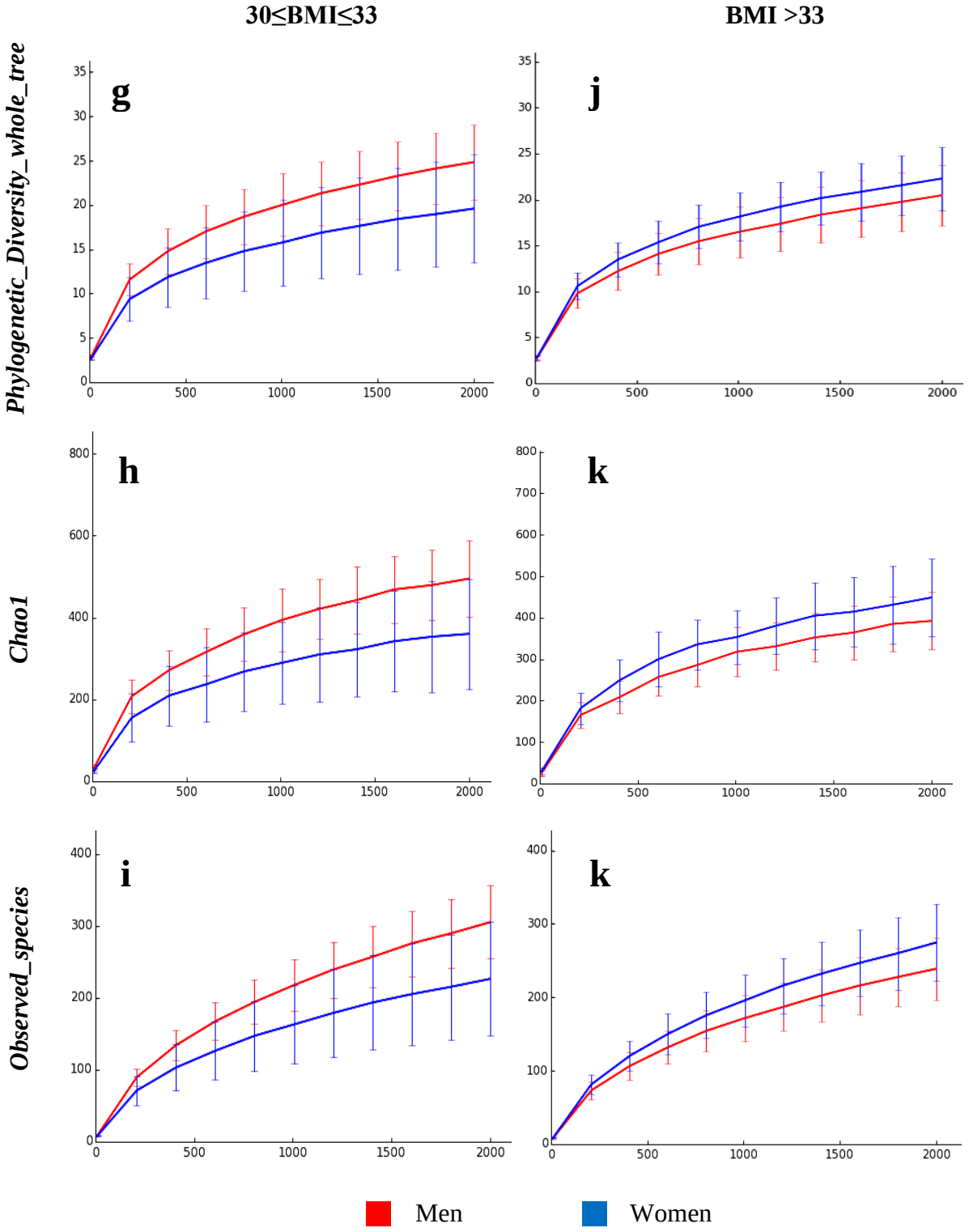
3. PAPER 3

S1 Fig. Evaluation of microbial diversity using a variety of alpha diversity metrics



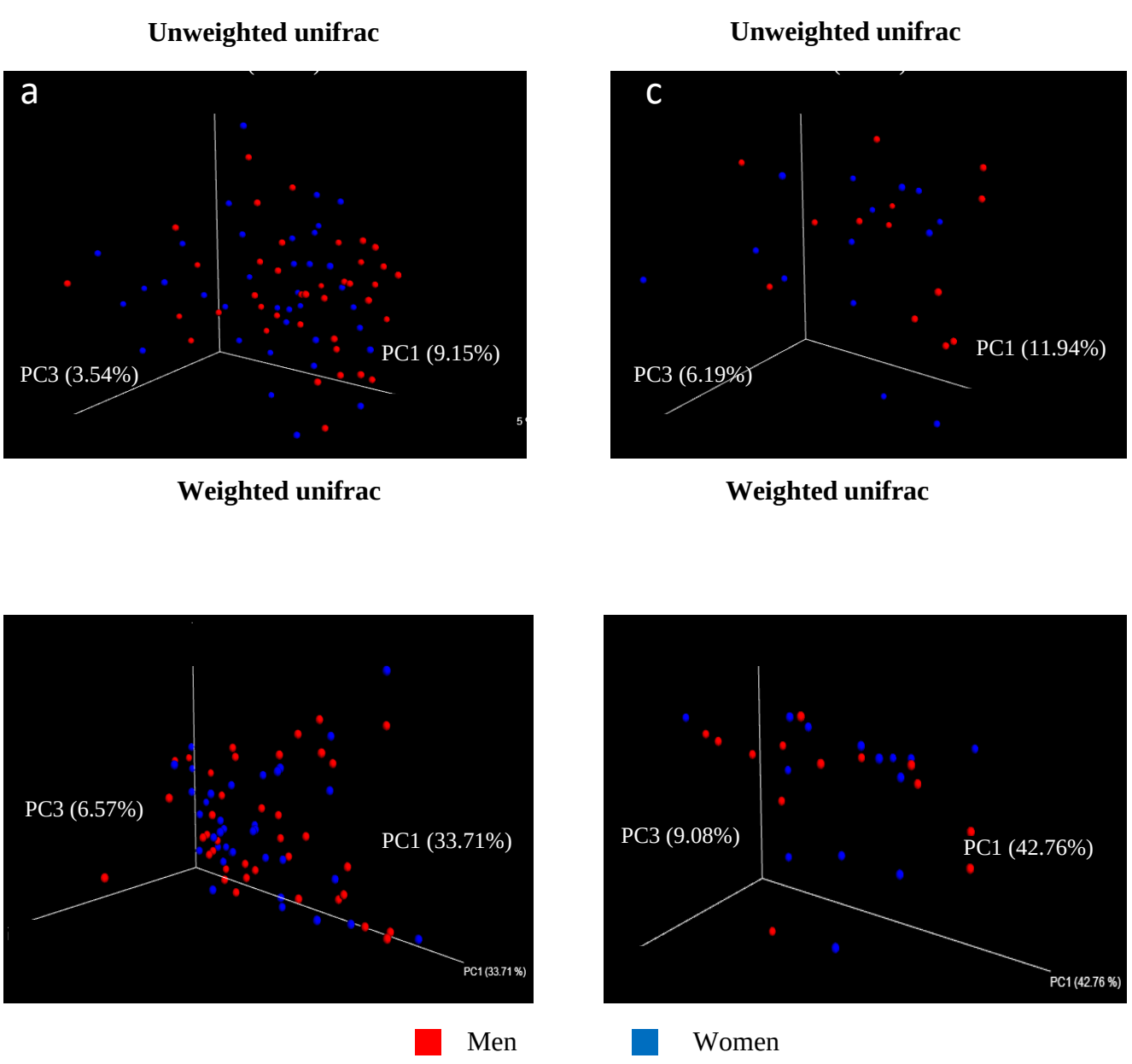
Rarefaction curves were generated using phylogenetic metrics (a,d,g,j) and non phylogenetic metrics (b,c,e,f,h,i,k,l). Horizontal and vertical axes represent rarefaction depth and alpha diversity values, respectively. Error bars correspond to standard deviation for alpha diversity values at each rarefaction depth. Rarefaction curves for gut microbiome richness estimated by gender in all subject (a,b,c), in subject with a BMI lower than 30 (d,e,f), in subject with BMI equal or greater than 30 and equal or less than 33 (g,h,i), and in subject with a BMI greater than 33 (j,k.l).

S1 Fig. Evaluation of microbial diversity using a variety of alpha diversity metrics



Rarefaction curves were generated using phylogenetic metrics (a,d,g,j) and non phylogenetic metrics (b,c,e,f,h,i,k,l). Horizontal and vertical axes represent rarefaction depth and alpha diversity values, respectively. Error bars correspond to standard deviation for alpha diversity values at each rarefaction depth. Rarefaction curves for gut microbiome richness estimated by gender in all subject (a,b,c), in subject with a BMI lower than 30 (d,e,f), in subject with BMI equal or greater than 30 and equal or less than 33 (g,h,i), and in subject with a BMI greater than 33 (j,k.l).

S2 Fig. Evaluation of microbial diversity through beta-diversity, including unweighted and weighted UniFrac measures.

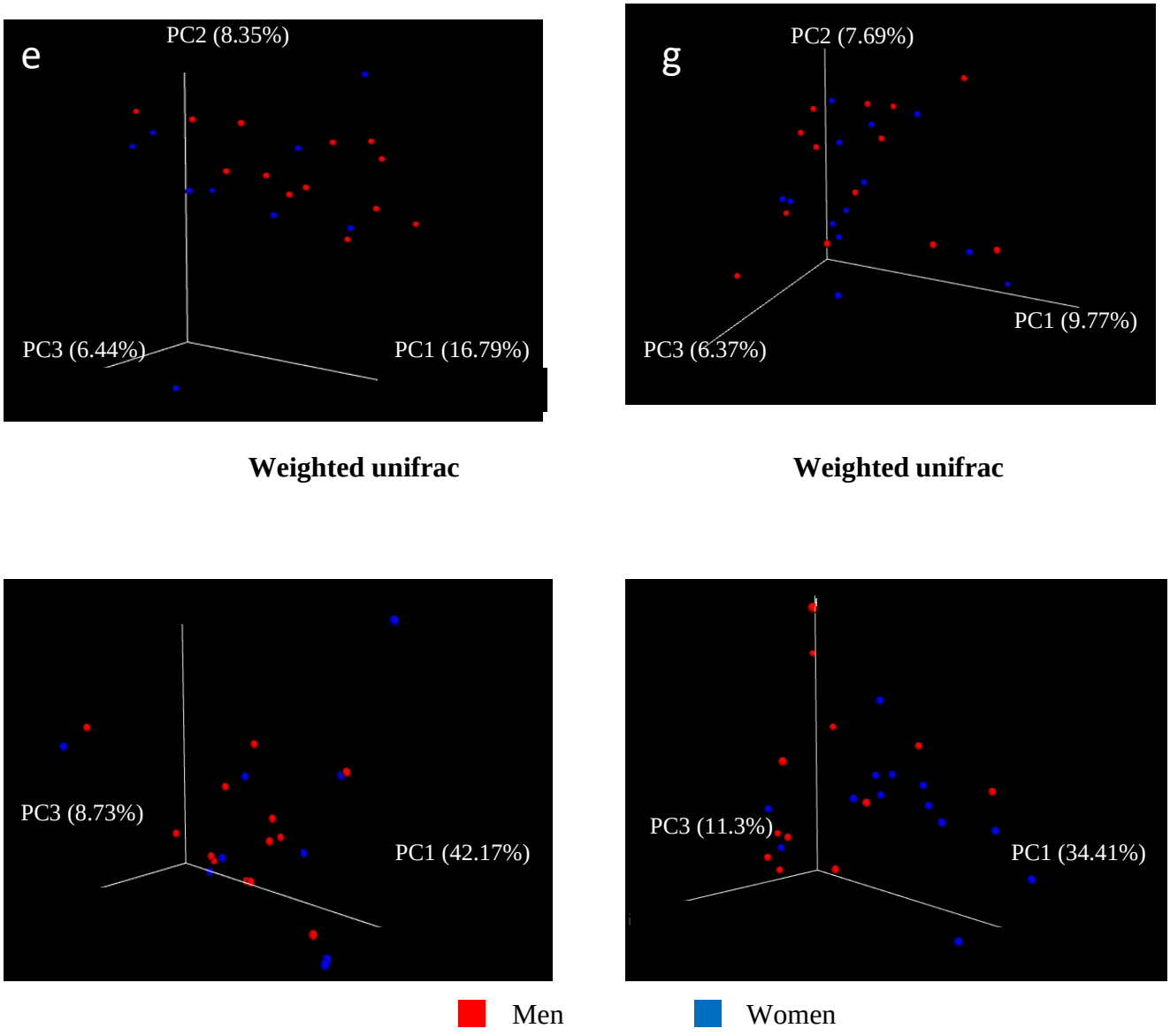


3D PCoA Plots were generated using quantitative measures (unweighted unifrac) and qualitative measures (weighted unifrac). Proportion of variance explained by each principal coordinate axis is denoted in the corresponding axis label. Beta diversity was estimated by gender in all subject (a,b), in subject with a BMI lower than 30 (c,d), in subject with BMI equal or greater than 30 and equal or less than 33 (e,f), and in subject with a BMI greater than 33 (g,h).

S2 Fig. Evaluation of microbial diversity through beta-diversity, including unweighted and weighted UniFrac measures.

30≤BMI≤33 **BMI >33**

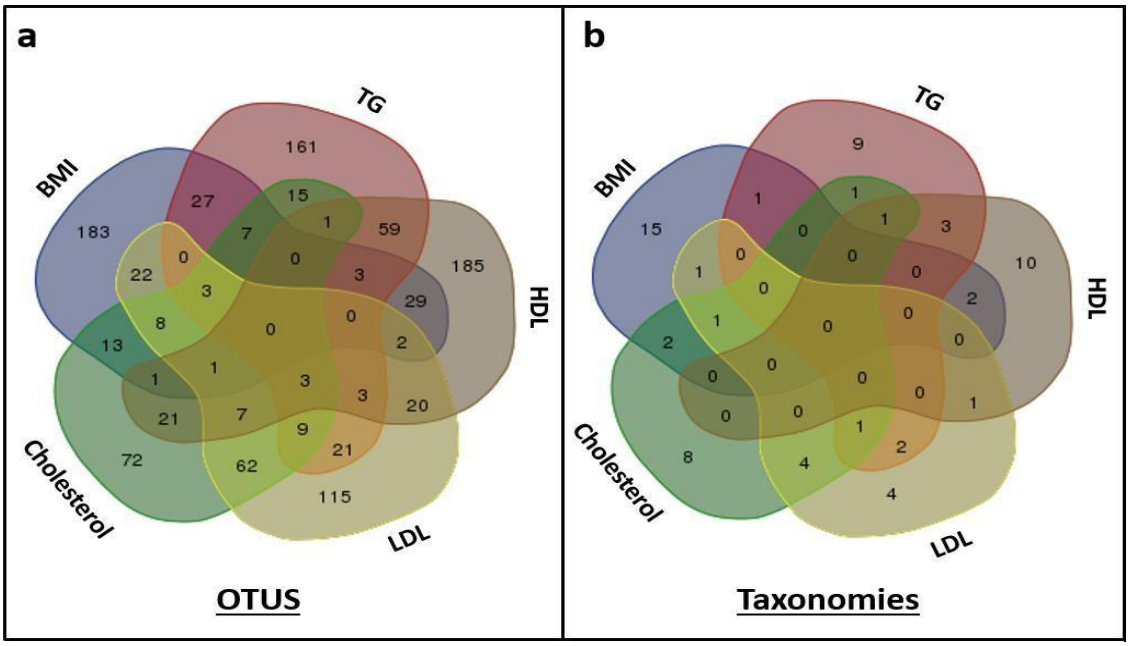
Unweighted unifrac **Unweighted unifrac**



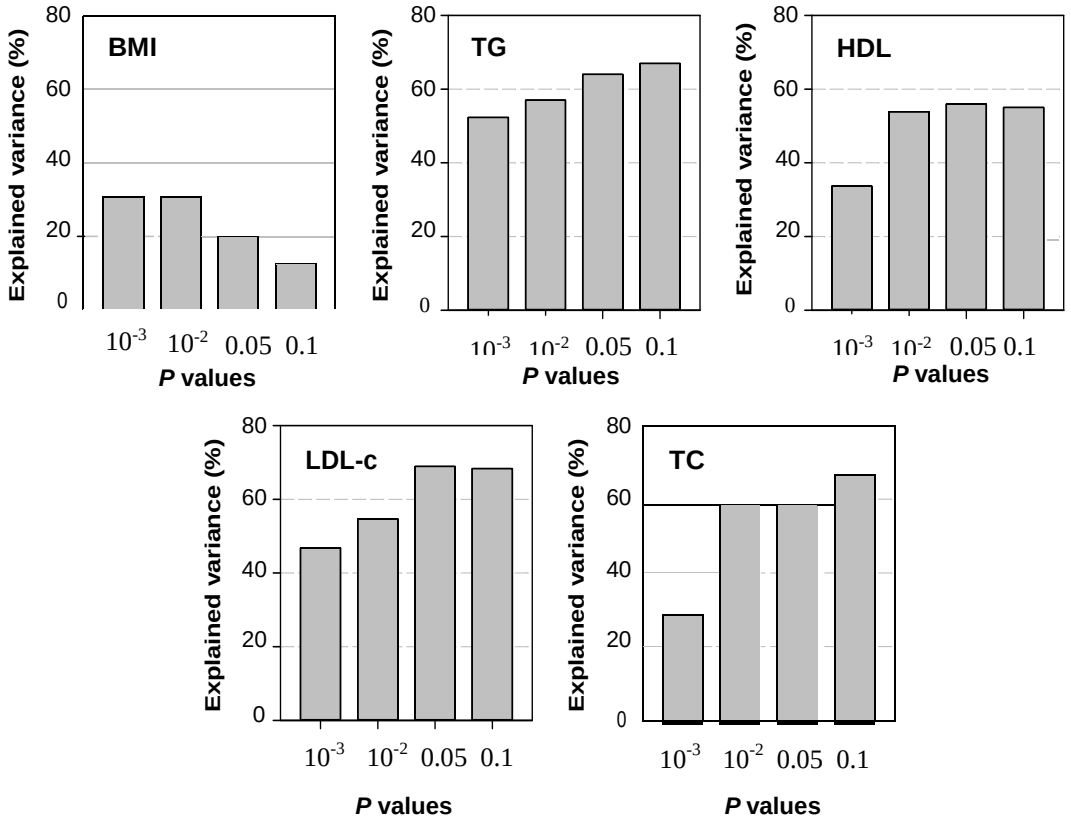
Weighted unifrac **Weighted unifrac**

Men Women

3D PCoA Plots were generated using quantitative measures (unweighted unifrac) and qualitative measures (weighted unifrac). Proportion of variance explained by each principal coordinate axis is denoted in the corresponding axis label. Beta diversity was estimated by gender in all subject (a,b), in subject with a BMI lower than 30 (c,d), in subject with BMI equal or greater than 30 and equal or less than 33 (e,f), and in subject with a BMI greater than 33 (g,h).



S3 Fig. The number of OTUs (a) or taxonomies (b) associated with TG, HDL, LDL,cholesterol and BMI at FDR < 0.05, and their overlaps with each other. BMI indicates body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and TC, total cholesterol.



S4 Fig. Contribution of the gut microbiome to body mass index and lipids. Variation explained by gut microbes at different levels of significance. BMI indicates body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and TC, total cholesterol.

Age (y)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	61.15±1.27	63.23±1.98	58.92±2.42	61.31±2.17
	Women	60.31±1.40	60.15±2.63	62.40±2.31	58.85±2.32
	P-value	0.654	0.359	0.322	0.447
BMI (kg/m2)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	31.44±0.60	27.58±0.58	31.41±0.28	35.33±0.69
	Women	31.75±0.90	27.03±0.84	31.40±0.30	36.73±1.35
	P-value	0.772	0.596	0.974	0.362
Glucose (mg/dl)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	129.33±7.67	132.28±13.78	138.69±16.72	116.91±8.31
	Women	111.46±6.66	106.08±7.07	98.40±8.05	128.17±15.70
	P-value	0.088	0.102	0.620	0.536
TG (mg/dl)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	136.50±11.03	124.85±21.00	143.69±18.35	141.33±18.97
	Women	154.75±12.63	135.31±17.67	168.30±24.90	163.77±23.82
	P-value	0.281	0.706	0.424	0.480
HDL-c (mg/dl)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	39.26±1.16	39.85±2.05	40.77±1.92	37.15±2.07
	Women	44.17±1.80	46.17±3.64	46.50±3.06	40.54±2.51
	P-value	0.022	0.137	0.113	0.309
LDL-c (mg/dl)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	86.53±3.47	76.62±4.24	95.31±6.04	87.75±6.77
	Women	87.22±4.29	94.15±9.42	87.10±7.55	80.38±4.37
	P-value	0.900	0.102	0.400	0.376
Total Cholesterol (mg/dl)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	155.13±4.48	141.85±5.18	165.15±7.90	158.38±8.82
	Women	159.85±3.58	159.45±7.61	167.70±5.97	154.15±4.93
	P-value	0.422	0.062	0.810	0.679
Systolic pressure (mm Hg)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	139.69±2.81	141.38±4.67	138.08±5.30	139.62±4.96
	Women	140.92±2.93	139.08±4.84	144.40±6.10	140.08±4.81
	P-value	0.764	0.735	0.442	0.947
Diastolic pressure (mm Hg)		***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
	Men	80.08±1.56	79.62±2.56	81.62±3.39	79.00±2.20
	Women	74.94±1.83	75.31±3.69	73.33±2.40	75.69±2.97
	P-value	0.035	0.347	0.087	0.380

S1 Table. Metabolic characteristic of the participants in the study. Values correspond to the mean±SEM of the main metabolic variables. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI < 30 group, 13 men and 13 women; 30 ≤ BMI ≤ 33 group, 13 men and 10 women; and BMI > 33 group, 13 men and 13 women.

Mediterranean diet score	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	8.41±0.30	8.46±0.50	8.15±0.53	8.62±0.55
Women	7.83±0.27	8.31±0.57	7.70±0.33	7.46±0.40
P-value	0.157	0.841	0.508	0.103
Low-fat diet score	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	3.77±0.24	4.23±0.44	3.92±0.42	3.15±0.37
Women	4.11±0.28	3.85±0.54	4.50±0.37	4.08±0.51
P-value	0.360	0.587	0.328	0.158
Fiber intake (g/d)	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	24.59±1.06	24.25±2.02	23.80±1.55	25.72±2.01
Women	24.67±1.34	24.36±1.80	24.12±3.01	25.40±2.45
P-value	0.961	0.967	0.919	0.922

S2 Table. Dietary assessment of the participant in the study. Values correspond to the mean±SEM of a 14-item questionnaire to assess adherence to the Mediterranean Diet and a 9-point score to assess adherence to low-fat diet. Fiber intake was calculated using the Spanish food composition tables. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI < 30 group, 13 men and 13 women; 30 ≤ BMI ≤ 33 group, 13 men and 10 women; and BMI > 33 group, 13 men and 13 women.

Total energy (Kcal)	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	2274.73±74.73	2279.81±151.85	2196.30±92.75	2348.09±142.95
Women	1888.88±57.95	1850.11±87.98	1982.05±115.75	1855.99±104.30
P-value	<0.001	0.022	0.158	0.010
Proteins (% of E)	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	18.22±0.52	18.11±0.82	18.80±1.01	17.77±0.89
Women	18.97±0.49	19.72±0.99	18.87±0.75	18.30±0.76
P-value	0.301	0.222	0.957	0.654
Carbohydrates (% of E)	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	40.91±1.35	38.49±2.93	41.33±2.04	42.92±1.93
Women	44.50±1.17	43.21±2.05	44.72±2.64	45.62±1.62
P-value	0.050	0.199	0.313	0.295
Fat (% of E)	***All Subjects***	***BMI < 30***	***30 ≤ BMI ≤ 33***	***BMI > 33***
Men	37.52±1.26	38.43±2.89	38.19±1.77	35.94±1.82
Women	35.97±0.97	36.43±1.51	35.74±2.48	35.67±1.31
P-value	0.337	0.546	0.418	0.907

S3 Table. Macronutrients intake of the participant in the study. Values correspond to the mean±SEM. Macronutrient percentage from total energy (E) intake was calculated using the Spanish food composition tables and food frequency questionnaires. The statistical differences between groups were evaluated by One-way ANOVA. N, 39 men and 36 women. BMI < 30 group, 13 men and 13 women; 30 < BMI < 33 group, 13 men and 10 women; and BMI > 33 group, 13 men and 13 women.

S4 Table. OTUs associated with Body mass index (a), triglycerides (b); HDL, high-density lipoprotein (c); LDL, low-density lipoprotein (d); and TC, total cholesterol (e) at FDR < 0.05 level.

Table S4a. Associated taxonomies with Body mass index (BMI) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
4212012	64	11	1.18	1.18371	1.52943	0.77395	0.44149	13.27102	1.92120	6.90768	**0.00012**	3.25878	**0.00112**	3.83894	**0.00012**
188348	65	10	4.90	5.29614	1.45878	3.63051	**0.00053**	2.72684	1.22619	2.22382	0.06153	3.77371	**0.00016**	3.77371	**0.00016**
4094259	51	24	6.17	-0.82249	1.15194	-0.71401	0.47753	1.86710	0.43689	4.27359	**0.00034**	2.03236	**0.04212**	3.58447	**0.00034**
4386018	57	18	11.89	4.24149	1.15012	3.68788	**0.00044**	0.33440	0.81125	0.41220	0.68602	2.77310	**0.00555**	3.51750	**0.00044**
165118	35	40	75.98	3.61296	0.99069	3.64691	**0.00050**	-0.12879	0.33146	-0.38856	0.69983	2.18922	**0.02858**	3.48157	**0.00050**
1952	7	68	202.57	6.45276	1.80900	3.56704	**0.00065**	0.27777	0.24021	1.15636	0.25177	3.22251	**0.00127**	3.41124	**0.00065**
4305923	60	15	42.80	2.93900	1.29645	2.26696	**0.02640**	1.15966	0.38415	3.01874	**0.01069**	3.37506	**0.00074**	3.37506	**0.00074**
183439	24	51	94.39	3.72449	1.06210	3.50672	**0.00079**	-0.20164	0.24648	-0.81806	0.41736	1.80093	0.07171	3.35790	**0.00079**
4227110	71	4	11.75	7.83335	2.24623	3.48733	**0.00084**	-2.09345	1.82709	-1.14579	0.45681	1.83608	0.06635	3.34071	**0.00084**
3134492	13	62	139.89	4.53815	1.31139	3.46057	**0.00091**	0.04086	0.24156	0.16914	0.86627	2.46452	**0.01372**	3.31696	**0.00091**
182656	64	11	2.55	-0.42446	1.51451	-0.28026	0.78008	-3.18090	0.66621	-4.77461	**0.00140**	-2.45635	**0.01404**	-3.19459	**0.00140**
4420570	64	11	16.27	4.73987	1.43516	3.30267	**0.00149**	-0.11364	0.56197	-0.20222	0.84479	2.10734	**0.03509**	3.17600	**0.00149**
839964	40	35	12.34	3.36831	1.02351	3.29093	**0.00155**	-0.13990	0.46583	-0.30033	0.76587	2.02775	**0.04259**	3.16546	**0.00155**
182911	39	36	7.17	3.27313	1.00115	3.26936	**0.00165**	0.74047	0.62182	1.19081	0.24222	3.05154	**0.00228**	3.14609	**0.00165**
213566	37	38	25.39	3.23748	1.00138	3.23302	**0.00185**	0.21706	0.40638	0.53414	0.59662	2.57575	**0.01000**	3.11339	**0.00185**
182603	71	4	2.00	5.80901	2.28794	2.53898	**0.01328**	6.28148	0.58718	10.69769	0.05934	3.08431	**0.00204**	3.08431	**0.00204**
229228	67	8	75.88	3.54541	1.68508	2.10400	**0.03887**	1.57907	0.48188	3.27692	**0.02203**	3.07974	**0.00207**	3.07974	**0.00207**
4321810	71	4	7.75	-7.10250	2.23202	-3.18209	**0.00216**	-1.89414	2.93973	-0.64432	0.63562	-2.50407	**0.01228**	-3.06745	**0.00216**
4378683	52	23	15.17	-3.46660	1.08996	-3.18049	**0.00217**	-0.29298	0.64184	-0.45647	0.65297	-2.48593	**0.01292**	-3.06600	**0.00217**
181432	34	41	60.73	3.16982	1.00887	3.14195	**0.00244**	-0.32718	0.35245	-0.92831	0.35911	1.49488	0.13494	3.03115	**0.00244**
227697	70	5	34.20	0.88382	2.14621	0.41181	0.68171	1.33111	0.06889	19.32277	**0.00267**	2.41391	**0.01578**	3.00365	**0.00267**
174516	43	32	22.66	3.10525	1.04540	2.97039	**0.00404**	0.59400	0.42570	1.39533	0.17351	2.99536	**0.00274**	2.99536	**0.00274**
2654263	56	19	5.68	3.56723	1.16986	3.04928	**0.00321**	1.89119	1.40638	1.34473	0.19747	2.99518	**0.00274**	2.99518	**0.00274**
356827	69	6	35.67	0.22292	1.99023	0.11201	0.91113	1.56080	0.17094	9.13053	**0.00278**	2.19418	**0.02822**	2.99143	**0.00278**
302049	58	17	3.94	-3.68755	1.20392	-3.06295	**0.00308**	0.38525	0.61742	0.62397	0.54268	-1.66220	0.09647	-2.95947	**0.00308**
197761	68	7	2.71	2.55979	1.82439	1.40309	0.16489	4.99352	0.91195	5.47562	**0.00541**	2.94872	**0.00319**	2.94872	**0.00319**
4457427	25	50	41.32	0.03437	1.13869	0.03018	0.97600	0.89259	0.29069	3.07058	**0.00355**	2.08322	**0.03723**	2.91604	**0.00355**
177222	43	32	5.00	-1.74233	1.06438	-1.63694	0.10601	-0.88400	0.34026	-2.59802	**0.01458**	-2.87015	**0.00410**	-2.87015	**0.00410**
158722	64	11	3.91	0.92343	1.51876	0.60802	0.54509	4.61883	1.17022	3.94698	**0.00425**	2.44929	**0.01431**	2.85869	**0.00425**
4429981	61	14	38.07	3.82990	1.29883	2.94873	**0.00430**	0.15568	0.77070	0.20199	0.84361	2.15847	**0.03089**	2.85526	**0.00430**
307113	63	12	1.67	-4.07104	1.38137	-2.94711	**0.00432**	1.04041	1.14275	0.91044	0.38632	-1.40535	0.15992	-2.85377	**0.00432**
4045882	63	12	1.67	-4.07104	1.38137	-2.94711	**0.00432**	1.04041	1.14275	0.91044	0.38632	-1.40535	0.15992	-2.85377	**0.00432**
188079	51	24	5.08	2.55770	1.10864	2.30707	**0.02393**	1.50412	0.80515	1.86814	0.07576	2.85253	**0.00434**	2.85253	**0.00434**
179291	68	7	11.29	0.20829	1.84245	0.11305	0.91030	-0.87150	0.14974	-5.82007	**0.00434**	-1.93726	0.05271	-2.85235	**0.00434**
366147	62	13	6.31	3.95561	1.34499	2.94100	**0.00440**	-0.53398	0.81869	-0.65224	0.52895	1.56877	0.11670	2.84819	**0.00440**
167950	70	5	4.80	5.96385	2.02914	2.93910	**0.00442**	-0.17127	2.85008	-0.06009	0.95754	1.97510	**0.04826**	2.84645	**0.00442**
338301	59	16	11.13	-1.34893	1.31826	-1.02326	0.30961	1.85743	0.54159	3.42958	**0.00448**	1.29129	0.19660	2.84219	**0.00448**
3799784	70	5	1.40	0.32812	2.15060	0.15257	0.87916	6.44509	0.43697	14.74943	**0.00457**	2.11300	**0.03460**	2.83621	**0.00457**
4387246	70	5	6.60	5.97544	2.04157	2.92689	**0.00458**	2.88063	1.97320	1.45988	0.28175	2.76596	**0.00568**	2.83526	**0.00458**
320915	69	6	19.33	-0.44244	1.97606	-0.22390	0.82347	-2.27599	0.29909	-7.60971	**0.00471**	-2.15620	**0.03107**	-2.82624	**0.00471**
4428929	69	6	1.83	4.03243	1.91627	2.10431	**0.03884**	3.89414	1.26576	3.07652	0.05428	2.82164	**0.00478**	2.82164	**0.00478**
179508	63	12	1.67	2.17952	1.43812	1.51553	0.13402	4.23522	1.36835	3.09513	**0.01282**	2.81931	**0.00481**	2.81931	**0.00481**
1614788	32	43	7.74	3.00910	1.03487	2.90771	**0.00484**	-0.85397	0.40207	-2.12393	**0.03991**	0.53952	0.58953	2.81768	**0.00484**
4472174	70	5	3.20	-5.34952	2.17440	-2.46023	**0.01629**	-1.62037	0.61095	-2.65221	0.11761	-2.80534	**0.00503**	-2.80534	**0.00503**
4357713	55	20	2.90	3.31381	1.14659	2.89014	**0.00509**	-0.70073	0.90806	-0.77167	0.45090	1.44790	0.14765	2.80155	**0.00509**
363430	67	8	71.88	-0.11196	1.73495	-0.06453	0.94872	-0.50663	0.10825	-4.68001	**0.00543**	-2.01133	**0.04429**	-2.78014	**0.00543**
147702	30	45	27.76	1.94792	1.07011	1.82030	0.07287	0.86086	0.39029	2.20568	**0.03293**	2.77649	**0.00549**	2.77649	**0.00549**
188676	22	53	17.13	1.41192	1.16990	1.20688	0.23143	1.01935	0.35951	2.83537	**0.00659**	2.76734	**0.00565**	2.76734	**0.00565**
186772	35	40	5.33	2.90910	1.02040	2.85092	**0.00568**	0.50955	0.55488	0.91830	0.36441	2.59686	**0.00941**	2.76551	**0.00568**
189548	70	5	2.60	5.72272	2.08610	2.74327	**0.00767**	-4.39082	0.33377	-13.15518	**0.00573**	-0.06840	0.94547	-2.76291	**0.00573**
187180	33	42	14.98	1.54183	1.06927	1.44194	0.15365	-1.00692	0.34567	-2.91298	**0.00590**	-0.93812	0.34818	-2.75344	**0.00590**
4391326	69	6	14.17	-5.33730	1.88204	-2.83591	**0.00593**	0.05655	0.64273	0.08799	0.93543	-1.88846	0.05896	-2.75169	**0.00593**
847934	67	8	1.88	4.66489	1.64624	2.83365	**0.00597**	-0.26458	2.67134	-0.09904	0.92495	1.87766	0.06043	2.74961	**0.00597**
2700883	18	57	11.93	1.97753	1.24813	1.58440	0.11749	-0.95597	0.33571	-2.84756	**0.00622**	-0.82775	0.40781	-2.73602	**0.00622**
183457	55	20	10.75	2.41614	1.17725	2.05236	**0.04377**	1.23952	0.62674	1.97772	0.06440	2.73342	**0.00627**	2.73342	**0.00627**
267718	64	11	2.82	-2.98471	1.47242	-2.02708	**0.04636**	-2.93536	1.38583	-2.11812	0.06702	-2.70373	**0.00686**	-2.70373	**0.00686**
2272791	64	11	2.82	-2.98471	1.47242	-2.02708	**0.04636**	-2.93536	1.38583	-2.11812	0.06702	-2.70373	**0.00686**	-2.70373	**0.00686**
197624	43	32	5.97	-2.86551	1.02988	-2.78236	**0.00689**	0.22237	0.60356	0.36844	0.71522	-1.65284	0.09836	-2.70232	**0.00689**
187388	62	13	2.62	3.73970	1.34581	2.77876	**0.00695**	0.41188	2.09437	0.19666	0.84803	2.04398	**0.04096**	2.69899	**0.00695**
207487	47	28	5.14	2.92705	1.05388	2.77742	**0.00698**	0.44096	0.82750	0.53288	0.59882	2.27960	**0.02263**	2.69775	**0.00698**
182647	63	12	29.67	-2.33134	1.43793	-1.62131	0.10932	-0.76240	0.28888	-2.63913	**0.02695**	-2.69652	**0.00701**	-2.69652	**0.00701**
194297	53	22	35.55	-1.28784	1.16778	-1.10281	0.27378	-0.87342	0.28922	-3.01994	**0.00704**	-2.67935	**0.00738**	-2.69478	**0.00704**
4467347	71	4	3.50	4.51960	2.33792	1.93317	0.05715	-6.97488	0.07929	-87.96415	**0.00724**	-0.55406	0.57954	-2.68574	**0.00724**
299302	66	9	3.33	-4.31559	1.56760	-2.75299	**0.00747**	-0.06452	1.84304	-0.03501	0.97321	-1.91538	0.05544	-2.67518	**0.00747**
3530697	37	38	6.55	2.82965	1.03043	2.74610	**0.00761**	-0.28976	0.55311	-0.52388	0.60366	1.52004	0.12850	2.66880	**0.00761**
346253	69	6	2.67	-3.36047	1.95590	-1.71812	0.09007	-2.06861	0.59181	-3.49540	**0.03961**	-2.65362	**0.00796**	-2.65362	**0.00796**
189450	66	9	2.44	-4.32220	1.58378	-2.72904	**0.00798**	0.10965	0.98529	0.11128	0.91502	-1.80051	0.07178	-2.65301	**0.00798**
347243	71	4	2.25	-0.27906	2.41506	-0.11555	0.90833	-2.24329	0.02838	-79.03470	**0.00805**	-1.95510	0.05057	-2.64978	**0.00805**
196061	68	7	5.57	3.87536	1.86405	2.07900	**0.04118**	4.46167	1.99053	2.24145	0.08848	2.64824	**0.00809**	2.64824	**0.00809**
180215	70	5	2.00	5.60382	2.06372	2.71540	**0.00828**	1.36171	3.62512	0.37563	0.74329	2.09861	**0.03585**	2.64038	**0.00828**
161423	6	69	36.12	-5.10888	1.88283	-2.71341	**0.00833**	-0.03913	0.28604	-0.13680	0.89161	-1.96209	**0.04975**	-2.63854	**0.00833**
4368216	18	57	399.56	2.45075	1.22200	2.00552	**0.04867**	0.25780	0.14391	1.79140	0.07883	2.63680	**0.00837**	2.63680	**0.00837**
4451906	44	31	4.68	1.53600	1.08983	1.40939	0.16302	-2.00576	0.70703	-2.83689	**0.00837**	-0.87795	0.37997	-2.63658	**0.00837**
2953981	67	8	3.50	-0.86319	1.73213	-0.49834	0.61977	2.47816	0.58813	4.21362	**0.00838**	1.51336	0.13019	2.63640	**0.00838**
279048	70	5	26.80	-5.61723	2.07793	-2.70329	**0.00856**	0.78834	1.36483	0.57761	0.62189	-1.51036	0.13095	-2.62915	**0.00856**
185763	3	72	91.36	-0.14715	2.73972	-0.05371	0.95731	0.68401	0.25277	2.70604	**0.00857**	1.82088	0.06863	2.62864	**0.00857**
814442	50	25	69.36	1.80770	1.12916	1.60093	0.11377	0.78838	0.34598	2.27872	**0.03275**	2.62806	**0.00859**	2.62806	**0.00859**

Table S4a. Associated taxonomies with Body mass index (BMI) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
193644	59	16	2.25	0.54398	1.30575	0.41661	0.67820	3.01894	0.98078	3.07810	**0.00881**	2.14553	**0.03191**	2.61932	**0.00881**
186559	68	7	2.29	2.77604	1.87488	1.48065	0.14306	6.11929	1.75793	3.48097	**0.02533**	2.61687	**0.00887**	2.61687	**0.00887**
4403113	71	4	1.75	6.10114	2.27387	2.68315	**0.00904**	-5.01366	0.79608	-6.29796	0.10025	0.68365	0.49420	2.61048	**0.00904**
179760	67	8	2.75	4.44370	1.66469	2.66938	**0.00939**	0.46870	1.44088	0.32529	0.75813	2.05459	**0.03992**	2.59769	**0.00939**
4383924	68	7	2.29	-4.69991	1.76138	-2.66831	**0.00941**	0.94467	1.05375	0.89648	0.42067	-1.26674	0.20525	-2.59670	**0.00941**
2699610	45	30	2.03	2.78080	1.04443	2.66250	**0.00956**	0.62498	0.98588	0.63393	0.53146	2.27482	**0.02292**	2.59130	**0.00956**
197988	42	33	14.00	1.68549	1.06088	1.58877	0.11649	1.04809	0.47858	2.19000	**0.03644**	2.58921	**0.00962**	2.58921	**0.00962**
184426	46	29	5.07	2.80765	1.05562	2.65971	**0.00963**	0.09584	0.64757	0.14800	0.88349	1.93412	0.05310	2.58871	**0.00963**
4438983	49	26	30.96	-0.44387	1.13651	-0.39056	0.69728	-1.12546	0.39977	-2.81525	**0.00982**	-2.10089	**0.03565**	-2.58212	**0.00982**
197460	68	7	1.86	0.37402	1.84209	0.20304	0.83967	2.11479	0.45795	4.61792	**0.00990**	1.96698	**0.04919**	2.57942	**0.00990**
322835	38	37	7.84	1.35967	1.06246	1.27973	0.20475	1.65720	0.66344	2.49790	**0.01749**	2.57692	**0.00997**	2.57692	**0.00997**
4326573	70	5	11.00	5.40927	2.05529	2.63188	**0.01038**	-1.42962	1.91698	-0.74577	0.53355	1.37195	0.17008	2.56283	**0.01038**
4354235	10	65	52.98	3.97183	1.51090	2.62878	**0.01047**	0.09018	0.26262	0.34339	0.73246	2.05188	**0.04018**	2.55995	**0.01047**
327218	29	46	7.09	2.06293	1.07324	1.92214	0.05854	1.06951	0.60459	1.76898	0.08399	2.55944	**0.01048**	2.55944	**0.01048**
195294	53	22	2.59	1.80765	1.20818	1.49618	0.13898	2.50751	1.08815	2.30437	**0.03266**	2.55680	**0.01056**	2.55680	**0.01056**
186888	51	24	8.71	-2.89134	1.10528	-2.61593	**0.01083**	-0.15710	0.66074	-0.23777	0.81437	-1.96773	**0.04910**	-2.54799	**0.01083**
366451	66	9	4.89	1.08229	1.64550	0.65773	0.51281	3.03207	0.83338	3.63829	**0.01086**	2.26397	**0.02358**	2.54727	**0.01086**
182142	70	5	1.60	5.39933	2.06729	2.61179	**0.01095**	-0.19330	5.23494	-0.03692	0.97390	1.77584	0.07576	2.54413	**0.01095**
4350477	57	18	5.28	-3.16682	1.21481	-2.60683	**0.01110**	0.13757	0.84575	0.16266	0.87296	-1.68264	0.09245	-2.53951	**0.01110**
841907	52	23	37.48	-2.89859	1.11508	-2.59946	**0.01132**	0.06219	0.35625	0.17456	0.86318	-1.66899	0.09512	-2.53264	**0.01132**
313593	25	50	6.04	2.83083	1.08934	2.59866	**0.01134**	-0.70017	0.52193	-1.34150	0.18621	0.85561	0.39222	2.53190	**0.01134**
191660	69	6	1.67	4.91088	1.89013	2.59817	**0.01136**	-5.72879	1.19539	-4.79241	**0.01728**	0.10663	0.91508	2.53145	**0.01136**
193161	71	4	1.25	-3.27708	2.35336	-1.39251	0.16805	-14.03829	0.64380	-21.80544	**0.02918**	-2.51702	**0.01184**	-2.51702	**0.01184**
177515	14	61	56.00	3.40074	1.31714	2.58191	**0.01186**	-0.20488	0.32163	-0.63701	0.52663	1.33156	0.18300	2.51628	**0.01186**
4232048	66	9	1.89	0.42156	1.66417	0.25332	0.80075	3.62250	1.03070	3.51460	**0.01260**	1.94259	0.05207	2.49485	**0.01260**
321063	68	7	2.43	4.32975	1.78212	2.42955	**0.01761**	2.38980	1.78193	1.34113	0.25097	2.49019	**0.01277**	2.49019	**0.01277**
2829179	38	37	331.81	2.70483	1.06660	2.53594	**0.01338**	0.23528	0.22186	1.06049	0.29640	2.48728	**0.01287**	2.48728	**0.01287**
4456702	63	12	1.92	-0.37885	1.46176	-0.25918	0.79624	-6.07388	1.97268	-3.07900	**0.01316**	-1.93577	0.05290	-2.47937	**0.01316**
4484075	38	37	4.03	2.25627	1.04077	2.16788	**0.03347**	1.01713	0.72557	1.40184	0.17003	2.47379	**0.01337**	2.47379	**0.01337**
187751	45	30	57.10	0.82696	1.09118	0.75786	0.45101	0.58955	0.22314	2.64209	**0.01354**	2.27897	**0.02267**	2.46922	**0.01354**
180771	61	14	26.00	2.76157	1.33939	2.06181	**0.04284**	0.89786	0.56987	1.57555	0.14343	2.46669	**0.01364**	2.46669	**0.01364**
3443092	68	7	1.71	3.09503	1.88560	1.64141	0.10507	4.15042	1.63068	2.54521	0.06363	2.45756	**0.01399**	2.45756	**0.01399**
3785400	69	6	56.67	-3.22392	1.93797	-1.66355	0.10055	-0.70250	0.25114	-2.79729	0.06801	-2.45166	**0.01422**	-2.45166	**0.01422**
4301511	33	42	11.90	1.66505	1.06256	1.56702	0.12149	0.95054	0.48087	1.97673	0.05517	2.45086	**0.01425**	2.45086	**0.01425**
194215	57	18	4.33	1.46236	1.26517	1.15586	0.25156	1.54079	0.59768	2.57794	**0.02100**	2.44274	**0.01458**	2.44274	**0.01458**
2423305	57	18	7.61	-0.35599	1.26202	-0.28208	0.77869	-2.03083	0.73683	-2.75618	**0.01470**	-1.92379	0.05438	-2.43963	**0.01470**
1522739	71	4	2.25	-3.11914	2.35569	-1.32409	0.18966	-3.25676	0.16729	-19.46738	**0.03267**	-2.43786	**0.01477**	-2.43786	**0.01477**
179583	67	8	1.75	-0.37365	1.73444	-0.21543	0.83004	2.50601	0.69359	3.61311	**0.01533**	1.56259	0.11815	2.42449	**0.01533**
199279	55	20	1.95	-0.20446	1.23433	-0.16565	0.86890	-1.69420	0.63328	-2.67530	**0.01598**	-1.82044	0.06869	-2.40943	**0.01598**
270094	23	52	35.77	1.76807	1.14326	1.54651	0.12636	0.58372	0.30352	1.92315	0.06028	2.40934	**0.01598**	2.40934	**0.01598**
188047	12	63	20.89	3.49434	1.41705	2.46593	**0.01605**	0.31878	0.34067	0.93574	0.35316	2.35911	**0.01832**	2.40780	**0.01605**
189356	67	8	2.50	3.33224	1.72170	1.93544	0.05686	2.18251	1.22496	1.78170	0.13490	2.40375	**0.01623**	2.40375	**0.01623**
189877	70	5	2.40	5.31492	2.16467	2.45530	**0.01649**	2.26269	6.79115	0.33318	0.77068	1.90163	0.05722	2.39783	**0.01649**
326626	56	19	6.32	2.42131	1.19792	2.02126	**0.04697**	-2.02396	0.75736	-2.67240	**0.01669**	-0.28775	0.77354	-2.39350	**0.01669**
171559	61	14	1.57	0.80844	1.37471	0.58808	0.55832	-4.85406	1.72505	-2.81387	**0.01685**	-1.27604	0.20194	-2.38993	**0.01685**
194001	50	25	3.64	2.65948	1.09225	2.43485	**0.01738**	1.01235	1.06522	0.95037	0.35225	2.33972	**0.01930**	2.37863	**0.01738**
4469576	29	46	37.48	-1.84447	1.08061	-1.70687	0.09216	-0.56929	0.33307	-1.70923	0.09462	-2.37281	**0.01765**	-2.37281	**0.01765**
196371	69	6	3.33	-1.04039	1.99530	-0.52142	0.60367	-3.59689	0.75903	-4.73883	**0.01782**	-2.04247	**0.04110**	-2.36936	**0.01782**
177032	70	5	2.20	-3.75968	2.20948	-1.70161	0.09314	-3.65150	1.21213	-3.01245	0.09479	-2.36861	**0.01786**	-2.36861	**0.01786**
4410097	68	7	4.14	-3.20432	1.80202	-1.77818	0.07960	-2.54222	1.24918	-2.03511	0.11156	-2.36474	**0.01804**	-2.36474	**0.01804**
354850	62	13	2.77	0.44971	1.44839	0.31049	0.75708	-2.41990	0.85712	-2.82331	**0.01806**	-1.45319	0.14617	-2.36443	**0.01806**
4362300	70	5	2.60	1.07810	2.17201	0.49636	0.62115	4.93291	0.67380	7.32100	**0.01815**	2.02002	**0.04338**	2.36252	**0.01815**
194373	68	7	9.43	4.32862	1.79688	2.40896	**0.01856**	-0.58165	0.95461	-0.60930	0.57523	1.26850	0.20462	2.35429	**0.01856**
195937	27	48	9.63	2.59412	1.08004	2.40188	**0.01889**	-0.86391	0.44131	-1.95761	0.05650	0.31144	0.75547	2.34764	**0.01889**
158711	71	4	36.00	0.56522	2.39650	0.23585	0.81422	0.41946	0.01292	32.47746	**0.01960**	1.81655	0.06929	2.33400	**0.01960**
192244	58	17	5.65	2.94711	1.23888	2.37884	**0.02002**	-0.56350	0.90197	-0.62474	0.54219	1.21371	0.22486	2.32595	**0.02002**
1992	14	61	12.31	-1.38668	1.36911	-1.01284	0.31453	-0.82306	0.35135	-2.34255	**0.02261**	-2.32341	**0.02016**	-2.32341	**0.02016**
3203801	45	30	7.33	-1.61979	1.07734	-1.50351	0.13708	-0.99999	0.53477	-1.86994	0.07237	-2.32180	**0.02024**	-2.32180	**0.02024**
759816	68	7	5.43	-3.58119	1.79231	-1.99808	**0.04949**	-5.84242	3.75454	-1.55610	0.19467	-2.30607	**0.02111**	-2.30607	**0.02111**
4300690	42	33	100.88	0.73970	1.08021	0.68477	0.49569	-0.68648	0.28208	-2.43363	**0.02112**	-1.14875	0.25066	-2.30587	**0.02112**
185597	55	20	4.15	2.82025	1.19641	2.35726	**0.02113**	-1.06295	0.95894	-1.10847	0.28310	0.87132	0.38358	2.30561	**0.02113**
189708	67	8	2.25	0.35494	1.73802	0.20422	0.83876	4.30512	1.29985	3.31201	**0.02119**	1.77343	0.07616	2.30452	**0.02119**
4409280	16	59	162.36	1.37326	1.29732	1.05854	0.29335	0.58237	0.25718	2.26446	**0.02744**	2.30238	**0.02131**	2.30238	**0.02131**
3943182	17	58	10.81	-2.64395	1.25613	-2.10484	**0.03880**	-0.39565	0.33018	-1.19827	0.23595	-2.29917	**0.02150**	-2.29917	**0.02150**
3474081	70	5	2.00	2.44971	2.12748	1.15147	0.25335	-2.83956	0.42764	-6.64013	**0.02194**	-0.81262	0.41644	-2.29146	**0.02194**
730939	71	4	2.50	-1.57077	2.37819	-0.66049	0.51105	11.28613	0.38976	28.95683	**0.02198**	1.15511	0.24805	2.29078	**0.02198**
2148365	51	24	7.92	-2.59118	1.10688	-2.34098	**0.02201**	-0.21287	0.79136	-0.26899	0.79056	-1.80725	0.07072	-2.29026	**0.02201**
4480359	28	47	8.09	2.49700	1.06786	2.33831	**0.02215**	-0.05023	0.52469	-0.09572	0.92418	1.55038	0.12105	2.28775	**0.02215**
175922	69	6	2.67	-3.16682	1.94133	-1.63126	0.10720	-6.18878	2.68938	-2.30119	0.10487	-2.28577	**0.02227**	-2.28577	**0.02227**
146554	20	55	91.85	1.53237	1.20040	1.27655	0.20586	0.57057	0.28390	2.00977	**0.04966**	2.28248	**0.02246**	2.28248	**0.02246**
111135	70	5	11.00	-0.27657	2.14717	-0.12881	0.89787	0.74552	0.11415	6.53115	**0.02265**	1.52094	0.12827	2.27929	**0.02265**
311950	43	32	19.56	2.20664	1.05652	2.08860	**0.04028**	0.59773	0.50018	1.19502	0.24176	2.27791	**0.02273**	2.27791	**0.02273**
189292	64	11	6.00	3.41102	1.46566	2.32729	**0.02277**	-0.78963	1.21611	-0.64931	0.53433	1.17093	0.24163	2.27735	**0.02277**
368236	67	8	2.13	-1.74632	1.72487	-1.01243	0.31472	-2.47027	0.79614	-3.10279	**0.02677**	-2.27706	**0.02278**	-2.27706	**0.02278**
195465	41	34	3.88	0.91119	1.07966	0.84396	0.40149	-1.40900	0.58815	-2.39567	**0.02281**	-1.01650	0.30939	-2.27652	**0.02281**
289734	19	56	17.04	-0.09343	1.23150	-0.07587	0.93973	-0.82653	0.35264	-2.34383	**0.02287**	-1.66256	0.09640	-2.27561	**0.02287**
4458576	23	52	72.92	2.61906	1.12687	2.32420	**0.02294**	0.00514	0.30655	0.01678	0.98668	1.62007	0.10522	2.27443	**0.02294**

Table S4a. Associated taxonomies with Body mass index (BMI) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
363442	70	5	1.60	4.81240	2.07152	2.32312	**0.02300**	-5.75146	3.09677	-1.85725	0.20440	0.71014	0.47762	2.27341	**0.02300**
3544699	68	7	4.43	-4.12458	1.77573	-2.32275	**0.02302**	-1.68556	1.78519	-0.94419	0.39853	-2.20427	**0.02751**	-2.27306	**0.02302**
187924	46	29	5.93	2.46750	1.06246	2.32244	**0.02304**	-1.37151	0.68744	-1.99509	0.05661	0.25914	0.79553	2.27277	**0.02304**
179319	67	8	2.50	-3.93430	1.69674	-2.31874	**0.02325**	2.58751	1.67415	1.54557	0.18287	-0.66278	0.50747	-2.26927	**0.02325**
211750	71	4	14.25	4.34059	2.32791	1.86459	0.06632	1.62204	0.44496	3.64533	0.17045	2.26773	**0.02335**	2.26773	**0.02335**
302333	63	12	2.50	1.24252	1.45756	0.85247	0.39678	-3.03195	1.11513	-2.71893	**0.02365**	-1.00083	0.31691	-2.26278	**0.02365**
47365	70	5	6.00	0.40116	2.16074	0.18566	0.85323	1.32934	0.20837	6.37964	**0.02370**	1.73025	0.08358	2.26196	**0.02370**
181140	59	16	3.06	-0.90595	1.30813	-0.69255	0.49082	1.97458	0.77120	2.56039	**0.02372**	1.11200	0.26614	2.26161	**0.02372**
4301298	44	31	50.84	0.64005	1.09457	0.58475	0.56054	-0.76522	0.32021	-2.38978	**0.02383**	-1.18638	0.23547	-2.25983	**0.02383**
198449	20	55	15.67	-1.01631	1.21113	-0.83915	0.40416	-0.76039	0.32669	-2.32760	**0.02386**	-2.18749	**0.02871**	-2.25937	**0.02386**
158217	66	9	3.67	3.70550	1.60688	2.30603	**0.02399**	0.84191	2.00813	0.41925	0.68963	1.87852	0.06031	2.25727	**0.02399**
189793	71	4	1.25	3.90422	2.37044	1.64704	0.10391	11.34500	2.14122	5.29837	0.11876	2.25299	**0.02426**	2.25299	**0.02426**
3926480	22	53	23.74	2.61708	1.13732	2.30109	**0.02428**	-0.37098	0.31678	-1.17109	0.24711	0.77443	0.43868	2.25260	**0.02428**
199190	69	6	2.00	3.41440	1.94475	1.75570	0.08339	3.59704	1.84765	1.94682	0.14673	2.25037	**0.02443**	2.25037	**0.02443**
4425571	30	45	21.82	2.44928	1.06638	2.29681	**0.02454**	-0.08789	0.34188	-0.25707	0.79838	1.40934	0.15873	2.24855	**0.02454**
275237	42	33	229.64	2.39206	1.04159	2.29655	**0.02456**	-0.09576	0.24420	-0.39213	0.69774	1.31517	0.18845	2.24831	**0.02456**
329313	70	5	1.20	0.64305	2.18224	0.29468	0.76909	-4.88817	0.78182	-6.25227	**0.02464**	-1.38129	0.16719	-2.24700	**0.02464**
198183	16	59	11.10	2.95129	1.28689	2.29335	**0.02475**	-0.26743	0.37831	-0.70691	0.48255	1.09113	0.27522	2.24529	**0.02475**
363646	58	17	13.29	2.83368	1.23684	2.29107	**0.02489**	-0.03179	0.55117	-0.05769	0.95481	1.54606	0.12209	2.24313	**0.02489**
174638	66	9	18.22	3.65503	1.59636	2.28961	**0.02498**	-0.19769	0.69955	-0.28260	0.78698	1.39407	0.16330	2.24175	**0.02498**
3887769	55	20	1.95	1.25025	1.21369	1.03012	0.30640	2.11529	0.90633	2.33390	**0.03213**	2.23840	**0.02520**	2.23840	**0.02520**
1000547	51	24	2.75	2.09717	1.12130	1.87029	0.06551	1.50036	1.09645	1.36837	0.18566	2.23821	**0.02521**	2.23821	**0.02521**
3251419	63	12	4.67	-3.22263	1.41065	-2.28450	**0.02529**	-0.61673	1.33946	-0.46043	0.65613	-1.89659	0.05788	-2.23692	**0.02529**
175373	70	5	1.60	-4.83026	2.11722	-2.28142	**0.02548**	1.03933	1.36105	0.76362	0.52488	-1.13007	0.25845	-2.23400	**0.02548**
4446669	60	15	12.40	-2.09376	1.31798	-1.58861	0.11653	-1.07164	0.62558	-1.71302	0.11240	-2.23233	**0.02559**	-2.23233	**0.02559**
4463108	70	5	4.80	0.52223	2.15575	0.24225	0.80928	-2.77526	0.45415	-6.11090	**0.02575**	-1.40617	0.15968	-2.22998	**0.02575**
4396297	33	42	7.98	2.37548	1.04473	2.27377	**0.02596**	-0.96391	0.47032	-2.04949	**0.04718**	0.17119	0.86407	2.22676	**0.02596**
688923	63	12	3.08	0.22256	1.48564	0.14981	0.88133	4.23127	1.59176	2.65823	**0.02612**	1.67844	0.09326	2.22440	**0.02612**
192437	26	49	16.29	2.46691	1.08727	2.26890	**0.02627**	0.27343	0.44793	0.61042	0.54459	1.99974	**0.04553**	2.22216	**0.02627**
197539	69	6	4.50	-1.49488	1.96640	-0.76021	0.44961	1.35457	0.33397	4.05598	**0.02701**	1.02910	0.30343	2.21143	**0.02701**
193763	63	12	3.17	0.88719	1.48485	0.59750	0.55205	3.49114	1.32360	2.63762	**0.02702**	1.98409	**0.04725**	2.21124	**0.02702**
816702	48	27	33.30	2.43313	1.07862	2.25578	**0.02712**	-0.03957	0.39577	-0.09997	0.92120	1.49257	0.13555	2.20974	**0.02712**
193873	65	10	1.10	2.85822	1.55592	1.83700	0.07034	7.95997	5.47236	1.45458	0.18911	2.20826	**0.02723**	2.20826	**0.02723**
184990	66	9	4.89	-1.68044	1.64473	-1.02171	0.31034	-1.10093	0.40593	-2.71210	**0.03501**	-2.20814	**0.02723**	-2.20814	**0.02723**
185814	24	51	7.24	2.51825	1.11956	2.24932	**0.02755**	-0.53974	0.40819	-1.32229	0.19234	0.63634	0.52455	2.20362	**0.02755**
3475269	67	8	2.38	-1.80463	1.72210	-1.04793	0.29818	-2.89091	1.03444	-2.79467	**0.03823**	-2.20101	**0.02774**	-2.20101	**0.02774**
308322	69	6	4.00	0.72569	1.97573	0.36730	0.71447	-2.50120	0.62343	-4.01203	**0.02779**	-1.29711	0.19459	-2.20025	**0.02779**
368490	59	16	12.25	1.10087	1.30605	0.84290	0.40208	1.11135	0.44876	2.47646	**0.02779**	2.14825	**0.03169**	2.20017	**0.02779**
198754	70	5	2.80	3.96227	2.09578	1.89060	0.06270	2.37909	1.31360	1.81112	0.21182	2.19901	**0.02788**	2.19901	**0.02788**
3946926	69	6	3.17	4.32411	1.92719	2.24374	**0.02792**	-0.21009	2.34592	-0.08956	0.93428	1.49616	0.13461	2.19834	**0.02792**
234443	67	8	6.88	2.12644	1.73491	1.22568	0.22431	2.16106	0.88425	2.44396	0.05837	2.19773	**0.02797**	2.19773	**0.02797**
191148	71	4	2.50	4.26313	2.33001	1.82966	0.07144	6.27986	1.95612	3.21038	0.19224	2.19674	**0.02804**	2.19674	**0.02804**
2530636	61	14	176.79	0.30809	1.37636	0.22384	0.82352	0.75212	0.29747	2.52834	**0.02806**	1.71086	0.08711	2.19650	**0.02806**
176269	9	66	145.11	0.43719	1.64749	0.26537	0.79148	0.43873	0.19524	2.24717	**0.02814**	1.73927	0.08199	2.19532	**0.02814**
363389	65	10	304.50	3.41274	1.52613	2.23620	**0.02844**	0.03187	0.32816	0.09712	0.92535	1.61566	0.10617	2.19119	**0.02844**
4433737	62	13	7.54	0.71895	1.42096	0.50596	0.61443	-1.24564	0.48863	-2.54922	**0.02890**	-1.18869	0.23456	-2.18481	**0.02890**
1835779	59	16	2.38	0.79107	1.30860	0.60451	0.54740	1.86683	0.76037	2.45517	**0.02893**	1.97006	**0.04883**	2.18443	**0.02893**
182653	70	5	8.80	-4.66877	2.09579	-2.22769	**0.02903**	0.20035	0.42913	0.46687	0.68651	-1.25832	0.20828	-2.18312	**0.02903**
164259	66	9	3.33	3.55504	1.60008	2.22179	**0.02944**	-0.40275	2.07998	-0.19363	0.85285	1.40859	0.15896	2.17753	**0.02944**
2831841	54	21	3.67	1.03230	1.18882	0.86835	0.38809	1.31375	0.55603	2.36274	**0.02960**	2.14853	**0.03167**	2.17539	**0.02960**
176108	64	11	2.18	0.39567	1.52815	0.25892	0.79643	-5.30171	2.00659	-2.64215	**0.02961**	-1.35570	0.17520	-2.17521	**0.02961**
361702	71	4	2.00	-4.84593	2.33321	-2.07694	**0.04138**	-5.12441	2.63994	-1.94111	0.30285	-2.17088	**0.02994**	-2.17088	**0.02994**
199677	54	21	2.90	-0.22591	1.19633	-0.18883	0.85075	-1.69433	0.72054	-2.35148	**0.03029**	-1.66485	0.09594	-2.16629	**0.03029**
178183	71	4	1.25	-1.25179	2.38028	-0.52590	0.60057	15.34949	0.74591	20.57833	**0.03091**	1.15586	0.24774	2.15820	**0.03091**
4094866	57	18	5.89	-2.67099	1.21434	-2.19954	**0.03105**	-0.62953	0.71924	-0.87527	0.39523	-2.12598	**0.03350**	-2.15642	**0.03105**
588471	64	11	315.64	-0.55796	1.51512	-0.36826	0.71376	-0.65723	0.25172	-2.61096	**0.03108**	-1.78390	0.07444	-2.15600	**0.03108**
4421070	52	23	2.96	2.26007	1.15937	1.94940	0.05514	1.41776	1.21794	1.16407	0.25810	2.15572	**0.03111**	2.15572	**0.03111**
4305372	70	5	27.60	-1.21748	2.14394	-0.56787	0.57189	-0.78765	0.14285	-5.51383	**0.03135**	-1.92180	0.05463	-2.15256	**0.03135**
4278525	48	27	3.67	-2.26326	1.08473	-2.08648	**0.04048**	-0.59482	0.58923	-1.00949	0.32281	-2.14787	**0.03172**	-2.14787	**0.03172**
193778	66	9	3.00	1.88940	1.63297	1.15703	0.25108	3.38041	1.45818	2.31824	0.05959	2.14361	**0.03206**	2.14361	**0.03206**
258099	64	11	4.64	3.21945	1.47293	2.18575	**0.03209**	-1.67410	1.72358	-0.97129	0.35985	0.86810	0.38534	2.14332	**0.03209**
2331530	71	4	1.50	-1.01006	2.38413	-0.42366	0.67308	-0.81432	0.04125	-19.74055	**0.03222**	-1.81272	0.06987	-2.14165	**0.03222**
194129	71	4	2.00	-0.45280	2.39683	-0.18892	0.85069	5.46473	0.27753	19.69075	**0.03230**	1.38056	0.16742	2.14064	**0.03230**
4452400	71	4	2.50	-5.04780	2.31312	-2.18225	**0.03235**	-2.03554	9.30343	-0.21879	0.86287	-1.63534	0.10198	-2.14000	**0.03235**
573110	67	8	2.88	-2.25023	1.71461	-1.31238	0.19356	-1.78425	0.83198	-2.14457	0.08483	-2.13792	**0.03252**	-2.13792	**0.03252**
4315974	68	7	2.71	3.01408	1.80834	1.66677	0.09991	-4.69667	1.46471	-3.20655	**0.03270**	-0.34682	0.72872	-2.13580	**0.03270**
197540	69	6	4.33	-4.18345	1.92522	-2.17297	**0.03307**	-0.97879	2.09938	-0.46623	0.67282	-1.80557	0.07099	-2.13118	**0.03307**
4329572	69	6	3.00	4.15493	1.91259	2.17240	**0.03312**	-2.09920	1.80300	-1.16428	0.32848	0.81563	0.41471	2.13065	**0.03312**
4412540	23	52	179.63	2.12686	1.13670	1.87108	0.06540	-0.50253	0.22955	-2.18917	**0.03338**	-0.20151	0.84030	-2.12751	**0.03338**
184342	68	7	1.86	3.59109	1.80301	1.99172	0.05020	-4.53746	1.42970	-3.17372	**0.03374**	-0.11661	0.90717	-2.12320	**0.03374**
209475	70	5	2.40	4.50800	2.08268	2.16452	**0.03374**	-1.65411	3.95890	-0.41782	0.71666	1.24467	0.21325	2.12315	**0.03374**
182255	42	33	4.58	1.52074	1.08288	1.40435	0.16451	-1.52637	0.68726	-2.22094	**0.03405**	-0.51579	0.60600	-2.11948	**0.03405**
180999	35	40	7.23	2.24833	1.04256	2.15655	**0.03438**	0.04691	0.58245	0.08053	0.93625	1.55250	0.12054	2.11558	**0.03438**
1046624	71	4	34.25	-0.53296	2.39660	-0.22238	0.82465	-1.08484	0.05882	-18.44235	**0.03449**	-1.65174	0.09859	-2.11435	**0.03449**
168071	30	45	10.31	2.29478	1.06653	2.15162	**0.03478**	-0.03537	0.47409	-0.07461	0.94088	1.44018	0.14982	2.11089	**0.03478**
4390319	49	26	4.85	-1.15249	1.11730	-1.03149	0.30576	-1.34852	0.65304	-2.06499	0.05038	-2.10783	**0.03505**	-2.10783	**0.03505**
178708	50	25	2.28	2.37874	1.10968	2.14362	**0.03544**	-0.62700	1.21803	-0.51477	0.61185	1.12843	0.25914	2.10328	**0.03544**

Table S4a. Associated taxonomies with Body mass index (BMI) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
184925	44	31	20.10	2.26155	1.05596	2.14171	**0.03560**	0.34729	0.49075	0.70768	0.48500	1.97972	**0.04773**	2.10146	**0.03560**
110192	60	15	20.60	-2.78505	1.30157	-2.13976	**0.03576**	0.42000	0.48975	0.85758	0.40792	-0.89948	0.36840	-2.09960	**0.03576**
308544	62	13	3.15	-2.46808	1.39302	-1.77175	0.08067	-1.07461	0.82531	-1.30206	0.22209	-2.09857	**0.03585**	-2.09857	**0.03585**
1726408	70	5	40.80	-3.88068	2.10091	-1.84714	0.06884	-1.79682	1.12478	-1.59749	0.25125	-2.09786	**0.03592**	-2.09786	**0.03592**
577170	14	61	25.00	-0.41519	1.38814	-0.29910	0.76573	-0.68982	0.32135	-2.14666	**0.03601**	-1.69333	0.09039	-2.09676	**0.03601**
330469	49	26	7.15	1.46921	1.11305	1.31998	0.19102	-1.06352	0.47785	-2.22562	**0.03613**	-0.55716	0.57742	-2.09551	**0.03613**
4399109	53	22	48.32	2.43712	1.14303	2.13216	**0.03641**	-0.31419	0.51856	-0.60590	0.55175	1.05870	0.28973	2.09237	**0.03641**
179826	60	15	2.20	-2.81273	1.32039	-2.13023	**0.03657**	-1.39380	1.58240	-0.88081	0.39572	-2.07877	**0.03764**	-2.09053	**0.03657**
218710	67	8	3.75	-2.70719	1.71509	-1.57845	0.11884	-2.70149	1.65583	-1.63151	0.16371	-2.08762	**0.03683**	-2.08762	**0.03683**
158415	64	11	104.45	2.82740	1.50078	1.88395	0.06361	0.28307	0.24261	1.16679	0.27690	2.08047	**0.03748**	2.08047	**0.03748**
1820776	64	11	40.09	3.11897	1.47180	2.11916	**0.03753**	-0.61859	0.57961	-1.06726	0.31700	0.76321	0.44534	2.07999	**0.03753**
122049	54	21	13.14	1.88374	1.17350	1.60523	0.11282	0.67765	0.48177	1.40659	0.17658	2.07677	**0.03782**	2.07677	**0.03782**
842594	70	5	2.60	-2.86921	2.14027	-1.34058	0.18427	-3.23578	1.16522	-2.77696	0.10890	-2.07244	**0.03822**	-2.07244	**0.03822**
178151	71	4	3.25	-4.07035	2.33554	-1.74279	0.08564	-1.17728	0.43574	-2.70182	0.22567	-2.07213	**0.03825**	-2.07213	**0.03825**
336559	19	56	226.68	2.52453	1.19640	2.11010	**0.03833**	0.06934	0.20970	0.33067	0.74220	1.69727	0.08965	2.07136	**0.03833**
289452	68	7	1.00	-3.77236	1.78818	-2.10961	**0.03837**	0.00000	0.00000	0.00000	1.00000	-1.46434	0.14310	-2.07089	**0.03837**
334336	45	30	6.80	1.18768	1.08547	1.09416	0.27753	1.06640	0.55554	1.91958	0.06554	2.07003	**0.03845**	2.07003	**0.03845**
4424598	51	24	3.33	2.35244	1.11805	2.10405	**0.03887**	-1.73924	1.06467	-1.63359	0.11725	0.35297	0.72411	2.06559	**0.03887**
184114	25	50	16.10	2.34300	1.11674	2.09808	**0.03941**	-0.19876	0.38336	-0.51848	0.60655	1.09241	0.27465	2.05990	**0.03941**
190490	65	10	5.00	-1.62254	1.56411	-1.03735	0.30304	-1.17713	0.52581	-2.23869	0.06019	-2.05719	**0.03967**	-2.05719	**0.03967**
195805	67	8	11.75	-2.28720	1.71636	-1.33258	0.18687	-1.13928	0.59315	-1.92073	0.11282	-2.05452	**0.03993**	-2.05452	**0.03993**
113542	45	30	17.70	-1.30428	1.08400	-1.20321	0.23284	-0.65992	0.37170	-1.77543	0.08710	-2.05342	**0.04003**	-2.05342	**0.04003**
185583	22	53	26.92	2.44084	1.16824	2.08933	**0.04021**	0.00494	0.33680	0.01468	0.98835	1.46100	0.14402	2.05156	**0.04021**
2341726	69	6	2.00	4.04615	1.93934	2.08636	**0.04049**	2.29635	4.02793	0.57011	0.60850	1.81085	0.07016	2.04873	**0.04049**
179695	71	4	2.50	-2.60565	2.36955	-1.09964	0.27515	1.54608	0.09866	15.67005	**0.04057**	0.67642	0.49877	2.04788	**0.04057**
4364405	41	34	10.06	-0.72159	1.09568	-0.65857	0.51227	-1.04422	0.48876	-2.13647	**0.04064**	-1.91093	0.05601	-2.04716	**0.04064**
4434579	62	13	83.77	2.86908	1.37671	2.08401	**0.04071**	0.39284	0.47891	0.82027	0.43118	2.00370	**0.04510**	2.04648	**0.04071**
4346374	70	5	2.40	4.37709	2.10059	2.08374	**0.04073**	-9.62428	4.72320	-2.03766	0.17847	0.49551	0.62024	2.04623	**0.04073**
216710	28	47	7.55	0.26297	1.12599	0.23355	0.81600	1.31078	0.62517	2.09667	**0.04181**	1.60382	0.10875	2.03545	**0.04181**
175612	70	5	65.20	2.38008	2.21210	1.07594	0.28555	-1.76498	0.37282	-4.73417	**0.04184**	-0.68390	0.49404	-2.03513	**0.04184**
2298935	20	55	31.00	2.43613	1.17654	2.07058	**0.04198**	0.11246	0.31250	0.35986	0.72041	1.69111	0.09082	2.03368	**0.04198**
1945397	66	9	9.00	-3.31040	1.60219	-2.06617	**0.04241**	-0.17723	1.70936	-0.10368	0.92080	-1.50535	0.13223	-2.02947	**0.04241**
4333652	67	8	2.13	0.69519	1.73325	0.40109	0.68954	-2.65548	0.98279	-2.70199	**0.04268**	-1.15069	0.24986	-2.02680	**0.04268**
4457064	59	16	11.19	-2.73032	1.32370	-2.06264	**0.04276**	0.29097	0.94391	0.30826	0.76277	-1.21922	0.22276	-2.02609	**0.04276**
3648884	62	13	5.62	-2.82765	1.37547	-2.05577	**0.04343**	-0.51233	1.25193	-0.40923	0.69100	-1.70910	0.08743	-2.01953	**0.04343**
4460786	61	14	5.00	-1.10821	1.37029	-0.80874	0.42133	-1.49831	0.65685	-2.28105	**0.04346**	-1.99646	**0.04588**	-2.01930	**0.04346**
189007	67	8	2.00	-0.98619	1.73488	-0.56845	0.57150	-7.39027	2.75546	-2.68205	**0.04371**	-1.82623	0.06782	-2.01683	**0.04371**
4445673	49	26	34.08	2.24509	1.09393	2.05232	**0.04377**	0.04805	0.31897	0.15065	0.88157	1.53104	0.12576	2.01624	**0.04377**
1736067	67	8	3.38	3.52071	1.71649	2.05111	**0.04390**	-0.56620	1.98127	-0.28578	0.78651	1.23337	0.21744	2.01509	**0.04390**
181466	69	6	3.17	-1.91269	1.96122	-0.97525	0.33270	-4.42934	1.50382	-2.94539	0.06025	-2.01360	**0.04405**	-2.01360	**0.04405**
4460021	54	21	8.24	-0.49680	1.19418	-0.41602	0.67863	-1.84935	0.85438	-2.16455	**0.04411**	-1.71642	0.08609	-2.01306	**0.04411**
193129	50	25	5.52	0.62969	1.14732	0.54884	0.58481	-1.27098	0.59662	-2.13031	**0.04457**	-1.03400	0.30114	-2.00866	**0.04457**
189208	55	20	3.80	0.53183	1.22099	0.43557	0.66445	-2.07842	0.95904	-2.16718	**0.04471**	-1.11268	0.26585	-2.00734	**0.04471**
1146771	69	6	2.83	-2.99570	1.94728	-1.53841	0.12833	-4.52146	2.66476	-1.69676	0.18831	-2.00557	**0.04490**	-2.00557	**0.04490**
354334	66	9	2.89	0.89699	1.64555	0.54510	0.58737	-6.66461	2.63811	-2.52628	**0.04490**	-1.03442	0.30094	-2.00555	**0.04490**
185575	14	61	187.34	2.82522	1.38650	2.03767	**0.04526**	0.03489	0.26164	0.13337	0.89436	1.50969	0.13112	2.00224	**0.04526**
583134	71	4	2.50	-4.73459	2.32651	-2.03506	**0.04553**	0.75144	1.36740	0.54954	0.68010	-1.12248	0.26166	-1.99975	**0.04553**
114348	67	8	7.25	1.72984	1.72293	1.00401	0.31874	4.89145	2.10018	2.32906	0.06729	1.99884	**0.04563**	1.99884	**0.04563**
2774254	69	6	13.50	-3.90064	1.91989	-2.03170	**0.04588**	-0.77310	0.87221	-0.88637	0.44070	-1.95695	0.05035	-1.99654	**0.04588**
845396	69	6	4.00	3.54972	1.92945	1.83976	0.06993	3.20869	2.64739	1.21202	0.31225	1.99609	**0.04592**	1.99609	**0.04592**
198248	56	19	23.58	0.75399	1.23875	0.60867	0.54466	-1.08786	0.50268	-2.16411	**0.04593**	-0.98308	0.32557	-1.99607	**0.04593**
180826	45	30	4.87	2.16642	1.06713	2.03014	**0.04604**	0.38402	0.72907	0.52673	0.60268	1.77880	0.07527	1.99505	**0.04604**
4434334	51	24	9.75	2.26822	1.11790	2.02899	**0.04616**	-0.12231	0.69743	-0.17537	0.86247	1.28744	0.19794	1.99395	**0.04616**
3211875	25	50	7.50	-0.22260	1.13740	-0.19571	0.84539	-1.06646	0.52118	-2.04622	**0.04635**	-1.54657	0.12197	-1.99218	**0.04635**
25461	70	5	1.60	-1.34377	2.14117	-0.62758	0.53226	15.08178	3.37368	4.47042	**0.04657**	0.96564	0.33422	1.99018	**0.04657**
4472721	4	71	28.08	-0.06419	2.43931	-0.02632	0.97908	-0.71691	0.35412	-2.02450	**0.04685**	-1.42404	0.15444	-1.98767	**0.04685**
184534	66	9	14.67	-2.23082	1.63543	-1.36405	0.17680	-1.14200	0.68155	-1.67561	0.14483	-1.98605	**0.04703**	-1.98605	**0.04703**
125270	61	14	3.50	-2.71407	1.34532	-2.01742	**0.04738**	-0.33335	1.00853	-0.33053	0.74721	-1.63003	0.10310	-1.98289	**0.04738**
2932557	61	14	2.79	2.40449	1.42134	1.69171	0.09503	-2.14154	0.96296	-2.22392	**0.04803**	-0.21751	0.82781	-1.97706	**0.04803**
2119418	32	43	269.07	1.37740	1.07161	1.28535	0.20279	0.37847	0.24449	1.54802	0.12949	1.97265	**0.04854**	1.97265	**0.04854**
1139021	55	20	43.05	1.37799	1.20764	1.14106	0.25763	-0.95896	0.45177	-2.12265	**0.04877**	-0.59296	0.55320	-1.97060	**0.04877**
158625	70	5	2.80	-4.19743	2.09648	-2.00213	**0.04904**	0.59973	2.34912	0.25530	0.82235	-1.23300	0.21757	-1.96826	**0.04904**
519880	70	5	3.80	2.48061	2.15851	1.14922	0.25427	-27.04683	6.23030	-4.34118	**0.04918**	-0.58476	0.55871	-1.96702	**0.04918**
4436552	18	57	852.02	2.44127	1.22104	1.99934	**0.04935**	0.05112	0.13330	0.38351	0.70285	1.65963	0.09699	1.96559	**0.04935**
4379889	55	20	4.70	1.58269	1.20524	1.31318	0.19329	-1.56247	0.73936	-2.11329	**0.04966**	-0.46806	0.63974	-1.96284	**0.04966**
199491	69	6	4.00	2.28134	1.96908	1.15858	0.25045	2.97205	1.28844	2.30670	0.10435	1.96108	**0.04987**	1.96108	**0.04987**
592616	60	15	2.67	2.61590	1.31187	1.99402	**0.04994**	-2.77350	1.30007	-2.13335	0.05423	0.02511	0.97997	1.96050	**0.04994**
167373	4	71	50.25	3.52286	2.35587	1.49535	0.13919	0.42360	0.32433	1.30606	0.19593	1.96012	**0.04998**	1.96012	**0.04998**

[a] Statistics associated to the two part model for association analysis between the trait and OTUs adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe represented by an OTU with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the OTU for the subjects where that OTU is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per OTU-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Supplemental Information

Table S4b. OTUs associated with Triglycerides at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
195123	59	16	3.75	-2.21729	24.85185	-0.08922	0.92915	48.95492	8.02948	6.09690	**0.00004**	2.84996	**0.00437**	4.11935	**0.00004**
2388088	58	17	3.82	95.10042	21.79403	4.36360	**0.00004**	39.05425	31.13143	1.25450	0.23020	3.74494	**0.00018**	4.09631	**0.00004**
178151	71	4	3.25	172.47685	40.31464	4.27827	**0.00006**	-248.98487	0.60620	-410.73177	**0.00155**	0.60777	0.54334	4.02468	**0.00006**
4442508	71	4	6.50	171.59973	40.95988	4.18946	**0.00008**	157.78946	81.41094	1.93818	0.30324	3.52082	**0.00043**	3.94968	**0.00008**
3327894	3	72	136.31	-193.62657	46.67167	-4.14870	**0.00009**	-1.56333	3.09463	-0.50518	0.61505	-3.12399	**0.00178**	-3.91510	**0.00009**
4437746	70	5	10.80	151.71499	36.61591	4.14342	**0.00009**	19.86678	73.27994	0.27111	0.81173	2.93366	**0.00335**	3.91062	**0.00009**
163494	59	16	3.44	19.66282	24.77447	0.79367	0.42999	128.77073	24.77472	5.19767	**0.00017**	3.21483	**0.00131**	3.75726	**0.00017**
177100	71	4	1.25	131.18782	42.91750	3.05674	**0.00314**	506.00000	16.58312	30.51295	**0.02086**	3.72249	**0.00020**	3.72249	**0.00020**
175729	54	21	4.29	-44.07819	22.18797	-1.98658	0.05078	-32.84108	8.43923	-3.89148	**0.00107**	-3.69459	**0.00022**	-3.69459	**0.00022**
174439	61	14	3.29	-54.76893	25.21380	-2.17218	**0.03314**	-27.31647	6.88011	-3.97036	**0.00219**	-3.67198	**0.00024**	-3.67198	**0.00024**
182653	70	5	8.80	142.58930	37.43193	3.80930	**0.00029**	1.45473	93.77599	0.01551	0.98903	2.57188	**0.01011**	3.62344	**0.00029**
318970	67	8	1.63	26.44487	34.24850	0.77215	0.44255	203.03638	23.93529	8.48272	**0.00037**	3.05864	**0.00222**	3.55768	**0.00037**
583134	71	4	2.50	136.03998	42.37249	3.21057	**0.00198**	261.23319	25.75264	10.14394	0.06256	3.50406	**0.00046**	3.50406	**0.00046**
4421273	40	35	9.49	-44.42512	19.87763	-2.23493	**0.02853**	-15.02770	5.07366	-2.96190	**0.00572**	-3.50243	**0.00046**	-3.50243	**0.00046**
3302039	68	7	2.29	102.11586	33.20857	3.07498	**0.00297**	175.24412	64.28930	2.72587	0.05267	3.47051	**0.00052**	3.47051	**0.00052**
187178	51	24	56.92	25.94119	21.60963	1.20045	0.23390	33.32894	8.43313	3.95214	**0.00073**	3.23079	**0.00123**	3.37866	**0.00073**
130763	70	5	9.20	133.13555	37.76472	3.52539	**0.00074**	73.01093	95.72293	0.76273	0.52531	2.83523	**0.00458**	3.37444	**0.00074**
519763	70	5	11.40	129.62156	37.96771	3.41400	**0.00105**	105.28224	75.14789	1.40100	0.29622	3.05477	**0.00225**	3.27552	**0.00105**
4020502	17	58	96.33	-78.91159	23.14056	-3.41010	**0.00107**	0.41016	3.01656	0.13597	0.89234	-2.21798	**0.02656**	-3.27205	**0.00107**
4379646	67	8	1.75	103.46286	30.53319	3.38854	**0.00114**	128.10938	312.41797	0.41006	0.69874	2.57375	**0.01006**	3.25282	**0.00114**
571642	40	35	5.00	-65.66006	19.57723	-3.35390	**0.00127**	-5.44005	7.57639	-0.71803	0.47795	-2.77997	**0.00544**	-3.22188	**0.00127**
170462	68	7	4.00	109.33597	32.83151	3.33021	**0.00137**	24.93031	105.12404	0.23715	0.82419	2.42031	**0.01551**	3.20068	**0.00137**
4444262	65	10	13.70	92.70458	27.91868	3.32052	**0.00141**	-20.86735	25.49646	-0.81844	0.44007	1.71115	0.08705	3.19200	**0.00141**
4378683	52	23	15.17	67.83950	20.54066	3.30269	**0.00149**	0.75949	16.67221	0.04555	0.96412	2.27759	**0.02275**	3.17601	**0.00149**
4469032	70	5	5.80	124.62234	37.97335	3.28184	**0.00159**	-59.90431	113.98038	-0.52557	0.65165	1.91330	0.05571	3.15730	**0.00159**
194089	68	7	2.14	95.52322	33.55399	2.84685	**0.00575**	248.08042	111.32062	2.22852	0.08976	3.15259	**0.00162**	3.15259	**0.00162**
309391	71	4	3.00	137.43994	42.12865	3.26239	**0.00169**	210.19327	157.74733	1.33247	0.40986	2.80294	**0.00506**	3.13982	**0.00169**
1835985	68	7	4.71	60.51589	34.16059	1.77151	0.08071	217.96757	43.58857	5.00057	**0.00749**	3.12608	**0.00177**	3.12608	**0.00177**
151870	70	5	4.80	125.13938	38.57312	3.24421	**0.00179**	-18.51976	99.83160	-0.18551	0.86994	2.09285	**0.03636**	3.12347	**0.00179**
4428929	69	6	1.83	113.13588	34.92739	3.23917	**0.00182**	-67.74654	141.44090	-0.47897	0.66470	1.89894	0.05757	3.11893	**0.00182**
191043	70	5	4.20	122.23330	38.16084	3.20311	**0.00203**	36.30152	169.23000	0.21451	0.85003	2.31613	**0.02055**	3.08643	**0.00203**
326662	70	5	1.40	121.82848	38.09906	3.19768	**0.00206**	-132.36471	222.86241	-0.59393	0.61279	1.82110	0.06859	3.08153	**0.00206**
194053	71	4	1.50	136.71756	43.00021	3.17946	**0.00218**	-381.49057	268.41610	-1.42127	0.39034	1.55993	0.11878	3.06508	**0.00218**
362955	66	9	16.44	-4.71435	31.37827	-0.15024	0.88099	41.96079	8.22838	5.09952	**0.00222**	2.05702	**0.03968**	3.05877	**0.00222**
186352	29	46	8.22	-62.79353	19.85743	-3.16222	**0.00229**	-3.54220	6.64189	-0.53331	0.59656	-2.53062	**0.01139**	-3.04949	**0.00229**
166689	69	6	4.00	110.94030	35.10651	3.16011	**0.00231**	-3.79122	117.39124	-0.03230	0.97626	2.13393	**0.03285**	3.04758	**0.00231**
191361	21	54	17.31	-66.88559	21.18813	-3.15675	**0.00233**	-2.84727	5.68569	-0.50078	0.61868	-2.50476	**0.01225**	-3.04454	**0.00233**
168439	44	31	33.58	33.56790	21.09612	1.59119	0.11595	25.12707	8.50586	2.95409	**0.00629**	3.04354	**0.00234**	3.04354	**0.00234**
183619	70	5	2.20	119.41571	38.42977	3.10738	**0.00270**	25.12318	131.64275	0.19084	0.86627	2.24027	**0.02507**	2.99982	**0.00270**
4343184	63	12	2.75	81.05727	26.17627	3.09659	**0.00279**	-53.77380	48.28355	-1.11371	0.29426	1.37265	0.16986	2.99003	**0.00279**
185802	31	44	91.45	-51.23176	19.71249	-2.59895	**0.01134**	-6.18315	3.56937	-1.73228	0.09074	-2.98658	**0.00282**	-2.98658	**0.00282**
179384	68	7	1.86	74.04184	33.77213	2.19239	**0.03158**	196.52116	67.13172	2.92740	**0.04293**	2.95151	**0.00316**	2.95151	**0.00316**
4331360	22	53	13.06	-0.47368	22.36086	-0.02118	0.98316	-26.35196	8.49753	-3.10113	**0.00317**	-2.10175	**0.03557**	-2.95122	**0.00317**
4391326	69	6	14.17	107.80488	35.36310	3.04851	**0.00322**	-30.98809	61.95396	-0.50018	0.65134	1.76382	0.07776	2.94633	**0.00322**
4468466	18	57	118.93	-41.07753	23.88759	-1.71962	0.08980	-8.62870	3.44402	-2.50542	**0.01528**	-2.91482	**0.00356**	-2.91482	**0.00356**
174571	64	11	2.82	72.60399	27.51598	2.63861	**0.01020**	87.88975	52.13012	1.68597	0.13029	2.88645	**0.00390**	2.88645	**0.00390**
230479	70	5	4.00	114.34613	38.40503	2.97737	**0.00396**	25.79741	144.49669	0.17853	0.87475	2.14895	**0.03164**	2.88145	**0.00396**
300374	43	32	6.13	-41.32386	20.28601	-2.03706	**0.04532**	-14.82788	6.82569	-2.17236	**0.03814**	-2.88143	**0.00396**	-2.88143	**0.00396**
169364	61	14	6.64	29.93819	25.80473	1.16018	0.24981	74.83746	20.62621	3.62827	**0.00397**	2.85067	**0.00436**	2.88064	**0.00397**
199524	61	14	1.29	43.37672	26.09974	1.66196	0.10087	186.45194	65.29559	2.85551	**0.01564**	2.86931	**0.00411**	2.86931	**0.00411**
4423553	37	38	12.08	-47.18898	19.60270	-2.40727	**0.01864**	-10.61398	6.07017	-1.74855	0.08914	-2.86568	**0.00416**	-2.86568	**0.00416**
176269	9	66	145.11	-87.11626	29.47532	-2.95557	**0.00422**	-1.38344	2.83137	-0.48861	0.62681	-2.36720	**0.01792**	-2.86151	**0.00422**
192462	20	55	44.05	-8.98919	22.91413	-0.39230	0.69600	17.92372	5.99021	2.99217	**0.00423**	1.74643	0.08074	2.86055	**0.00423**
4450415	70	5	2.60	100.83604	39.35611	2.56214	**0.01250**	198.67864	76.91674	2.58304	0.12286	2.85722	**0.00427**	2.85722	**0.00427**
168071	30	45	10.31	-58.05155	19.67982	-2.94980	**0.00429**	-1.37593	7.74837	-0.17758	0.85991	-2.14446	**0.03200**	-2.85624	**0.00429**
292041	71	4	1.50	13.80766	45.10377	0.30613	0.76039	-153.36851	1.04496	-146.77020	**0.00434**	-1.80139	0.07164	-2.85252	**0.00434**
2182669	70	5	6.80	7.27226	41.10219	0.17693	0.86006	-16.60887	1.10668	-15.00779	**0.00441**	-1.88862	0.05894	-2.84721	**0.00441**
185864	56	19	4.95	39.36651	23.08040	1.70563	0.09239	56.64785	21.84122	2.59362	**0.01959**	2.84044	**0.00451**	2.84044	**0.00451**
174288	49	26	3.69	-59.12423	20.26925	-2.91694	**0.00471**	8.63900	9.37280	0.92171	0.36625	-1.35950	0.17399	-2.82614	**0.00471**
185034	31	44	11.86	-35.19822	20.25176	-1.73803	0.08648	-15.02325	6.37969	-2.35485	**0.02340**	-2.81508	**0.00488**	-2.81508	**0.00488**
2157225	65	10	2.40	81.87876	28.23526	2.89988	**0.00495**	27.18942	78.15937	0.34787	0.73816	2.22369	**0.02617**	2.81049	**0.00495**
4483337	17	58	50.33	7.76230	24.43743	0.31764	0.75168	14.64031	5.01851	2.91726	**0.00510**	2.20392	**0.02753**	2.80038	**0.00510**
184036	68	7	1.57	38.20940	35.06843	1.08957	0.27954	245.58041	44.22114	5.55346	**0.00515**	2.74298	**0.00609**	2.79780	**0.00515**
181756	70	5	4.20	110.91458	38.52101	2.87933	**0.00524**	-113.72411	189.80861	-0.59915	0.60990	1.61320	0.10670	2.79162	**0.00524**
4453609	9	66	370.65	-84.83721	29.71645	-2.85489	**0.00562**	-0.91090	3.23240	-0.28180	0.77902	-2.15650	**0.03104**	-2.76916	**0.00562**
191110	69	6	1.00	101.05523	35.48670	2.84769	**0.00574**	0.00000	0.00000	0.00000	1.00000	1.95341	0.05077	2.76254	**0.00574**
195756	71	4	1.50	121.69993	42.79332	2.84390	**0.00580**	-89.79246	366.18606	-0.24521	0.84692	1.81443	0.06961	2.75905	**0.00580**
165928	69	6	4.83	2.78407	37.47675	0.07429	0.94099	-59.29387	8.46649	-7.00336	**0.00598**	-1.89148	0.05856	-2.74898	**0.00598**
701221	66	9	2.11	-27.85417	31.08167	-0.89616	0.37315	-58.28232	14.04988	-4.14824	**0.00602**	-2.57183	**0.01012**	-2.74653	**0.00602**
180090	69	6	1.00	99.98104	35.49136	2.81705	**0.00625**	0.00000	0.00000	0.00000	1.00000	1.93346	0.05318	2.73432	**0.00625**
183579	57	18	3.67	50.03141	23.22741	2.15398	**0.03459**	43.66729	23.34783	1.87029	0.08109	2.72770	**0.00638**	2.72770	**0.00638**
197708	68	7	3.29	93.03935	33.23527	2.79942	**0.00657**	77.71388	136.47246	0.56945	0.59951	2.29326	**0.02183**	2.71806	**0.00657**
3692427	69	6	4.83	102.06442	36.76204	2.77635	**0.00700**	9.28890	90.54426	0.10259	0.92476	1.97368	**0.04842**	2.69677	**0.00700**
265871	7	68	161.65	-83.30525	33.52179	-2.48511	**0.01528**	-4.96050	3.54269	-1.40021	0.16621	-2.69427	**0.00705**	-2.69427	**0.00705**
2575651	65	10	7.10	78.63575	28.37572	2.77123	**0.00710**	-48.29897	53.94330	-0.89537	0.40033	1.30886	0.19058	2.69204	**0.00710**
110060	70	5	2.00	107.79014	39.14467	2.75364	**0.00746**	-97.10638	314.88457	-0.30839	0.78694	1.70094	0.08895	2.67577	**0.00746**
213870	64	11	5.00	75.05376	27.27095	2.75215	**0.00749**	-0.24862	45.13530	-0.00551	0.99574	1.88731	0.05912	2.67440	**0.00749**
187210	69	6	1.83	97.78547	35.61370	2.74573	**0.00762**	-62.97015	157.75652	-0.39916	0.71649	1.63010	0.10308	2.66846	**0.00762**
2532173	29	46	9.89	-54.33160	19.83908	-2.73862	**0.00777**	-1.28905	5.39564	-0.23891	0.81231	-2.05013	**0.04035**	-2.66188	**0.00777**
197988	42	33	14.00	-53.17653	19.45338	-2.73354	**0.00788**	-4.97914	6.33993	-0.78536	0.43840	-2.42684	**0.01523**	-2.65718	**0.00788**

Table S4b. OTUs associated with Triglycerides at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
749837	61	14	2.71	67.39075	24.78876	2.71860	**0.00821**	61.09484	73.43342	0.83198	0.42312	2.43554	**0.01487**	2.64335	**0.00821**
190639	24	51	24.22	-47.30604	21.03556	-2.24886	**0.02758**	-7.42920	4.76555	-1.55894	0.12558	-2.64101	**0.00827**	-2.64101	**0.00827**
1010113	54	21	14.67	2.02378	22.58537	0.08961	0.92885	13.11867	4.43086	2.96075	**0.00837**	1.92764	0.05390	2.63680	**0.00837**
158950	69	6	5.83	92.56582	36.10961	2.56347	**0.01245**	81.23794	52.42039	1.54974	0.21898	2.63632	**0.00838**	2.63632	**0.00838**
4473788	66	9	2.11	80.38916	29.73540	2.70348	**0.00856**	-224.40974	86.06161	-2.60755	**0.04025**	0.40884	0.68266	2.62934	**0.00856**
132892	60	15	3.40	65.06886	24.21174	2.68749	**0.00894**	43.43479	50.67772	0.85708	0.40819	2.43357	**0.01495**	2.61450	**0.00894**
196982	58	17	2.88	-31.17575	24.04551	-1.29653	0.19893	40.77553	13.46519	3.02822	**0.00903**	0.93780	0.34835	2.61085	**0.00903**
4412540	23	52	179.63	-5.67105	22.03160	-0.25741	0.79760	-13.80607	5.08265	-2.71631	**0.00910**	-2.02580	**0.04279**	-2.60845	**0.00910**
336627	56	19	2.84	14.63388	23.29516	0.62819	0.53186	79.54714	26.82041	2.96592	**0.00910**	2.28628	**0.02224**	2.60813	**0.00910**
4450214	31	44	39.32	-27.13816	20.72865	-1.30921	0.19463	-18.00920	7.26243	-2.47977	**0.01735**	-2.59954	**0.00934**	-2.59954	**0.00934**
193534	68	7	2.43	88.73102	33.25662	2.66807	**0.00942**	26.57816	107.71742	0.24674	0.81726	1.99938	**0.04557**	2.59648	**0.00942**
188966	70	5	2.20	103.46094	38.79245	2.66704	**0.00944**	-246.62377	131.58052	-1.87432	0.20174	0.93260	0.35103	2.59552	**0.00944**
825808	43	32	39.38	-40.55821	19.96602	-2.03136	**0.04591**	-7.34414	4.24532	-1.72994	0.09427	-2.59473	**0.00947**	-2.59473	**0.00947**
4407515	58	17	5.06	61.82362	23.20214	2.66457	**0.00951**	16.04877	27.04859	0.59333	0.56242	2.24327	**0.02488**	2.59322	**0.00951**
782953	15	60	127.50	-64.10696	24.30549	-2.63755	**0.01023**	3.17170	3.00913	1.05403	0.29632	-1.07745	0.28128	-2.56811	**0.01023**
194488	71	4	2.50	-58.87229	45.04644	-1.30692	0.19540	-93.92308	2.88128	-32.59769	**0.01952**	-2.56691	**0.01026**	-2.56691	**0.01026**
180215	70	5	2.00	-16.75253	40.98337	-0.40876	0.68393	33.63396	3.45490	9.73514	**0.01039**	1.52420	0.12746	2.56266	**0.01039**
173863	31	44	5.07	0.12133	21.18528	0.00573	0.99545	-29.39359	10.94683	-2.68512	**0.01042**	-1.80735	0.07071	-2.56169	**0.01042**
179695	71	4	2.50	113.53189	43.22146	2.62675	**0.01053**	51.62255	309.10137	0.16701	0.89465	1.90246	0.05711	2.55806	**0.01053**
147969	35	40	69.65	-40.64780	19.98958	-2.03345	**0.04569**	-6.27839	3.79455	-1.65458	0.10647	-2.55441	**0.01064**	-2.55441	**0.01064**
190301	56	19	3.21	-58.46819	22.37850	-2.61270	**0.01093**	0.98536	9.07411	0.10859	0.91488	-1.72399	0.08471	-2.54498	**0.01093**
183147	70	5	1.60	101.73995	39.01166	2.60794	**0.01107**	247.54682	320.13402	0.77326	0.52025	2.25107	**0.02438**	2.54054	**0.01107**
190100	70	5	1.60	101.91415	39.09090	2.60711	**0.01109**	156.77801	190.05281	0.82492	0.49615	2.27712	**0.02278**	2.53977	**0.01109**
2407149	33	42	5.76	-51.15777	19.62291	-2.60704	**0.01109**	-2.30358	6.35314	-0.36259	0.71887	-2.05038	**0.04033**	-2.53971	**0.01109**
187751	45	30	57.10	26.28048	20.51239	1.28120	0.20423	11.23512	4.55952	2.46410	**0.02039**	2.53755	**0.01116**	2.53755	**0.01116**
194471	63	12	1.92	47.53048	27.19976	1.74746	0.08482	106.06336	50.00205	2.12118	0.06292	2.53367	**0.01129**	2.53367	**0.01129**
199286	40	35	9.26	-31.24569	20.12071	-1.55291	0.12483	-14.49630	6.82252	-2.12477	**0.04142**	-2.52725	**0.01150**	-2.52725	**0.01150**
3747551	68	7	1.57	87.79126	33.86141	2.59266	**0.01153**	-83.50580	122.59605	-0.68115	0.53317	1.34572	0.17839	2.52631	**0.01153**
206513	51	24	5.46	-31.68956	21.44267	-1.47787	0.14380	-18.59210	8.25479	-2.25228	**0.03513**	-2.52340	**0.01162**	-2.52340	**0.01162**
172233	63	12	2.00	48.06567	27.16067	1.76968	0.08101	112.31128	54.64587	2.05526	0.07002	2.51492	**0.01191**	2.51492	**0.01191**
4350477	57	18	5.28	59.39946	23.02421	2.57987	**0.01192**	-17.68972	26.12239	-0.67719	0.50860	1.31052	0.19002	2.51438	**0.01192**
4405104	39	36	18.58	-22.27857	20.13056	-1.10670	0.27211	-23.49909	9.06066	-2.59353	**0.01406**	-2.51311	**0.01197**	-2.51311	**0.01197**
325850	63	12	69.92	68.47034	26.64869	2.56937	**0.01226**	3.15025	20.56974	0.15315	0.88166	1.87627	0.06062	2.50458	**0.01226**
189937	59	16	2.38	45.52820	24.54553	1.85485	0.06771	75.73118	41.03841	1.84537	0.08788	2.49864	**0.01247**	2.49864	**0.01247**
365621	70	5	2.40	100.81869	39.55011	2.54914	**0.01293**	-602.90982	406.31388	-1.48385	0.27611	0.98754	0.32338	2.48569	**0.01293**
3236435	37	38	40.37	-49.74473	19.54899	-2.54462	**0.01308**	6.70704	5.33173	1.25795	0.21674	-0.88121	0.37820	-2.48147	**0.01308**
2565100	67	8	6.25	38.95856	32.90137	1.18410	0.24027	179.87547	53.39242	3.36893	**0.01991**	2.47650	**0.01327**	2.47650	**0.01327**
4326869	71	4	2.75	-51.57389	44.72479	-1.15314	0.25267	-264.64863	7.72278	-34.26855	**0.01857**	-2.47339	**0.01338**	-2.47339	**0.01338**
197499	33	42	8.17	-50.11530	19.77543	-2.53422	**0.01345**	-1.32462	6.47480	-0.20458	0.83896	-1.89149	0.05856	-2.47175	**0.01345**
3507744	68	7	4.57	-20.35545	34.80143	-0.58490	0.56044	59.06433	13.99864	4.21929	**0.01349**	1.33537	0.18175	2.47069	**0.01349**
178242	46	29	7.38	-50.73766	20.07670	-2.52719	**0.01369**	6.94856	6.90439	1.00640	0.32350	-1.04502	0.29601	-2.46518	**0.01369**
196371	69	6	3.33	91.58361	36.28301	2.52415	**0.01380**	51.63088	136.83958	0.37731	0.73105	1.98418	**0.04724**	2.46233	**0.01380**
310380	70	5	4.00	-34.23186	41.37962	-0.82726	0.41082	-241.29679	28.90696	-8.34736	**0.01405**	-2.31820	**0.02044**	-2.45599	**0.01405**
4405423	60	15	4.40	4.75524	25.77278	0.18451	0.85413	-51.18804	17.82959	-2.87096	**0.01406**	-1.60638	0.10819	-2.45561	**0.01406**
302049	58	17	3.94	48.30662	23.55919	2.05044	**0.04396**	46.90615	30.37866	1.54405	0.14488	2.45530	**0.01408**	2.45530	**0.01408**
195081	48	27	6.33	-50.82587	20.28079	-2.50611	**0.01447**	11.31309	7.16308	1.57936	0.12734	-0.65110	0.51498	-2.44545	**0.01447**
184465	58	17	1.94	34.91331	24.11439	1.44782	0.15201	82.67188	37.16405	2.22451	**0.04307**	2.44340	**0.01455**	2.44340	**0.01455**
2700883	18	57	11.93	24.68613	23.86645	1.03434	0.30444	16.66597	6.68503	2.49303	**0.01576**	2.43338	**0.01496**	2.43338	**0.01496**
189334	70	5	3.00	97.46562	39.13854	2.49027	**0.01507**	6.63829	223.16518	0.02975	0.97897	1.73735	0.08233	2.43062	**0.01507**
4449427	31	44	29.41	49.24041	19.79426	2.48761	**0.01518**	0.87771	8.31831	0.10551	0.91648	1.79110	0.07328	2.42813	**0.01518**
183681	43	32	5.41	-49.00989	19.71313	-2.48615	**0.01523**	0.24300	9.87756	0.02460	0.98054	-1.69873	0.08937	-2.42676	**0.01523**
2017729	51	24	3.92	-8.27528	22.25828	-0.37178	0.71115	68.63617	26.04498	2.63529	**0.01547**	1.45018	0.14701	2.42119	**0.01547**
191792	19	56	14.52	-55.41859	22.38435	-2.47577	**0.01565**	1.85411	5.15189	0.35989	0.72036	-1.45597	0.14540	-2.41703	**0.01565**
4457427	25	50	41.32	-51.16154	20.70227	-2.47130	**0.01583**	-1.66139	4.10426	-0.40480	0.68746	-1.99060	**0.04652**	-2.41284	**0.01583**
180421	62	13	3.85	36.64920	26.81960	1.36651	0.17603	109.53120	46.94106	2.33338	**0.04181**	2.39603	**0.01657**	2.39603	**0.01657**
4479397	70	5	1.60	56.79051	40.10896	1.41591	0.16111	62.48330	14.09396	4.43334	**0.04730**	2.39352	**0.01669**	2.39352	**0.01669**
184996	70	5	2.80	-48.81048	40.66515	-1.20030	0.23396	-39.03632	6.78003	-5.75754	**0.02887**	-2.38686	**0.01699**	-2.38686	**0.01699**
322835	38	37	7.84	-47.77160	19.55197	-2.44331	**0.01701**	-4.88765	6.97818	-0.70042	0.48843	-2.17745	**0.02945**	-2.38658	**0.01701**
4301511	33	42	11.90	-47.90802	19.66652	-2.43602	**0.01733**	-4.48482	6.89823	-0.65014	0.51941	-2.13827	**0.03249**	-2.37973	**0.01733**
323135	48	27	24.15	-47.42779	20.40843	-2.32393	**0.02296**	-7.18108	6.46540	-1.11069	0.27770	-2.37564	**0.01752**	-2.37564	**0.01752**
315846	67	8	20.50	-6.10638	33.58215	-0.18183	0.85622	16.45910	4.73879	3.47327	**0.01779**	1.54775	0.12168	2.37003	**0.01779**
349246	69	6	2.00	90.47741	37.33718	2.42325	**0.01790**	-88.28282	79.25003	-1.11398	0.34650	1.00857	0.31318	2.36773	**0.01790**
187038	69	6	1.50	-66.35625	36.56331	-1.81483	0.07371	-19.97471	9.25157	-2.15906	0.11968	-2.36491	**0.01803**	-2.36491	**0.01803**
4370941	45	30	11.83	-31.16896	20.93498	-1.48885	0.14090	-12.51587	6.40912	-1.95282	0.06128	-2.36453	**0.01805**	-2.36453	**0.01805**
307113	63	12	1.67	43.88454	27.20315	1.61322	0.11107	135.41651	69.71354	1.94247	0.08398	2.34863	**0.01884**	2.34863	**0.01884**
4045882	63	12	1.67	43.88454	27.20315	1.61322	0.11107	135.41651	69.71354	1.94247	0.08398	2.34863	**0.01884**	2.34863	**0.01884**
1820513	43	32	67.81	47.53789	19.80276	2.40057	**0.01896**	-9.49668	8.62952	-1.10049	0.28017	0.89553	0.37051	2.34640	**0.01896**
214036	39	36	11.44	46.95873	19.58069	2.39822	**0.01907**	-10.46376	11.18549	-0.93548	0.35634	1.00538	0.31471	2.34419	**0.01907**
4390839	68	7	1.29	-53.88049	34.42758	-1.56504	0.12196	77.17136	20.30473	3.80066	**0.01909**	0.56364	0.57300	2.34371	**0.01909**
208790	68	7	16.43	58.24674	34.22957	1.70165	0.09314	68.61499	32.52459	2.10963	0.10253	2.34178	**0.01919**	2.34178	**0.01919**
189007	67	8	2.00	64.30472	32.04142	2.00692	**0.04851**	194.32692	125.13089	1.55299	0.18113	2.34061	**0.01925**	2.34061	**0.01925**
193915	44	31	3.48	0.68101	20.77383	0.03278	0.97394	40.14230	16.17838	2.48123	**0.01937**	1.67660	0.09362	2.33840	**0.01937**
181059	28	47	150.64	-48.15260	20.19395	-2.38451	**0.01974**	-2.44281	2.82059	-0.86606	0.39115	-2.25483	**0.02414**	-2.33129	**0.01974**
189924	61	14	4.57	-60.08368	25.20948	-2.38338	**0.01979**	-2.45341	5.09056	-0.48195	0.63929	-1.97914	**0.04780**	-2.33022	**0.01979**
1102370	45	30	18.37	-25.48541	20.72501	-1.22969	0.22282	-16.62155	7.67561	-2.16550	**0.03935**	-2.31901	**0.02039**	-2.31901	**0.02039**
4102199	51	24	3.08	20.17826	22.59833	0.89291	0.37488	48.77255	19.44815	2.50782	**0.02043**	2.26679	**0.02340**	2.31836	**0.02043**
179864	64	11	3.64	63.99355	28.01553	2.28422	**0.02531**	66.21111	59.69590	1.10914	0.29960	2.31503	**0.02061**	2.31503	**0.02061**
3235048	48	27	23.07	-48.38194	20.44409	-2.36655	**0.02065**	-2.16861	6.11485	-0.35465	0.72595	-1.88436	0.05952	-2.31437	**0.02065**
150412	70	5	10.20	93.33865	39.45395	2.36576	**0.02069**	-0.98306	45.35544	-0.02167	0.98468	1.62240	0.10472	2.31363	**0.02069**
3138798	31	44	219.93	18.62128	20.47824	0.90932	0.36621	11.09369	4.61090	2.40597	**0.02072**	2.27447	**0.02294**	2.31299	**0.02072**

Supplemental Information

Table S4b. OTUs associated with Triglycerides at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
186133	60	15	12.33	-45.98717	24.77520	-1.85618	0.06752	-8.12557	5.27906	-1.53921	0.14970	-2.31139	**0.02081**	-2.31139	**0.02081**
198754	70	5	2.80	-56.93224	40.10147	-1.41970	0.16001	-21.44762	5.64250	-3.80108	0.06277	-2.30933	**0.02093**	-2.30933	**0.02093**
4079463	59	16	4.19	-22.29402	24.91801	-0.89470	0.37393	-24.19937	9.22253	-2.62394	**0.02103**	-2.26035	**0.02380**	-2.30748	**0.02103**
316732	14	61	101.10	7.89002	26.03172	0.30309	0.76269	-11.71085	4.93732	-2.37190	**0.02104**	-1.41802	0.15618	-2.30733	**0.02104**
3997242	71	4	1.00	103.78485	44.00119	2.35868	**0.02106**	0.00000	0.00000	0.00000	1.00000	1.63127	0.10283	2.30696	**0.02106**
187883	64	11	2.45	-39.76123	28.54934	-1.39272	0.16799	-28.67722	13.13143	-2.18386	0.06050	-2.30223	**0.02132**	-2.30223	**0.02132**
198930	67	8	2.88	-41.32565	32.50083	-1.27153	0.20763	-54.06759	20.58156	-2.62699	**0.04670**	-2.29745	**0.02159**	-2.29745	**0.02159**
4458576	23	52	72.92	-0.53224	22.12432	-0.02406	0.98087	14.75022	6.21511	2.37328	**0.02160**	1.60754	0.10794	2.29738	**0.02160**
3903651	21	54	29.11	29.56909	22.39833	1.32015	0.19097	12.75872	6.44541	1.97950	0.05317	2.29192	**0.02191**	2.29192	**0.02191**
196664	21	54	29.11	29.56909	22.39833	1.32015	0.19097	12.75872	6.44541	1.97950	0.05317	2.29192	**0.02191**	2.29192	**0.02191**
4447950	14	61	232.70	17.30970	26.17808	0.66123	0.51058	8.25941	3.51021	2.35297	**0.02204**	2.08432	**0.03713**	2.28973	**0.02204**
2403301	57	18	52.33	-38.44138	23.67794	-1.62351	0.10885	-8.67227	4.98683	-1.73903	0.10250	-2.28835	**0.02212**	-2.28835	**0.02212**
192244	58	17	5.65	-54.76460	23.49365	-2.33104	**0.02256**	0.90976	8.61323	0.10562	0.91738	-1.53948	0.12369	-2.28088	**0.02256**
179267	45	30	6.03	-40.35553	20.16209	-2.00156	**0.04910**	-8.31587	6.45589	-1.28811	0.20864	-2.28044	**0.02258**	-2.28044	**0.02258**
198587	23	52	10.27	-37.36723	21.56507	-1.73277	0.08742	-10.25507	6.66537	-1.53856	0.13035	-2.27824	**0.02271**	-2.27824	**0.02271**
189997	71	4	1.50	1.15733	45.13493	0.02564	0.97961	166.77451	6.09576	27.35910	**0.02326**	1.62261	0.10467	2.26916	**0.02326**
4463532	50	25	4.52	21.41429	21.45106	0.99829	0.32148	38.86307	16.39041	2.37109	**0.02691**	2.26569	**0.02347**	2.26569	**0.02347**
198956	46	29	2.59	-33.35409	20.74317	-1.60796	0.11222	-17.80628	10.66404	-1.66975	0.10697	-2.26293	**0.02364**	-2.26293	**0.02364**
180352	70	5	4.00	-68.84537	39.89800	-1.72553	0.08872	22.36105	3.50075	6.38750	**0.02364**	0.39646	0.69176	2.26287	**0.02364**
232222	70	5	6.60	-35.97630	40.59184	-0.88629	0.37841	21.25173	3.33615	6.37014	**0.02377**	0.97582	0.32915	2.26085	**0.02377**
191483	60	15	2.33	20.45718	25.24011	0.81050	0.42032	59.95420	23.17769	2.58672	**0.02380**	2.16812	**0.03015**	2.26032	**0.02380**
4329132	69	6	2.83	63.65980	36.87160	1.72653	0.08854	202.90912	100.63059	2.01638	0.13714	2.25545	**0.02411**	2.25545	**0.02411**
158855	67	8	11.63	-39.80098	32.51657	-1.22402	0.22493	19.67791	6.15779	3.19561	**0.02411**	0.73667	0.46132	2.25532	**0.02411**
183686	51	24	11.63	44.78100	21.09007	2.12332	**0.03716**	16.24957	14.36342	1.13132	0.27068	2.25246	**0.02429**	2.25246	**0.02429**
840376	64	11	17.09	0.58765	28.66581	0.02050	0.98370	32.59523	11.80323	2.76155	**0.02462**	1.60358	0.10881	2.24738	**0.02462**
174443	52	23	5.65	-39.41158	21.55842	-1.82813	0.07167	-16.19527	11.34105	-1.42802	0.16872	-2.24686	**0.02465**	-2.24686	**0.02465**
4469007	13	62	62.66	-4.60304	26.81843	-0.17164	0.86420	-11.39746	4.96020	-2.29778	**0.02514**	-1.70435	0.08832	-2.23929	**0.02514**
35260	69	6	14.50	-69.94902	36.46915	-1.91803	0.05907	-10.08927	6.17893	-1.63285	0.20101	-2.23894	**0.02516**	-2.23894	**0.02516**
552235	68	7	4.57	76.96918	33.66694	2.28619	**0.02519**	4.23333	82.63947	0.05123	0.96160	1.61692	0.10590	2.23852	**0.02519**
4398588	63	12	4.00	-10.40146	27.71216	-0.37534	0.70851	-32.59845	12.17097	-2.67838	**0.02527**	-1.84630	0.06485	-2.23720	**0.02527**
175148	50	25	5.04	-47.39022	20.87941	-2.26971	**0.02622**	-5.99276	7.73416	-0.77484	0.44668	-2.10993	**0.03486**	-2.22293	**0.02622**
180307	70	5	1.80	89.15115	39.32119	2.26725	**0.02638**	607.14019	587.78010	1.03294	0.41018	2.15256	**0.03135**	2.22060	**0.02638**
4299126	56	19	41.21	37.91930	22.98491	1.64975	0.10335	23.75111	14.91958	1.59194	0.13096	2.21972	**0.02644**	2.21972	**0.02644**
186732	18	57	11.37	1.13647	23.83635	0.04768	0.96210	17.89702	7.84218	2.28215	**0.02645**	1.60309	0.10891	2.21960	**0.02645**
2497335	29	46	20.37	-13.31593	20.76661	-0.64122	0.52342	16.20784	7.06461	2.29423	**0.02672**	1.11542	0.26467	2.21554	**0.02672**
208539	67	8	3.38	71.79815	31.74409	2.26178	**0.02673**	-64.47749	60.90414	-1.05867	0.33819	0.88931	0.37384	2.21542	**0.02673**
4469576	29	46	37.48	17.66814	20.76826	0.85073	0.39774	14.66200	6.39131	2.29405	**0.02673**	2.16448	**0.03043**	2.21537	**0.02673**
197890	60	15	4.20	43.02557	24.95242	1.72430	0.08894	47.54074	31.16646	1.52538	0.15308	2.21306	**0.02689**	2.21306	**0.02689**
188047	12	63	20.89	-60.93498	27.00489	-2.25644	**0.02708**	-4.19129	4.87253	-0.86019	0.39311	-2.16683	**0.03025**	-2.21037	**0.02708**
176062	41	34	33.12	-36.87512	20.92547	-1.76221	0.08228	-8.99778	6.34090	-1.41901	0.16587	-2.20844	**0.02721**	-2.20844	**0.02721**
194696	69	6	2.50	82.79768	36.84148	2.24740	**0.02768**	-196.58317	112.94388	-1.74054	0.18014	0.60915	0.54242	2.20181	**0.02768**
263518	41	34	22.88	-28.78536	20.23656	-1.42244	0.15922	-9.69650	5.54645	-1.74824	0.09032	-2.19303	**0.02831**	-2.19303	**0.02831**
191180	68	7	2.14	75.36356	33.72509	2.23464	**0.02854**	-219.62505	120.35642	-1.82479	0.14208	0.51028	0.60986	2.18972	**0.02854**
179826	60	15	2.20	14.66326	25.72088	0.57009	0.57039	100.85475	40.56138	2.48647	**0.02862**	1.94893	0.05130	2.18873	**0.02862**
180257	69	6	2.50	77.14763	36.31339	2.12450	**0.03706**	129.26654	107.05085	1.20752	0.31375	2.18670	**0.02876**	2.18670	**0.02876**
291090	65	10	19.70	64.37866	28.85791	2.23088	**0.02880**	-0.61572	28.62875	-0.02151	0.98344	1.53117	0.12573	2.18615	**0.02880**
180462	68	7	2.29	75.31237	33.75962	2.23084	**0.02881**	-232.69295	106.65093	-2.18182	0.09455	0.36363	0.71613	2.18611	**0.02881**
190980	71	4	2.00	97.60779	43.75834	2.23061	**0.02882**	261.58737	367.86899	0.71109	0.60649	1.90989	0.05615	2.18589	**0.02882**
186468	62	13	3.46	44.48865	26.27467	1.69321	0.09474	53.20290	34.67023	1.53454	0.15591	2.18489	**0.02890**	2.18489	**0.02890**
175535	13	62	10.56	-59.11020	26.52919	-2.22812	**0.02900**	-5.67483	6.21380	-0.91326	0.36482	-2.18478	**0.02890**	-2.18478	**0.02890**
177040	58	17	4.71	11.46621	24.26971	0.47245	0.63803	62.17285	25.60002	2.42862	**0.02922**	1.87451	0.06086	2.18051	**0.02922**
311947	52	23	38.52	-37.15756	21.55272	-1.72403	0.08899	-9.96520	6.95519	-1.43277	0.16737	-2.17890	**0.02934**	-2.17890	**0.02934**
183030	65	10	4.90	-64.18760	28.87632	-2.22285	**0.02937**	1.84904	8.02197	0.23050	0.82430	-1.38346	0.16652	-2.17853	**0.02937**
562244	67	8	18.25	-3.84209	32.90030	-0.11678	0.90736	22.59323	7.48547	3.01828	**0.02947**	1.45714	0.14508	2.17708	**0.02947**
195436	37	38	4.76	-43.80452	19.74541	-2.21847	**0.02968**	1.87247	7.96292	0.23515	0.81546	-1.37249	0.16991	-2.17438	**0.02968**
192127	38	37	4.57	-43.53959	19.62766	-2.21828	**0.02969**	0.04467	5.16672	0.00865	0.99315	-1.53132	0.12569	-2.17420	**0.02969**
189450	66	9	2.44	58.59327	30.73744	1.90625	0.06061	128.24232	96.21839	1.33283	0.23097	2.17379	**0.02972**	2.17379	**0.02972**
850841	68	7	17.14	-30.77180	34.89092	-0.88194	0.38074	7.79352	2.36625	3.29362	**0.03011**	0.91363	0.36091	2.16860	**0.03011**
3756485	44	31	5.19	-39.17592	20.29770	-1.93007	0.05754	-10.01165	8.40536	-1.19110	0.24361	-2.16743	**0.03020**	-2.16743	**0.03020**
211907	42	33	3.91	-44.44179	20.16538	-2.20387	**0.03073**	4.32890	6.42731	0.67352	0.50577	-1.05719	0.29042	-2.16052	**0.03073**
344525	35	40	4.93	-4.66459	20.32584	-0.22949	0.81914	25.76227	11.47582	2.24492	**0.03084**	1.36508	0.17223	2.15917	**0.03084**
358781	21	54	282.70	-47.44914	22.04635	-2.15224	**0.03473**	-2.02599	2.14130	-0.94615	0.34854	-2.15591	**0.03109**	-2.15591	**0.03109**
175520	69	6	2.33	81.00575	36.84814	2.19837	**0.03114**	-130.52129	125.14408	-1.04297	0.37361	0.89490	0.37084	2.15531	**0.03114**
849346	70	5	3.60	-66.05151	39.96355	-1.65279	0.10273	-38.54768	17.41775	-2.21313	0.15735	-2.15371	**0.03126**	-2.15371	**0.03126**
300662	71	4	4.00	-29.29696	45.16650	-0.64864	0.51863	-35.59448	1.77158	-20.09189	**0.03166**	-1.97576	**0.04818**	-2.14869	**0.03166**
350627	69	6	7.50	12.16603	37.35482	0.32569	0.74560	34.01557	8.92820	3.80990	**0.03179**	1.74760	0.08053	2.14704	**0.03179**
366794	42	33	5.21	-40.39501	19.89066	-2.03085	**0.04596**	-6.26896	5.91963	-1.05901	0.29805	-2.14703	**0.03179**	-2.14703	**0.03179**
329798	65	10	5.80	63.12643	28.89063	2.18501	**0.03214**	-12.35026	41.35487	-0.29864	0.77388	1.31192	0.18955	2.14263	**0.03214**
505670	65	10	4.00	63.21708	28.94208	2.18426	**0.03220**	-49.29793	61.30630	-0.80413	0.44777	0.97777	0.32819	2.14191	**0.03220**
4341886	63	12	1.92	40.45896	27.62267	1.46470	0.14736	123.71837	71.30404	1.73508	0.11675	2.13369	**0.03287**	2.13369	**0.03287**
185597	55	20	4.15	-22.77759	23.35955	-0.97509	0.33278	-28.81691	13.00726	-2.21545	**0.04067**	-2.13223	**0.03299**	-2.13223	**0.03299**
330414	69	6	2.83	62.52221	36.67955	1.70455	0.09259	170.95660	99.50689	1.71804	0.18429	2.12808	**0.03333**	2.12808	**0.03333**
4449054	21	54	13.20	-12.26756	22.77832	-0.53856	0.59185	-12.97377	5.94102	-2.18376	**0.03360**	-1.88157	0.05989	-2.12478	**0.03360**
194670	26	49	7.55	-39.08946	21.07954	-1.85438	0.06778	-7.67923	6.45195	-1.19022	0.24007	-2.12223	**0.03382**	-2.12223	**0.03382**
4446902	41	34	7.62	41.17358	19.82570	2.07678	**0.04139**	12.16886	12.46695	0.97609	0.33658	2.12170	**0.03386**	2.12170	**0.03386**
199421	54	21	2.95	-21.46166	22.44418	-0.95622	0.34216	-26.15673	11.83612	-2.20991	**0.04030**	-2.12169	**0.03386**	-2.12169	**0.03386**
182506	71	4	4.50	94.41359	43.76729	2.15717	**0.03433**	-32.00062	46.81307	-0.68358	0.61827	1.14400	0.25262	2.11617	**0.03433**
2203165	69	6	2.67	80.44687	37.30435	2.15650	**0.03439**	-72.55753	21.79962	-3.32839	**0.04477**	0.07690	0.93871	2.11553	**0.03439**
2724175	42	33	50.27	31.70736	20.13931	1.57440	0.11978	11.24396	7.63519	1.47265	0.15126	2.11481	**0.03445**	2.11481	**0.03445**
191738	66	9	5.78	-52.08477	30.68670	-1.69731	0.09396	19.17883	7.04598	2.72195	**0.03455**	0.31019	0.75642	2.11356	**0.03455**

Table S4b. OTUs associated with Triglycerides at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
180341	70	5	1.60	-40.02909	40.49389	-0.98852	0.32621	71.01187	13.55847	5.23745	**0.03458**	0.80010	0.42365	2.11329	**0.03458**
210095	69	6	2.83	58.72751	36.73604	1.59864	0.11428	340.34626	183.16459	1.85814	0.16012	2.10993	**0.03486**	2.10993	**0.03486**
197458	65	10	2.40	-38.12312	29.49843	-1.29238	0.20036	25.08899	9.66463	2.59596	**0.03563**	0.58021	0.56177	2.10108	**0.03563**
4427290	43	32	17.88	-25.33008	20.43265	-1.23969	0.21912	12.03329	5.46772	2.20079	**0.03587**	0.61488	0.53863	2.09845	**0.03587**
216550	67	8	34.75	43.04540	32.87647	1.30931	0.19459	24.77391	12.09462	2.04834	0.09584	2.09477	**0.03619**	2.09477	**0.03619**
184394	57	18	4.67	14.24159	24.28375	0.58647	0.55940	68.76863	29.89382	2.30043	**0.03619**	1.89397	0.05823	2.09474	**0.03619**
4439487	62	13	4.31	-51.23971	26.10853	-1.96257	0.05356	-9.66298	8.90679	-1.08490	0.30343	-2.09267	**0.03638**	-2.09267	**0.03638**
4479443	65	10	2.90	59.26078	29.01317	2.04255	**0.04476**	50.29553	49.41583	1.01780	0.34265	2.09010	**0.03661**	2.09010	**0.03661**
176318	46	29	31.45	-15.63404	20.78516	-0.75217	0.45440	-14.03128	6.38056	-2.19907	**0.03697**	-2.00410	**0.04506**	-2.08612	**0.03697**
186090	64	11	4.09	55.60762	28.47208	1.95306	0.05470	51.19910	46.58712	1.09900	0.30374	2.08575	**0.03700**	2.08575	**0.03700**
215231	42	33	3.91	-41.51987	19.83661	-2.09309	**0.03986**	-7.80261	8.65428	-0.90159	0.37446	-2.08123	**0.03741**	-2.08123	**0.03741**
185584	45	30	5.60	-40.45630	20.39153	-1.98398	0.05107	-8.51460	8.45440	-1.00712	0.32282	-2.07859	**0.03766**	-2.07859	**0.03766**
184525	65	10	1.70	-45.66193	29.43911	-1.55106	0.12527	-33.20005	21.10459	-1.57312	0.15969	-2.07828	**0.03768**	-2.07828	**0.03768**
4381749	55	20	4.10	-44.80400	22.33716	-2.00581	**0.04864**	-9.94617	10.03171	-0.99147	0.33536	-2.07547	**0.03794**	-2.07547	**0.03794**
191153	11	64	130.27	-58.75910	27.86037	-2.10906	**0.03842**	-2.52817	4.06322	-0.62221	0.53612	-1.90145	0.05724	-2.07036	**0.03842**
3474081	70	5	2.00	-28.65714	40.51467	-0.70733	0.48165	48.56801	9.80857	4.95159	**0.03845**	0.96618	0.33396	2.07004	**0.03845**
212686	61	14	3.07	45.41081	25.50414	1.78053	0.07921	51.28357	41.40454	1.23860	0.24127	2.06978	**0.03847**	2.06978	**0.03847**
176297	68	7	2.00	71.31843	33.83512	2.10782	**0.03853**	-176.10191	132.65282	-1.32754	0.25503	0.65829	0.51035	2.06919	**0.03853**
215468	68	7	3.57	-53.75607	34.38502	-1.56336	0.12235	-18.95715	11.25971	-1.68363	0.16754	-2.06837	**0.03861**	-2.06837	**0.03861**
4379889	55	20	4.70	16.60081	23.01108	0.72143	0.47298	53.69522	23.99379	2.23788	**0.03891**	1.96775	**0.04910**	2.06518	**0.03891**
195937	27	48	9.63	-43.32982	20.63266	-2.10006	**0.03923**	-4.16877	6.36709	-0.65474	0.51597	-1.91722	0.05521	-2.06179	**0.03923**
293883	58	17	13.82	-21.40866	24.10232	-0.88824	0.37737	21.43180	9.43769	2.27087	**0.03947**	0.83193	0.40545	2.05928	**0.03947**
4439469	10	65	24.65	13.20769	29.79212	0.44333	0.65886	13.05191	6.21000	2.10176	**0.03964**	1.76701	0.07723	2.05744	**0.03964**
188449	67	8	3.50	-66.96702	31.96559	-2.09497	**0.03969**	3.49068	9.46243	0.36890	0.72731	-1.20790	0.22708	-2.05694	**0.03969**
193709	21	54	7.06	-46.00061	21.97118	-2.09368	**0.03981**	7.83309	8.27586	0.94650	0.34836	-0.79049	0.42924	-2.05571	**0.03981**
3424669	69	6	2.33	75.98308	36.31415	2.09238	**0.03993**	-2.60735	184.99395	-0.01409	0.98964	1.44355	0.14887	2.05447	**0.03993**
361507	66	9	143.67	24.05141	31.08120	0.77383	0.44157	14.23496	5.45257	2.61069	**0.04008**	1.99576	**0.04596**	2.05289	**0.04008**
177828	63	12	3.08	-56.11179	26.86050	-2.08901	**0.04024**	-6.32096	9.09121	-0.69528	0.50443	-1.92247	0.05455	-2.05125	**0.04024**
2066056	59	16	7.56	-50.49231	24.17159	-2.08891	**0.04025**	4.21614	6.71089	0.62825	0.54072	-1.01783	0.30876	-2.05116	**0.04025**
184770	50	25	4.28	-44.20894	21.17769	-2.08752	**0.04038**	-4.96994	8.96057	-0.55465	0.58473	-1.83588	0.06638	-2.04984	**0.04038**
4324985	67	8	4.63	-66.51257	31.90392	-2.08478	**0.04064**	12.03707	10.76482	1.11819	0.31431	-0.73610	0.46167	-2.04722	**0.04064**
589329	67	8	1.63	-30.08565	32.66293	-0.92109	0.36008	-38.74674	14.86474	-2.60662	**0.04786**	-2.04622	**0.04074**	-2.04622	**0.04074**
953855	67	8	1.63	66.31854	32.05907	2.06864	**0.04217**	-65.16558	129.69108	-0.50247	0.63668	1.10272	0.27015	2.03182	**0.04217**
173876	45	30	23.07	-41.61592	20.12937	-2.06742	**0.04229**	4.14216	5.94896	0.69628	0.49220	-0.95025	0.34199	-2.03066	**0.04229**
193528	10	65	122.38	2.38509	29.83729	0.07994	0.93651	-11.32880	5.48366	-2.06592	**0.04302**	-1.37451	0.16928	-2.02350	**0.04302**
4332078	59	16	3.81	9.33070	24.91611	0.37448	0.70914	86.14523	38.42803	2.24173	**0.04306**	1.69432	0.09020	2.02313	**0.04306**
187868	68	7	1.71	-25.56174	34.75370	-0.73551	0.46442	44.95000	15.37254	2.92405	**0.04307**	0.91319	0.36114	2.02304	**0.04307**
177660	66	9	3.44	-63.03137	30.61994	-2.05851	**0.04316**	-8.00742	24.08623	-0.33245	0.75086	-1.65439	0.09805	-2.02215	**0.04316**
312770	67	8	4.88	-47.43889	32.37564	-1.46527	0.14720	-13.56219	8.19078	-1.65579	0.15867	-2.02166	**0.04321**	-2.02166	**0.04321**
198209	69	6	2.33	-26.88959	37.25051	-0.72186	0.47272	53.30309	15.79519	3.37464	**0.04326**	0.92144	0.35682	2.02117	**0.04326**
186866	48	27	10.22	41.31081	20.72631	1.99316	0.05003	15.79267	17.24843	0.91560	0.36898	2.02094	**0.04329**	2.02094	**0.04329**
199344	64	11	3.27	-36.48136	28.34375	-1.28710	0.20218	-36.13552	20.33738	-1.77680	0.11350	-2.02092	**0.04329**	-2.02092	**0.04329**
234443	67	8	6.88	-13.02119	33.15773	-0.39270	0.69570	-25.30199	9.40661	-2.68981	**0.04331**	-1.70544	0.08811	-2.02072	**0.04331**
3430935	69	6	17.33	-36.60956	37.24305	-0.98299	0.32890	-15.43985	5.25544	-2.93788	0.06061	-2.01711	**0.04368**	-2.01711	**0.04368**
199081	64	11	5.36	57.23552	27.91238	2.05054	**0.04395**	13.49510	44.39096	0.30401	0.76888	1.63227	0.10262	2.01454	**0.04395**
186997	40	35	4.14	-7.72703	20.30765	-0.38050	0.70470	30.51314	14.55102	2.09698	**0.04399**	1.15629	0.24756	2.01423	**0.04399**
553611	45	30	9.03	-41.39126	20.40109	-2.02888	**0.04617**	10.85141	5.13887	2.11163	**0.04411**	0.01355	0.98919	2.01300	**0.04411**
194672	36	39	8.36	-29.34645	20.01261	-1.46640	0.14689	-11.54542	8.12299	-1.42133	0.16383	-2.01024	**0.04441**	-2.01024	**0.04441**
4094866	57	18	5.89	44.46619	23.16957	1.91916	0.05893	27.48103	27.96941	0.98254	0.34142	2.00826	**0.04462**	2.00826	**0.04462**
4340358	62	13	4.85	-29.73159	26.60440	-1.11754	0.26748	-24.50329	12.75516	-1.92105	0.08366	-2.00727	**0.04472**	-2.00727	**0.04472**
185164	40	35	9.03	-31.55273	19.99885	-1.57773	0.11901	-9.25878	7.09041	-1.30582	0.20092	-2.00667	**0.04479**	-2.00667	**0.04479**
182431	52	23	7.65	-44.05041	21.57865	-2.04139	**0.04488**	-4.04268	7.58839	-0.53275	0.60008	-1.78904	0.07361	-2.00580	**0.04488**
147702	30	45	27.76	-10.87441	20.68496	-0.52572	0.60070	-16.19948	7.83595	-2.06733	**0.04490**	-1.78825	0.07374	-2.00557	**0.04490**
288651	26	49	32.88	-42.34579	20.76412	-2.03937	**0.04508**	9.72487	6.09121	1.59654	0.11722	-0.30922	0.75715	-2.00387	**0.04508**
539647	60	15	19.27	-50.23989	24.66023	-2.03728	**0.04530**	10.45396	5.96488	1.75258	0.10516	-0.26979	0.78732	-2.00188	**0.04530**
4480861	48	27	61.48	-41.98303	20.62267	-2.03577	**0.04545**	-0.50362	4.92345	-0.10229	0.91938	-1.48609	0.13725	-2.00043	**0.04545**
194104	70	5	3.20	81.33446	39.97806	2.03448	**0.04559**	123.22147	157.51848	0.78227	0.51597	1.87296	0.06107	1.99919	**0.04559**
4431545	44	31	4.13	22.37017	20.62569	1.08458	0.28173	32.33352	17.83162	1.81327	0.08052	1.99694	**0.04583**	1.99694	**0.04583**
370086	45	30	20.33	-41.86603	20.60485	-2.03185	**0.04586**	-1.28577	5.93926	-0.21649	0.83024	-1.56347	0.11794	-1.99669	**0.04586**
1860112	60	15	8.60	38.23811	25.05052	1.52644	0.13128	30.67974	22.01897	1.39333	0.18879	1.99633	**0.04590**	1.99633	**0.04590**
185949	70	5	3.60	-22.74211	40.57511	-0.56049	0.57688	38.85712	8.63679	4.49902	**0.04602**	1.01630	0.30949	1.99521	**0.04602**
306299	50	25	7.36	25.59270	21.43467	1.19399	0.23640	31.20253	18.25723	1.70905	0.10151	1.99517	**0.04602**	1.99517	**0.04602**
540055	49	26	15.96	-42.08913	20.73391	-2.02997	**0.04606**	0.77178	6.34430	0.12165	0.90423	-1.32552	0.18500	-1.99488	**0.04606**
1600780	69	6	3.67	62.57080	36.74218	1.70297	0.09289	91.30609	64.94745	1.40585	0.25444	1.99403	**0.04615**	1.99403	**0.04615**
190649	41	34	4.21	-40.34723	19.94520	-2.02290	**0.04680**	-6.19852	8.35478	-0.74191	0.46372	-1.92394	0.05436	-1.98813	**0.04680**
1726408	70	5	40.80	-38.11288	40.46609	-0.94185	0.34942	17.60601	3.99602	4.40589	**0.04785**	0.73751	0.46081	1.97872	**0.04785**
4300690	42	33	100.88	-40.19395	19.96728	-2.01299	**0.04786**	-1.69163	3.61119	-0.46844	0.64286	-1.72701	0.08417	-1.97865	**0.04786**
2506486	41	34	4.32	32.92910	20.02023	1.64479	0.10437	20.61654	17.32413	1.19005	0.24306	1.97381	**0.04840**	1.97381	**0.04840**
509621	68	7	255.00	68.13465	34.01385	2.00314	**0.04893**	-26.03839	26.53066	-0.98145	0.38194	0.77421	0.43880	1.96923	**0.04893**
190772	45	30	6.20	-26.28120	20.81020	-1.26290	0.21070	-15.50280	9.81137	-1.58008	0.12573	-1.96773	**0.04910**	-1.96773	**0.04910**
3195723	52	23	6.52	36.73227	21.68265	1.69409	0.09457	31.38633	27.62184	1.13629	0.26927	1.96329	**0.04961**	1.96329	**0.04961**
189708	67	8	2.25	-63.97242	32.04581	-1.99628	**0.04969**	9.52509	13.84101	0.68818	0.52196	-0.93503	0.34977	-1.96266	**0.04969**

[a] Statistics associated to the two part model for association analysis between the trait and OTUs adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe represented by an OTU with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the OTU for the subjects where that OTU is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per OTU-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S4c. OTUs associated with High-density lipoprotein (HDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
299302	66	9	3.33	17.62727	3.94662	4.46642	**0.00003**	1.96800	10.36870	0.18980	0.85572	3.08572	**0.00203**	4.18204	**0.00003**
345301	65	10	4.90	13.79417	3.94346	3.49798	**0.00081**	11.54877	3.40796	3.38876	**0.01162**	4.15331	**0.00003**	4.15331	**0.00003**
4324985	67	8	4.63	17.97815	4.18987	4.29086	**0.00005**	10.11734	8.87482	1.14001	0.30593	3.57730	**0.00035**	4.03528	**0.00005**
35260	69	6	14.50	19.50821	4.82356	4.04436	**0.00013**	9.70897	3.60326	2.69450	0.07413	3.96825	**0.00007**	3.96825	**0.00007**
177828	63	12	3.08	14.68128	3.55464	4.13017	**0.00010**	-3.83281	6.09315	-0.62904	0.54496	2.32922	**0.01985**	3.89935	**0.00010**
3826120	71	4	1.75	23.67715	5.82352	4.06578	**0.00012**	-7.51531	51.89484	-0.14482	0.90844	2.63713	**0.00836**	3.84446	**0.00012**
749837	61	14	2.71	2.37180	3.70953	0.63938	0.52460	-30.34829	5.25162	-5.77884	**0.00012**	-2.26537	**0.02349**	-3.83998	**0.00012**
189459	70	5	2.00	21.30268	5.26876	4.04321	**0.00013**	-10.82277	20.66139	-0.52382	0.65267	2.38656	**0.01701**	3.82516	**0.00013**
190980	71	4	2.00	22.50777	5.89703	3.81680	**0.00028**	-9.85350	45.46907	-0.21671	0.86414	2.44577	**0.01445**	3.62995	**0.00028**
218710	67	8	3.75	16.28590	4.31329	3.77575	**0.00033**	-0.92213	9.18546	-0.10039	0.92394	2.47401	**0.01336**	3.59425	**0.00033**
180352	70	5	4.00	19.73198	5.33380	3.69942	**0.00042**	-17.21734	16.48598	-1.04436	0.40595	1.90676	0.05655	3.52760	**0.00042**
925131	69	6	76.17	18.09452	4.89841	3.69396	**0.00043**	-25.20579	12.92924	-1.94952	0.14634	1.46389	0.14322	3.52282	**0.00043**
199335	66	9	2.78	14.88843	4.10316	3.62853	**0.00053**	18.45221	10.92839	1.68847	0.14229	3.48798	**0.00049**	3.48798	**0.00049**
589989	71	4	4.75	21.06672	6.18516	3.40601	**0.00108**	101.10681	16.04890	6.29992	0.10022	3.47346	**0.00051**	3.47346	**0.00051**
1599042	63	12	21.33	13.24711	3.64254	3.63677	**0.00052**	-3.13136	4.42606	-0.70748	0.49718	1.97546	**0.04822**	3.47266	**0.00052**
196724	70	5	1.60	15.60220	5.55728	2.80752	**0.00642**	30.49865	5.37812	5.67087	**0.02972**	3.46439	**0.00053**	3.46439	**0.00053**
186913	70	5	2.00	20.04026	5.52797	3.62525	**0.00054**	-13.29087	20.14783	-0.65967	0.57727	2.05425	**0.03995**	3.46253	**0.00054**
174443	52	23	5.65	8.23427	2.99858	2.74606	**0.00761**	7.66099	3.24795	2.35872	**0.02862**	3.43469	**0.00059**	3.43469	**0.00059**
174792	59	16	4.13	3.73691	3.57408	1.04556	0.29926	17.27877	3.87030	4.46445	**0.00064**	3.14886	**0.00164**	3.41514	**0.00064**
337161	62	13	9.46	12.35754	3.54168	3.48917	**0.00083**	0.01305	4.65352	0.00280	0.99782	2.36533	**0.01801**	3.34235	**0.00083**
323903	59	16	5.06	6.69106	3.44924	1.93986	0.05631	8.19809	2.47053	3.31835	**0.00555**	3.31063	**0.00093**	3.31063	**0.00093**
1044419	70	5	155.80	18.54577	5.38472	3.44415	**0.00096**	2.01872	10.11859	0.19951	0.86031	2.45956	**0.01391**	3.30236	**0.00096**
177567	67	8	2.38	12.89635	4.45258	2.89637	**0.00500**	24.42468	10.34620	2.36074	0.06469	3.29134	**0.00100**	3.29134	**0.00100**
187196	71	4	2.25	20.47051	5.98244	3.42177	**0.00103**	-31.69466	23.96901	-1.32232	0.41220	1.74119	0.08165	3.28244	**0.00103**
198422	45	30	6.37	-0.43555	2.96303	-0.14699	0.88355	5.73123	1.57951	3.62848	**0.00117**	2.19139	**0.02842**	3.24557	**0.00117**
186030	67	8	2.25	14.73935	4.37715	3.36734	**0.00122**	2.91703	14.12332	0.20654	0.84452	2.42538	**0.01529**	3.23389	**0.00122**
370357	69	6	2.33	17.25459	5.13028	3.36328	**0.00124**	2.17277	14.67361	0.14807	0.89168	2.38044	**0.01729**	3.23026	**0.00124**
3134492	13	62	139.89	3.53904	3.80994	0.92890	0.35605	-2.20122	0.65553	-3.35791	**0.00138**	-1.60931	0.10755	-3.19883	**0.00138**
310633	71	4	10.00	20.08285	6.03538	3.32752	**0.00138**	6.67886	13.00793	0.51345	0.69802	2.53587	**0.01122**	3.19827	**0.00138**
188316	68	7	2.29	15.46694	4.65931	3.31958	**0.00142**	23.96252	17.82645	1.34421	0.25005	3.06981	**0.00214**	3.19115	**0.00142**
291054	70	5	14.20	18.10862	5.45606	3.31899	**0.00142**	7.52789	8.81516	0.85397	0.48308	2.75204	**0.00592**	3.19063	**0.00142**
362955	66	9	16.44	8.42441	4.37383	1.92609	0.05804	-15.97840	2.96730	-5.38483	**0.00169**	-0.88026	0.37872	-3.14027	**0.00169**
4439360	7	68	95.34	4.66983	4.95560	0.94233	0.34917	-2.67298	0.81664	-3.27312	**0.00171**	-1.55632	0.11963	-3.13717	**0.00171**
179499	68	7	2.86	15.14678	4.68682	3.23178	**0.00186**	-11.49899	10.45738	-1.09961	0.33324	1.51650	0.12939	3.11227	**0.00186**
185597	55	20	4.15	3.55513	3.33415	1.06628	0.28986	9.93404	2.70091	3.67803	**0.00186**	2.94821	**0.00320**	3.11098	**0.00186**
181560	60	15	6.00	10.95721	3.39655	3.22598	**0.00189**	3.08662	4.32610	0.71349	0.48919	2.68605	**0.00723**	3.10705	**0.00189**
352747	67	8	2.88	14.01221	4.39544	3.18789	**0.00212**	-3.71022	9.21262	-0.40273	0.70379	1.90387	0.05693	3.07270	**0.00212**
299755	71	4	5.00	19.28252	6.07152	3.17590	**0.00220**	-9.61323	91.28508	-0.10531	0.93320	2.10579	**0.03522**	3.06186	**0.00220**
300662	71	4	4.00	-0.78656	6.47309	-0.12151	0.90362	3.95724	0.01401	282.37870	**0.00225**	2.07423	**0.03806**	3.05448	**0.00225**
4439487	62	13	4.31	11.32469	3.58970	3.15477	**0.00234**	6.50974	4.84996	1.34222	0.20920	3.03952	**0.00237**	3.04276	**0.00234**
193969	61	14	21.43	11.11664	3.52854	3.15049	**0.00237**	-0.55228	3.61776	-0.15266	0.88143	2.04335	**0.04102**	3.03888	**0.00237**
183822	68	7	2.00	14.76980	4.68894	3.14992	**0.00238**	-16.23800	15.42116	-1.05297	0.35176	1.49000	0.13622	3.03837	**0.00238**
319197	70	5	1.40	13.49346	5.60215	2.40862	**0.01857**	73.77130	18.10403	4.07486	0.05528	3.01981	**0.00253**	3.01981	**0.00253**
186851	60	15	7.20	10.65624	3.41245	3.12275	**0.00258**	-4.34971	4.61320	-0.94288	0.36434	1.48961	0.13633	3.01376	**0.00258**
4405146	49	26	51.81	8.81930	2.86746	3.07564	**0.00297**	-1.68362	1.32528	-1.27039	0.21665	1.22719	0.21975	2.97101	**0.00297**
4391262	49	26	15.23	-0.55207	3.10316	-0.17790	0.85930	2.47946	0.74916	3.30967	**0.00306**	1.96898	**0.04895**	2.96183	**0.00306**
184534	66	9	14.67	12.86452	4.21851	3.04954	**0.00321**	-9.39345	3.90109	-2.40790	0.05272	0.71424	0.47508	2.94727	**0.00321**
3785400	69	6	56.67	15.33582	5.02956	3.04914	**0.00321**	-10.07777	2.97832	-3.38371	**0.04297**	0.65261	0.51401	2.94690	**0.00321**
215468	68	7	3.57	14.22853	4.70705	3.02281	**0.00347**	3.04877	9.88238	0.30851	0.77310	2.27070	**0.02317**	2.92292	**0.00347**
337285	64	11	4.00	11.61347	3.86571	3.00423	**0.00366**	4.31380	6.99879	0.61636	0.55478	2.47246	**0.01342**	2.90597	**0.00366**
1102370	45	30	18.37	8.09690	2.83664	2.85440	**0.00563**	2.23568	1.63243	1.36954	0.18211	2.90125	**0.00372**	2.90125	**0.00372**
177503	62	13	2.85	10.31740	3.64967	2.82694	**0.00608**	8.29932	5.70825	1.45392	0.17662	2.89536	**0.00379**	2.89536	**0.00379**
175729	54	21	4.29	7.16654	3.14529	2.27849	**0.02567**	6.09643	3.07317	1.98376	0.06275	2.89363	**0.00381**	2.89363	**0.00381**
181826	60	15	3.47	6.92039	3.53113	1.95982	0.05389	10.50687	4.30979	2.43791	**0.03128**	2.88589	**0.00390**	2.88589	**0.00390**
177660	66	9	3.44	12.66617	4.24833	2.98145	**0.00391**	3.34835	17.35082	0.19298	0.85334	2.17084	**0.02994**	2.88517	**0.00391**
229348	64	11	13.64	11.46800	3.86893	2.96413	**0.00411**	-4.75054	3.29744	-1.44067	0.18764	1.09726	0.27253	2.86934	**0.00411**
105287	64	11	3.09	3.17775	4.15862	0.76413	0.44728	9.94492	2.50469	3.97053	**0.00412**	2.56615	**0.01028**	2.86914	**0.00412**
4111715	50	25	5.60	-1.73332	3.14098	-0.55184	0.58277	2.95515	0.92315	3.20115	**0.00412**	1.64007	0.10099	2.86876	**0.00412**
701221	66	9	2.11	10.67676	4.28582	2.49118	**0.01504**	21.00520	10.97443	1.91401	0.10413	2.86848	**0.00412**	2.86848	**0.00412**
4295707	49	26	67.85	8.52019	2.87618	2.96233	**0.00413**	0.40298	1.40831	0.28614	0.77733	2.22774	**0.02590**	2.86770	**0.00413**
2309802	69	6	4.50	11.57522	5.18436	2.23272	**0.02868**	17.16839	5.92114	2.89951	0.06253	2.86409	**0.00418**	2.86409	**0.00418**
293883	58	17	13.82	9.67151	3.27045	2.95724	**0.00420**	1.31984	2.69372	0.48997	0.63174	2.36337	**0.01811**	2.86304	**0.00420**
183681	43	32	5.41	8.17531	2.77312	2.94806	**0.00431**	0.95617	2.43591	0.39253	0.69753	2.29336	**0.02183**	2.85464	**0.00431**
4381054	55	20	3.00	7.26727	3.16353	2.29720	**0.02452**	6.97545	3.69598	1.88731	0.07631	2.84357	**0.00446**	2.84357	**0.00446**
180982	38	37	16.59	-8.01179	2.74122	-2.92271	**0.00463**	0.58902	0.69122	0.85215	0.40009	-1.40712	0.15939	-2.83143	**0.00463**
186077	62	13	4.69	10.01256	3.64270	2.74867	**0.00756**	5.39289	3.77800	1.42744	0.18392	2.82839	**0.00468**	2.82839	**0.00468**
185164	40	35	9.03	1.06025	2.90442	0.36505	0.71615	4.83973	1.60845	3.00894	**0.00508**	2.23849	**0.02519**	2.80208	**0.00508**
2123717	36	39	6.79	-6.53374	2.79690	-2.33606	**0.02228**	-1.51686	0.88321	-1.71743	0.09449	-2.79858	**0.00513**	-2.79858	**0.00513**
798581	39	36	7.00	7.89833	2.74777	2.87446	**0.00532**	-0.75431	1.70048	-0.44358	0.66024	1.65998	0.09692	2.78715	**0.00532**
195756	71	4	1.50	-9.57561	6.35063	-1.50782	0.13598	-3.12732	0.07072	-44.22135	**0.01439**	-2.78475	**0.00536**	-2.78475	**0.00536**
189235	68	7	8.29	13.56502	4.73816	2.86293	**0.00549**	8.14933	9.04302	0.90117	0.41845	2.53545	**0.01123**	2.77655	**0.00549**
849346	70	5	3.60	12.63987	5.62478	2.24718	**0.02769**	47.49714	14.93665	3.17991	0.08629	2.76967	**0.00561**	2.76967	**0.00561**
4356062	71	4	1.25	1.02975	6.49349	0.15858	0.87444	-14.95717	0.13392	-111.68710	**0.00570**	-1.84311	0.06531	-2.76456	**0.00570**
4439489	70	5	3.40	15.82230	5.56541	2.84297	**0.00581**	9.64684	22.54162	0.42796	0.71036	2.21294	**0.02690**	2.75819	**0.00581**
199344	64	11	3.27	9.18620	3.95143	2.32478	**0.02291**	12.49360	6.90701	1.80883	0.10808	2.74487	**0.00605**	2.74487	**0.00605**
197343	69	6	2.17	1.15183	5.45921	0.21099	0.83349	30.20840	4.40137	6.86341	**0.00633**	2.07906	**0.03761**	2.73002	**0.00633**
4333897	63	12	2.67	0.47799	3.95454	0.12087	0.90413	5.71353	1.62809	3.50934	**0.00663**	2.00504	**0.04496**	2.71510	**0.00663**

Table S4c. OTUs associated with High-density lipoprotein (HDL) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
172878	71	4	3.25	15.75979	6.20616	2.53938	**0.01327**	36.93982	10.66263	3.46442	0.17890	2.70166	**0.00690**	2.70166	**0.00690**
4323555	70	5	2.60	-3.10597	5.79985	-0.53553	0.59394	-10.01477	0.85345	-11.73452	**0.00718**	-2.27782	**0.02274**	-2.68819	**0.00718**
158855	67	8	11.63	7.73045	4.60594	1.67837	0.09761	-5.03253	1.15402	-4.36088	**0.00728**	-0.72621	0.46771	-2.68356	**0.00728**
191251	24	51	13.16	-0.89823	3.14401	-0.28570	0.77593	-2.38373	0.85158	-2.79920	**0.00736**	-2.09649	**0.03604**	-2.68026	**0.00736**
312586	69	6	3.00	13.96949	5.08851	2.74530	**0.00763**	10.51387	19.84381	0.52983	0.63294	2.22432	**0.02613**	2.66806	**0.00763**
253471	71	4	7.25	15.59584	6.19092	2.51915	**0.01398**	15.64514	4.80297	3.25739	0.18962	2.66533	**0.00769**	2.66533	**0.00769**
300374	43	32	6.13	6.03575	2.89543	2.08457	**0.04066**	3.52385	1.97762	1.78187	0.08525	2.66441	**0.00771**	2.66441	**0.00771**
191483	60	15	2.33	-5.00453	3.57526	-1.39977	0.16588	-6.44760	2.34774	-2.74630	**0.01772**	-2.65654	**0.00789**	-2.65654	**0.00789**
180312	51	24	4.92	3.71362	3.08292	1.20458	0.23231	6.91502	2.47323	2.79594	**0.01083**	2.64645	**0.00813**	2.64645	**0.00813**
113919	61	14	93.21	9.62924	3.54437	2.71677	**0.00825**	1.04774	1.86362	0.56220	0.58525	2.25382	**0.02421**	2.64165	**0.00825**
180136	45	30	7.27	3.01905	3.05016	0.98980	0.32558	7.16313	2.51740	2.84545	**0.00836**	2.55981	**0.01047**	2.63707	**0.00836**
168439	44	31	33.58	-7.60837	2.93351	-2.59361	**0.01150**	-0.99332	0.80851	-1.22858	0.22946	-2.63677	**0.00837**	-2.63677	**0.00837**
178015	65	10	2.70	11.02965	4.06985	2.71009	**0.00840**	-14.64466	12.28010	-1.19255	0.27190	1.08665	0.27719	2.63546	**0.00840**
4421273	40	35	9.49	5.87661	2.85487	2.05845	**0.04317**	2.75777	1.56814	1.75862	0.08820	2.63542	**0.00840**	2.63542	**0.00840**
2298935	20	55	31.00	0.23284	3.27730	0.07105	0.94356	-2.46648	0.90111	-2.73715	**0.00846**	-1.81178	0.07002	-2.63304	**0.00846**
4481861	69	6	5.17	13.78793	5.09539	2.70596	**0.00850**	-11.94548	5.46068	-2.18755	0.11652	0.75101	0.45264	2.63163	**0.00850**
3138798	31	44	219.93	-5.55266	2.86972	-1.93491	0.05693	-0.88308	0.47648	-1.85336	0.07104	-2.62273	**0.00872**	-2.62273	**0.00872**
176062	41	34	33.12	7.84513	2.91106	2.69494	**0.00876**	1.75235	1.59044	1.10180	0.27903	2.61906	**0.00882**	2.62141	**0.00876**
851733	59	16	70.75	-4.24930	3.50354	-1.21286	0.22915	-1.34752	0.46378	-2.90549	**0.01228**	-2.62097	**0.00877**	-2.62097	**0.00877**
368261	70	5	2.20	-5.40532	5.82918	-0.92729	0.35688	2.61971	0.24699	10.60668	**0.00877**	1.20172	0.22947	2.62082	**0.00877**
2250985	34	41	10.93	-5.34829	2.84594	-1.87927	0.06425	-1.85415	0.97858	-1.89473	0.06576	-2.60957	**0.00907**	-2.60957	**0.00907**
3903651	21	54	29.11	-8.28147	3.08903	-2.68093	**0.00910**	-0.58679	0.67143	-0.87394	0.38625	-2.45710	**0.01401**	-2.60841	**0.00910**
196664	21	54	29.11	-8.28147	3.08903	-2.68093	**0.00910**	-0.58679	0.67143	-0.87394	0.38625	-2.45710	**0.01401**	-2.60841	**0.00910**
111135	70	5	11.00	14.83936	5.54200	2.67762	**0.00918**	-0.27015	10.27993	-0.02628	0.98142	1.82579	0.06788	2.60534	**0.00918**
4468234	1	74	18.84	0.00000	0.00000	0.00000	1.00000	-2.35815	0.88092	-2.67692	**0.00922**	-1.84111	0.06560	-2.60373	**0.00922**
183698	66	9	4.00	11.54758	4.32898	2.66751	**0.00943**	-2.97801	10.77117	-0.27648	0.79146	1.64864	0.09922	2.59595	**0.00943**
305141	61	14	9.14	8.91168	3.56825	2.49749	**0.01479**	-8.67442	2.76678	-3.13521	**0.00949**	-0.11068	0.91187	-2.59391	**0.00949**
157470	64	11	2.73	5.43436	4.04985	1.34187	0.18386	15.76314	5.41324	2.91196	**0.01953**	2.59100	**0.00957**	2.59100	**0.00957**
360508	42	33	9.12	6.31233	2.82518	2.23431	**0.02857**	4.51120	2.97952	1.51407	0.14047	2.59043	**0.00959**	2.59043	**0.00959**
346639	66	9	4.67	11.36127	4.28058	2.65415	**0.00978**	0.81080	6.46756	0.12536	0.90433	1.91183	0.05590	2.58354	**0.00978**
177062	65	10	3.70	9.91395	4.10379	2.41580	**0.01824**	11.08487	7.79551	1.42196	0.19803	2.57947	**0.00990**	2.57947	**0.00990**
2506486	41	34	4.32	-7.37126	2.78195	-2.64967	**0.00990**	-0.68254	1.23900	-0.55088	0.58567	-2.20936	**0.02715**	-2.57938	**0.00990**
191660	69	6	1.67	11.08908	5.18745	2.13768	**0.03594**	30.49819	14.30504	2.13199	0.12278	2.57445	**0.01004**	2.57445	**0.01004**
1522739	71	4	2.25	3.72494	6.43723	0.57866	0.56463	-33.30790	0.53109	-62.71624	**0.01015**	-1.41046	0.15840	-2.57068	**0.01015**
3709990	63	12	3.08	-6.54969	3.96537	-1.65172	0.10295	-5.71672	2.46507	-2.31909	**0.04555**	-2.56697	**0.01026**	-2.56697	**0.01026**
180473	54	21	4.05	8.12393	3.08269	2.63534	**0.01029**	-0.97962	2.94750	-0.33236	0.74346	1.58305	0.11341	2.56605	**0.01029**
4483337	17	58	50.33	1.86103	3.48801	0.53355	0.59529	-1.81917	0.68811	-2.64373	**0.01066**	-1.43003	0.15271	-2.55355	**0.01066**
4444262	65	10	13.70	-3.56985	4.26389	-0.83723	0.40523	4.24689	1.23243	3.44594	**0.01075**	1.21509	0.22433	2.55071	**0.01075**
306299	50	25	7.36	-7.73511	2.95613	-2.61664	**0.01081**	1.81627	1.45256	1.25039	0.22429	-0.94289	0.34573	-2.54864	**0.01081**
4319785	66	9	131.67	11.20635	4.28371	2.61604	**0.01083**	2.86017	2.71133	1.05489	0.33209	2.48761	**0.01286**	2.54809	**0.01083**
308322	69	6	4.00	10.39503	5.20966	1.99534	**0.04979**	47.91467	20.42151	2.34628	0.10064	2.54805	**0.01083**	2.54805	**0.01083**
189960	44	31	4.84	-2.73631	2.94160	-0.93021	0.35537	-3.20956	1.17637	-2.72836	**0.01087**	-2.45442	**0.01411**	-2.54685	**0.01087**
182188	56	19	3.68	7.47559	3.21793	2.32311	**0.02300**	5.54774	3.99784	1.38768	0.18426	2.54640	**0.01088**	2.54640	**0.01088**
4480861	48	27	61.48	7.56556	2.89679	2.61170	**0.01096**	1.05257	1.54711	0.68035	0.50279	2.27275	**0.02304**	2.54405	**0.01096**
3746307	70	5	3.20	14.47322	5.57101	2.59795	**0.01137**	-17.13554	7.44351	-2.30208	0.14794	0.76677	0.44322	2.53124	**0.01137**
4377144	70	5	7.40	12.07259	5.63672	2.14178	**0.03560**	19.11823	8.06383	2.37086	0.14118	2.52644	**0.01152**	2.52644	**0.01152**
538322	45	30	6.60	1.97788	2.95556	0.66921	0.50550	5.09757	1.88707	2.70132	**0.01178**	2.25169	**0.02434**	2.51852	**0.01178**
4483037	54	21	2.67	-8.08739	3.13400	-2.58054	**0.01190**	0.70890	1.39505	0.50815	0.61752	-1.42527	0.15408	-2.51500	**0.01190**
192818	71	4	1.25	1.97686	6.48379	0.30489	0.76133	-13.64865	0.25640	-53.23167	**0.01196**	-1.56245	0.11818	-2.51338	**0.01196**
194371	59	16	3.44	8.73377	3.38712	2.57853	**0.01197**	2.15402	4.51317	0.47727	0.64109	2.10668	**0.03515**	2.51313	**0.01197**
107044	30	45	60.24	-2.35746	2.94709	-0.79993	0.42638	-1.81929	0.69381	-2.62217	**0.01212**	-2.33631	**0.01948**	-2.50865	**0.01212**
1607319	64	11	1.27	-6.56641	4.13925	-1.58638	0.11704	-9.45141	4.05103	-2.33309	**0.04793**	-2.50693	**0.01218**	-2.50693	**0.01218**
187267	53	22	8.50	6.39578	3.14383	2.03439	**0.04560**	4.59906	2.84294	1.61771	0.12221	2.50647	**0.01219**	2.50647	**0.01219**
1000547	51	24	2.75	-6.82274	3.00151	-2.27310	**0.02600**	-2.76388	2.03122	-1.36070	0.18803	-2.50496	**0.01225**	-2.50496	**0.01225**
2840201	65	10	3.90	10.49178	4.08379	2.56913	**0.01227**	-13.83767	7.73605	-1.78873	0.11680	0.66185	0.50807	2.50436	**0.01227**
199182	63	12	4.08	9.90358	3.85704	2.56766	**0.01231**	8.08833	8.86250	0.91265	0.38522	2.38387	**0.01713**	2.50299	**0.01231**
184940	68	7	6.14	12.24342	4.77070	2.56638	**0.01236**	3.61748	6.24903	0.57889	0.59370	2.14626	**0.03185**	2.50179	**0.01236**
188317	68	7	1.57	8.56320	4.94126	1.73300	0.08738	28.30567	11.39574	2.48388	0.06793	2.49954	**0.01244**	2.49954	**0.01244**
187917	40	35	10.09	2.18348	2.90103	0.75265	0.45411	3.79141	1.43198	2.64766	**0.01247**	2.29598	**0.02168**	2.49842	**0.01247**
182167	41	34	9.29	0.47862	2.96528	0.16141	0.87223	4.45152	1.68211	2.64639	**0.01267**	1.87654	0.06058	2.49300	**0.01267**
4333652	67	8	2.13	11.47846	4.49672	2.55263	**0.01281**	8.20165	15.44828	0.53091	0.61821	2.11237	**0.03465**	2.48895	**0.01281**
188764	66	9	1.67	2.69748	4.47432	0.60288	0.54848	28.74267	8.22038	3.49651	**0.01288**	2.18290	**0.02904**	2.48705	**0.01288**
182163	69	6	9.00	13.20879	5.18225	2.54885	**0.01294**	8.48868	7.89073	1.07578	0.36084	2.40359	**0.01623**	2.48542	**0.01294**
3154070	2	73	33.99	-15.99891	8.79779	-1.81851	0.07314	-1.30796	0.74992	-1.74415	0.08552	-2.48295	**0.01303**	-2.48295	**0.01303**
3014082	67	8	3.88	11.67212	4.59329	2.54113	**0.01320**	-3.78206	8.61745	-0.43888	0.67906	1.45979	0.14435	2.47820	**0.01320**
4467447	22	53	49.87	-3.73379	3.15895	-1.18197	0.24111	-1.74478	0.72525	-2.40577	**0.01988**	-2.47549	**0.01331**	-2.47549	**0.01331**
4446902	41	34	7.62	-4.96513	2.85771	-1.73745	0.08658	-2.03441	1.10325	-1.84402	0.07476	-2.47180	**0.01344**	-2.47180	**0.01344**
2920309	51	24	3.21	-3.97219	3.14119	-1.26455	0.21011	-3.15749	1.30947	-2.41128	**0.02514**	-2.46961	**0.01353**	-2.46961	**0.01353**
1566691	69	6	2.33	13.02357	5.16782	2.52013	**0.01395**	-10.67000	18.28895	-0.58341	0.60058	1.36825	0.17123	2.45857	**0.01395**
199279	55	20	1.95	3.50213	3.31541	1.05632	0.29435	9.81330	3.63561	2.69922	**0.01520**	2.45805	**0.01397**	2.45805	**0.01397**
3426658	41	34	2.44	-7.03669	2.79386	-2.51863	**0.01400**	-0.48422	1.35410	-0.35760	0.72307	-1.98805	**0.04681**	-2.45716	**0.01400**
171559	61	14	1.57	-8.08074	3.60556	-2.24119	**0.02810**	-5.16548	3.81768	-1.35304	0.20320	-2.45254	**0.01419**	-2.45254	**0.01419**
3472078	28	47	14.98	-7.21262	2.87659	-2.50735	**0.01442**	-0.66876	0.71172	-0.93964	0.35253	-2.38741	**0.01697**	-2.44662	**0.01442**
266210	69	6	12.50	1.79127	5.42939	0.32992	0.74242	-11.48507	2.24751	-5.11012	**0.01450**	-1.49625	0.13459	-2.44467	**0.01450**
340474	46	29	3.79	-5.12739	3.01990	-1.69787	0.09385	-2.05167	1.10604	-1.85496	0.07497	-2.44379	**0.01453**	-2.44379	**0.01453**
4349261	52	23	26.26	6.53165	3.06303	2.13242	**0.03638**	2.65885	1.88564	1.41005	0.17389	2.44124	**0.01464**	2.44124	**0.01464**
193852	67	8	2.00	11.27475	4.50803	2.50104	**0.01466**	2.86763	13.07623	0.21930	0.83509	1.87304	0.06106	2.44070	**0.01466**

Table S4c. OTUs associated with High-density lipoprotein (HDL) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
184770	50	25	4.28	7.45445	2.99046	2.49274	**0.01498**	-0.04027	2.62548	-0.01534	0.98790	1.70962	0.08734	2.43293	**0.01498**
188333	69	6	3.50	10.82773	5.18823	2.08698	**0.04043**	21.87608	11.93717	1.83260	0.16424	2.43263	**0.01499**	2.43263	**0.01499**
185802	31	44	91.45	5.89708	2.86324	2.05958	**0.04305**	1.29567	0.90151	1.43722	0.15824	2.42833	**0.01517**	2.42833	**0.01517**
172777	28	47	7.26	-6.33421	2.90813	-2.17810	**0.03267**	-1.09183	0.82947	-1.31629	0.19489	-2.42701	**0.01522**	-2.42701	**0.01522**
4480359	28	47	8.09	4.22748	2.95591	1.43018	0.15699	2.83832	1.36692	2.07643	**0.04373**	2.42676	**0.01523**	2.42676	**0.01523**
3265161	34	41	8.24	-4.41657	2.88149	-1.53274	0.12972	-2.37416	1.20472	-1.97071	0.05607	-2.42231	**0.01542**	-2.42231	**0.01542**
306315	64	11	3.18	9.82768	3.97811	2.47044	**0.01586**	0.15485	9.05292	0.01711	0.98677	1.71729	0.08593	2.41203	**0.01586**
4405423	60	15	4.40	-2.36862	3.67357	-0.64477	0.52112	5.79639	2.07330	2.79573	**0.01617**	1.24691	0.21243	2.40501	**0.01617**
845444	61	14	2.64	8.79374	3.57255	2.46147	**0.01623**	-4.70108	4.83064	-0.97318	0.35138	1.04065	0.29804	2.40362	**0.01623**
4202174	40	35	9.69	6.83659	2.79141	2.44915	**0.01675**	-1.45942	1.50997	-0.96652	0.34103	1.01818	0.30859	2.39206	**0.01675**
235212	69	6	44.00	12.63518	5.16029	2.44854	**0.01678**	-2.94776	10.72238	-0.27492	0.80123	1.51301	0.13028	2.39148	**0.01678**
339494	61	14	100.57	8.75624	3.57678	2.44808	**0.01680**	0.09176	2.73103	0.03360	0.97380	1.71395	0.08654	2.39105	**0.01680**
189820	68	7	2.71	2.71259	4.97842	0.54487	0.58753	9.01001	2.28522	3.94273	**0.01692**	2.07242	**0.03823**	2.38842	**0.01692**
179018	54	21	9.76	7.57805	3.11099	2.43590	**0.01733**	-0.83004	2.30205	-0.36057	0.72262	1.43165	0.15225	2.37961	**0.01733**
553611	45	30	9.03	3.86565	2.96297	1.30466	0.19617	-4.16320	1.64272	-2.53433	**0.01738**	-0.76800	0.44249	-2.37867	**0.01738**
4468466	18	57	118.93	1.43663	3.47915	0.41293	0.68089	1.57718	0.64471	2.44635	**0.01772**	1.96767	**0.04911**	2.37145	**0.01772**
198696	71	4	2.75	14.52341	6.21993	2.33498	**0.02234**	34.90798	16.77692	2.08071	0.28521	2.37113	**0.01773**	2.37113	**0.01773**
4405482	59	16	1.56	-6.74376	3.45311	-1.95295	0.05471	-3.80534	2.50841	-1.51703	0.15320	-2.36845	**0.01786**	-2.36845	**0.01786**
2388088	58	17	3.82	-8.13916	3.36848	-2.41627	**0.01822**	-0.62350	2.20203	-0.28315	0.78121	-1.86600	0.06204	-2.36117	**0.01822**
3232988	68	7	11.00	11.38190	4.79824	2.37210	**0.02036**	7.36985	6.33520	1.16332	0.30937	2.35902	**0.01832**	2.35902	**0.01832**
363646	58	17	13.29	-4.78305	3.42089	-1.39819	0.16635	-2.56578	1.21143	-2.11798	0.05255	-2.34944	**0.01880**	-2.34944	**0.01880**
198210	52	23	5.78	3.58029	3.16285	1.13198	0.26140	5.11932	2.16742	2.36194	**0.02843**	2.34361	**0.01910**	2.34361	**0.01910**
43950	67	8	2.38	-3.49443	4.72817	-0.73907	0.46227	12.28831	3.60741	3.40641	**0.01912**	1.13711	0.25549	2.34323	**0.01912**
177697	59	16	2.00	8.19570	3.42562	2.39247	**0.01935**	-5.00641	5.86493	-0.85362	0.40877	1.06966	0.28477	2.33878	**0.01935**
189083	40	35	32.46	6.71275	2.80792	2.39065	**0.01944**	-0.89712	1.31529	-0.68207	0.50010	1.17573	0.23970	2.33707	**0.01944**
4370025	59	16	7.63	-6.99729	3.53484	-1.97952	0.05158	3.03951	1.14141	2.66295	**0.01952**	0.27489	0.78340	2.33536	**0.01952**
4444277	64	11	102.55	9.39930	3.94447	2.38291	**0.01982**	-3.59030	1.99628	-1.79849	0.10981	0.51669	0.60537	2.32978	**0.01982**
177518	62	13	4.23	6.72113	3.74592	1.79425	0.07697	-11.83318	4.27767	-2.76627	**0.01991**	-0.39557	0.69242	-2.32796	**0.01991**
189937	59	16	2.38	-3.70750	3.56405	-1.04025	0.30171	-7.70806	3.01890	-2.55327	**0.02404**	-2.32583	**0.02003**	-2.32583	**0.02003**
178759	64	11	12.27	9.38274	3.94826	2.37643	**0.02014**	0.66146	4.60866	0.14353	0.88942	1.74140	0.08161	2.32368	**0.02014**
195214	66	9	3.44	9.25278	4.37167	2.11653	**0.03776**	9.80179	7.29770	1.34313	0.22780	2.32180	**0.02024**	2.32180	**0.02024**
2403301	57	18	52.33	7.85916	3.31846	2.36832	**0.02056**	1.24943	1.81053	0.69009	0.50068	2.11387	**0.03453**	2.31604	**0.02056**
1602805	48	27	5.70	-4.35234	2.97749	-1.46175	0.14816	-2.60140	1.36018	-1.91254	0.06781	-2.31387	**0.02068**	-2.31387	**0.02068**
175184	64	11	2.55	4.18140	4.12754	1.01305	0.31443	9.92349	3.55798	2.78908	**0.02359**	2.31201	**0.02078**	2.31201	**0.02078**
191043	70	5	4.20	-6.37491	5.78074	-1.10278	0.27379	13.11592	1.92312	6.82014	**0.02083**	0.86033	0.38961	2.31106	**0.02083**
182797	69	6	2.83	12.39385	5.24864	2.36135	**0.02092**	-23.37186	31.27050	-0.74741	0.50907	1.16615	0.24356	2.30947	**0.02092**
190676	61	14	24.79	8.58277	3.64596	2.35405	**0.02130**	-4.57878	2.91270	-1.57201	0.14425	0.59570	0.55138	2.30259	**0.02130**
213394	59	16	41.63	-6.66359	3.48260	-1.91340	0.05967	-0.88872	0.61335	-1.44895	0.17104	-2.29956	**0.02147**	-2.29956	**0.02147**
579541	63	12	7.67	8.97303	3.82887	2.34352	**0.02187**	-5.93500	5.81260	-1.02106	0.33389	0.93787	0.34831	2.29266	**0.02187**
3754778	68	7	18.86	11.27637	4.82182	2.33861	**0.02214**	1.56475	6.82810	0.22916	0.82998	1.76972	0.07677	2.28803	**0.02214**
195015	63	12	3.33	-3.88252	3.92690	-0.98870	0.32612	4.20029	1.52415	2.75582	**0.02226**	0.92198	0.35654	2.28584	**0.02226**
586453	64	11	7.36	-8.07804	3.99195	-2.02358	**0.04673**	-1.87272	1.39493	-1.34251	0.21628	-2.28061	**0.02257**	-2.28061	**0.02257**
864465	51	24	3.08	-1.79239	3.17444	-0.56463	0.57408	-5.91891	2.40542	-2.46066	**0.02262**	-2.00954	**0.04448**	-2.27986	**0.02262**
59563	66	9	17.89	5.66046	4.41243	1.28284	0.20366	3.61265	1.48528	2.43231	0.05100	2.27876	**0.02268**	2.27876	**0.02268**
157162	70	5	3.00	13.04286	5.60318	2.32776	**0.02274**	21.12212	20.42321	1.03422	0.40970	2.19359	**0.02826**	2.27779	**0.02274**
175612	70	5	65.20	-2.97478	6.02417	-0.49381	0.62295	-4.30214	0.66853	-6.43522	**0.02331**	-1.95165	0.05098	-2.26837	**0.02331**
2203165	69	6	2.67	-9.31685	5.39010	-1.72851	0.08818	8.65283	2.01919	4.28529	**0.02335**	0.39779	0.69079	2.26762	**0.02335**
188735	30	45	7.40	-6.71530	2.89885	-2.31654	**0.02338**	0.21021	1.08517	0.19371	0.84733	-1.46702	0.14237	-2.26720	**0.02338**
173965	60	15	2.40	-1.34340	3.62099	-0.37100	0.71172	6.70568	2.59112	2.58794	**0.02375**	1.33759	0.18103	2.26119	**0.02375**
4318284	69	6	28.83	-10.39242	5.20328	-1.99728	**0.04957**	-1.39834	0.89926	-1.55499	0.21779	-2.25994	**0.02382**	-2.25994	**0.02382**
177520	44	31	6.06	3.11699	2.93072	1.06356	0.29108	4.85956	2.15941	2.25041	**0.03246**	2.25878	**0.02390**	2.25878	**0.02390**
184990	66	9	4.89	9.98163	4.32606	2.30732	**0.02392**	-2.51763	6.97759	-0.36082	0.73060	1.35352	0.17589	2.25849	**0.02392**
193161	71	4	1.25	14.31682	6.22947	2.29824	**0.02445**	-111.51370	119.01938	-0.93694	0.52072	1.13680	0.25562	2.24991	**0.02445**
4302904	51	24	5.67	-3.75655	3.11089	-1.20755	0.23117	-3.22354	1.53170	-2.10454	**0.04755**	-2.24769	**0.02460**	-2.24769	**0.02460**
4414420	71	4	1.75	-5.06823	6.44214	-0.78673	0.43402	-5.17015	0.20021	-25.82325	**0.02464**	-2.14205	**0.03219**	-2.24699	**0.02464**
366147	62	13	6.31	-5.06882	3.80567	-1.33191	0.18709	-3.45260	1.65350	-2.08806	0.06334	-2.24582	**0.02472**	-2.24582	**0.02472**
184426	46	29	5.07	3.85419	2.95914	1.30247	0.19691	3.85344	1.96402	1.96202	0.06055	2.23955	**0.02512**	2.23955	**0.02512**
843569	69	6	10.83	11.89433	5.20935	2.28326	**0.02537**	-9.84103	4.74599	-2.07355	0.12980	0.50973	0.61024	2.23575	**0.02537**
194660	47	28	5.07	5.08739	2.93628	1.73260	0.08745	4.02719	2.69654	1.49347	0.14783	2.23181	**0.02563**	2.23181	**0.02563**
4332078	59	16	3.81	7.84483	3.44234	2.27893	**0.02564**	-6.71919	5.40126	-1.24400	0.23546	0.73910	0.45985	2.23165	**0.02564**
577294	65	10	2.10	-9.41964	4.14054	-2.27498	**0.02589**	0.95464	2.75436	0.34659	0.73908	-1.33986	0.18029	-2.22791	**0.02589**
186876	70	5	2.40	-1.56945	5.84146	-0.26867	0.78895	11.11952	1.82586	6.09001	**0.02592**	1.38575	0.16582	2.22742	**0.02592**
336281	67	8	50.00	10.35414	4.55428	2.27350	**0.02598**	-0.19698	3.63849	-0.05414	0.95892	1.53796	0.12406	2.22651	**0.02598**
182431	52	23	7.65	4.23692	3.13233	1.35264	0.18040	4.55016	2.38077	1.91122	0.07042	2.22650	**0.02598**	2.22650	**0.02598**
4332428	71	4	2.00	14.18813	6.25496	2.26830	**0.02631**	6.03493	125.71564	0.04800	0.96946	1.59797	0.11005	2.22159	**0.02631**
304973	62	13	3.31	-1.05887	3.97503	-0.26638	0.79071	-6.72079	2.58204	-2.60290	**0.02636**	-1.75805	0.07874	-2.22087	**0.02636**
4423553	37	38	12.08	5.50381	2.83890	1.93871	0.05646	2.05349	1.63508	1.25589	0.21747	2.22086	**0.02636**	2.22086	**0.02636**
181140	59	16	3.06	3.30982	3.53039	0.93752	0.35162	11.02287	4.45123	2.47636	**0.02780**	2.21434	**0.02681**	2.21434	**0.02681**
849440	70	5	1.80	5.15182	5.81277	0.88629	0.37841	15.16582	2.54595	5.95684	**0.02704**	2.18617	**0.02880**	2.21089	**0.02704**
153075	67	8	10.50	10.25088	4.54887	2.25350	**0.02727**	-0.17991	6.21917	-0.02893	0.97804	1.54153	0.12319	2.20758	**0.02727**
319411	71	4	2.25	11.01250	6.35162	1.73381	0.08723	20.98350	5.35173	3.92088	0.15898	2.20526	**0.02744**	2.20526	**0.02744**
34789	63	12	3.83	7.68997	3.86934	1.98741	0.05068	8.67432	6.99649	1.23981	0.24640	2.20145	**0.02770**	2.20145	**0.02770**
4450214	31	44	39.32	4.68731	2.94613	1.59101	0.11599	1.71498	1.09391	1.56776	0.12462	2.19732	**0.02800**	2.19732	**0.02800**
4381749	55	20	4.10	2.74327	3.26430	0.84039	0.40347	6.94677	2.89176	2.40226	**0.02800**	2.14448	**0.03199**	2.19732	**0.02800**
183454	67	8	15.75	10.24523	4.57518	2.23931	**0.02823**	-6.00090	5.39044	-1.11325	0.31624	0.84281	0.39933	2.19414	**0.02823**
181754	49	26	4.00	3.33480	3.05852	1.09033	0.27920	4.15113	1.94904	2.12983	**0.04411**	2.18863	**0.02862**	2.18863	**0.02862**
4470870	51	24	78.92	0.21710	3.13909	0.06916	0.94505	1.96145	0.83665	2.34442	**0.02897**	1.59300	0.11116	2.18393	**0.02897**

Table S4c. OTUs associated with High-density lipoprotein (HDL) at FDR < 0.05 [a] level (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
3756485	44	31	5.19	3.50648	2.94608	1.19022	0.23787	4.55065	2.29809	1.98019	0.05759	2.17730	**0.02946**	2.17730	**0.02946**
807548	70	5	5.60	10.68571	5.67208	1.88392	0.06361	21.58441	12.29857	1.75503	0.22134	2.17636	**0.02953**	2.17636	**0.02953**
184209	50	25	2.80	-6.61241	2.97864	-2.21994	**0.02957**	-1.79854	2.07964	-0.86483	0.39646	-2.13810	**0.03251**	-2.17578	**0.02957**
307984	65	10	3.30	-2.93791	4.34048	-0.67686	0.50066	5.97263	2.19926	2.71574	**0.02995**	1.05876	0.28971	2.17076	**0.02995**
4300127	65	10	324.60	7.80503	4.16574	1.87362	0.06504	2.54212	1.90217	1.33643	0.22321	2.16587	**0.03032**	2.16587	**0.03032**
157631	71	4	11.25	13.79636	6.26213	2.20314	**0.03079**	-16.73865	4.72058	-3.54589	0.17499	0.56816	0.56992	2.15984	**0.03079**
4365130	16	59	116.85	-7.64795	3.47312	-2.20204	**0.03087**	-0.35028	0.56412	-0.62094	0.53716	-1.96286	**0.04966**	-2.15879	**0.03087**
4212012	64	11	1.18	8.85015	4.02317	2.19980	**0.03103**	-6.41594	17.53517	-0.36589	0.72393	1.27523	0.20223	2.15666	**0.03103**
288521	63	12	8.83	-8.42247	3.83119	-2.19840	**0.03114**	1.67747	1.99684	0.84006	0.42263	-0.95704	0.33855	-2.15533	**0.03114**
192437	26	49	16.29	6.47436	2.94865	2.19570	**0.03134**	0.27846	1.31687	0.21146	0.83346	1.67092	0.09474	2.15277	**0.03134**
299441	69	6	9.83	8.95229	5.28125	1.69511	0.09438	5.40630	3.01901	1.79075	0.17126	2.15025	**0.03154**	2.15025	**0.03154**
362765	46	29	4.90	-4.11079	2.95822	-1.38962	0.16893	-2.09731	1.21699	-1.72335	0.09670	-2.14729	**0.03177**	-2.14729	**0.03177**
182735	59	16	4.38	-4.92118	3.61829	-1.36009	0.17805	-3.34295	1.83720	-1.81959	0.09192	-2.14406	**0.03203**	-2.14406	**0.03203**
2018038	61	14	3.21	7.87091	3.60415	2.18384	**0.03223**	-1.37558	5.22348	-0.26335	0.79715	1.33253	0.18269	2.14151	**0.03223**
840376	64	11	17.09	-2.23961	4.08825	-0.54782	0.58551	-4.97413	1.93267	-2.57370	**0.03294**	-1.89377	0.05826	-2.13285	**0.03294**
176753	68	7	3.14	0.59852	4.99631	0.11979	0.90498	10.31550	3.22550	3.19811	**0.03296**	1.59236	0.11130	2.13257	**0.03296**
175485	11	64	27.86	0.24025	4.12938	0.05818	0.95377	-1.87797	0.86189	-2.17891	**0.03321**	-1.46480	0.14297	-2.12952	**0.03321**
199286	40	35	9.26	1.84293	2.91521	0.63218	0.52927	3.36741	1.51428	2.22377	**0.03335**	1.94950	0.05124	2.12790	**0.03335**
19080	62	13	64.00	-4.49425	3.87853	-1.15875	0.25039	0.88311	0.35854	2.46310	**0.03350**	0.69060	0.48982	2.12606	**0.03350**
312882	57	18	4.50	-3.51069	3.41423	-1.02825	0.30727	3.88645	1.66078	2.34014	**0.03351**	0.78129	0.43463	2.12587	**0.03351**
1820776	64	11	40.09	4.86953	4.06504	1.19790	0.23488	6.64395	3.16643	2.09825	0.06913	2.12519	**0.03357**	2.12519	**0.03357**
3211875	25	50	7.50	-2.71437	3.06223	-0.88640	0.37835	-2.46462	1.12838	-2.18422	**0.03397**	-2.12229	**0.03381**	-2.12229	**0.03381**
186133	60	15	12.33	1.90868	3.61746	0.52763	0.59938	5.41269	2.26614	2.38850	**0.03423**	1.86864	0.06167	2.11737	**0.03423**
3235048	48	27	23.07	4.08237	2.99485	1.36313	0.17709	3.05976	1.79149	1.70794	0.10055	2.11563	**0.03438**	2.11563	**0.03438**
341730	67	8	2.50	-5.83037	4.64928	-1.25404	0.21388	-5.95694	2.72343	-2.18730	0.08037	-2.11531	**0.03440**	-2.11531	**0.03440**
184464	45	30	150.53	6.18295	2.87525	2.15041	**0.03488**	-0.34095	0.96056	-0.35494	0.72539	1.24342	0.21371	2.10973	**0.03488**
182874	55	20	43.10	3.28269	3.25793	1.00760	0.31702	-3.67520	1.60323	-2.29237	**0.03491**	-0.78400	0.43304	-2.10935	**0.03491**
183970	69	6	4.67	11.17740	5.20973	2.14548	**0.03529**	2.74858	10.32287	0.26626	0.80729	1.66097	0.09672	2.10505	**0.03529**
3186216	63	12	1.67	8.26326	3.85407	2.14404	**0.03541**	-9.06866	10.26657	-0.88332	0.40004	0.89246	0.37215	2.10367	**0.03541**
1943669	69	6	1.33	0.78150	5.35306	0.14599	0.88434	16.33430	4.50046	3.62947	**0.03601**	1.58556	0.11284	2.09685	**0.03601**
180216	56	19	47.32	-4.91385	3.34063	-1.47094	0.14567	2.00308	0.87643	2.28551	**0.03626**	0.45181	0.65141	2.09397	**0.03626**
363321	67	8	5.63	-1.28274	4.69365	-0.27329	0.78541	7.75375	2.73122	2.83893	**0.03629**	1.28790	0.19778	2.09364	**0.03629**
306704	70	5	8.60	2.81252	5.83498	0.48201	0.63126	-6.91384	1.35528	-5.10141	**0.03634**	-1.14064	0.25402	-2.09306	**0.03634**
4458576	23	52	72.92	-0.85770	3.16027	-0.27140	0.78686	-1.88586	0.87762	-2.14882	**0.03662**	-1.66906	0.09510	-2.09002	**0.03662**
4256470	6	69	44.28	-9.04712	5.24848	-1.72376	0.08904	-0.84505	0.67028	-1.26073	0.21184	-2.08524	**0.03705**	-2.08524	**0.03705**
193654	59	16	4.25	-1.42912	3.56035	-0.40140	0.68932	-5.63856	2.43016	-2.32024	**0.03723**	-1.75574	0.07913	-2.08321	**0.03723**
4060501	46	29	13.69	-6.15238	2.90154	-2.12039	**0.03742**	0.56037	1.20861	0.46365	0.64676	-1.14755	0.25115	-2.08116	**0.03742**
358798	51	24	5.58	-0.17732	3.20076	-0.05540	0.95597	4.98157	2.24645	2.21753	**0.03775**	1.43001	0.15271	2.07755	**0.03775**
2438203	43	32	7.66	-3.35223	2.90786	-1.15282	0.25280	-2.90890	1.56535	-1.85831	0.07330	-2.07502	**0.03799**	-2.07502	**0.03799**
858999	70	5	2.20	11.14017	5.66006	1.96821	0.05290	12.59902	9.55762	1.31822	0.31816	2.07467	**0.03802**	2.07467	**0.03802**
3856408	60	15	2.47	-0.64267	3.66249	-0.17547	0.86120	-5.19396	2.22862	-2.33057	**0.03803**	-1.59054	0.11171	-2.07452	**0.03803**
356745	35	40	7.15	3.71726	2.88326	1.28926	0.20144	3.06995	1.80846	1.69755	0.09799	2.07335	**0.03814**	2.07335	**0.03814**
301253	40	35	35.94	5.95000	2.81920	2.11053	**0.03829**	-0.77370	1.47576	-0.52427	0.60370	1.09791	0.27224	2.07177	**0.03829**
176104	38	37	14.35	1.22775	2.92650	0.41953	0.67608	3.30555	1.53556	2.15267	**0.03853**	1.75858	0.07865	2.06918	**0.03853**
174885	68	7	2.86	-7.13107	4.91203	-1.45176	0.15091	-3.83259	2.06005	-1.86044	0.13633	-2.06893	**0.03855**	-2.06893	**0.03855**
4329572	69	6	3.00	-10.91623	5.18565	-2.10508	**0.03877**	1.09123	2.89389	0.37708	0.73121	-1.21839	0.22308	-2.06658	**0.03877**
1646171	59	16	7.25	-7.22280	3.43481	-2.10282	**0.03898**	-1.05312	1.47800	-0.71253	0.48873	-1.94932	0.05126	-2.06442	**0.03898**
2953981	67	8	3.50	-9.55658	4.55855	-2.09641	**0.03956**	-1.22582	2.44128	-0.50212	0.63691	-1.78922	0.07358	-2.05831	**0.03956**
4438999	61	14	3.93	-2.03534	3.71226	-0.54828	0.58520	-4.66480	1.99923	-2.33330	**0.03964**	-1.84082	0.06565	-2.05750	**0.03964**
180107	61	14	3.93	-2.03534	3.71226	-0.54828	0.58520	-4.66480	1.99923	-2.33330	**0.03964**	-1.84082	0.06565	-2.05750	**0.03964**
555547	59	16	3.56	7.18998	3.43505	2.09312	**0.03986**	-2.14329	4.48764	-0.47760	0.64087	1.12337	0.26128	2.05518	**0.03986**
332732	47	28	16.50	-3.84262	2.96741	-1.29494	0.19948	1.69724	0.78310	2.16733	**0.03994**	0.54543	0.58546	2.05439	**0.03994**
206513	51	24	5.46	4.89791	3.05656	1.60242	0.11344	3.86221	2.83274	1.36342	0.18719	2.05192	**0.04018**	2.05192	**0.04018**
365717	62	13	3.69	-7.81546	3.74338	-2.08781	**0.04035**	3.28889	3.13608	1.04873	0.31899	-0.74499	0.45628	-2.05011	**0.04035**
4428313	48	27	160.15	0.37137	3.01981	0.12298	0.90247	-1.35312	0.62484	-2.16555	**0.04051**	-1.36190	0.17323	-2.04856	**0.04051**
355187	69	6	3.50	10.77307	5.19229	2.07482	**0.04158**	-4.19572	16.95523	-0.24746	0.82052	1.28046	0.20038	2.03772	**0.04158**
3186388	60	15	1.80	-7.29929	3.52238	-2.07226	**0.04182**	-0.91401	3.36567	-0.27157	0.79057	-1.62695	0.10375	-2.03528	**0.04182**
4379646	67	8	1.75	-9.45478	4.56467	-2.07130	**0.04192**	7.40625	15.61294	0.47437	0.65523	-1.12278	0.26153	-2.03436	**0.04192**
186624	68	7	1.14	-0.68673	4.98347	-0.13780	0.89078	34.09677	11.55594	2.95059	**0.04194**	1.34121	0.17985	2.03407	**0.04194**
725212	65	10	5.30	8.61283	4.16050	2.07014	**0.04203**	0.45977	3.07565	0.14949	0.88538	1.53966	0.12364	2.03326	**0.04203**
175642	71	4	3.25	6.76047	6.42130	1.05282	0.29594	68.61899	7.28972	9.41312	0.06738	2.03245	**0.04211**	2.03245	**0.04211**
4336939	61	14	9.07	7.52354	3.63754	2.06830	**0.04220**	3.54790	4.51954	0.78501	0.44902	1.97180	**0.04863**	2.03150	**0.04220**
192347	61	14	1.57	6.76390	3.71209	1.82213	0.07259	9.54382	8.43351	1.13165	0.28185	2.03052	**0.04230**	2.03052	**0.04230**
174831	43	32	34.69	5.89301	2.85264	2.06581	**0.04245**	-1.12526	1.26725	-0.88796	0.38187	0.81647	0.41423	2.02912	**0.04245**
308498	63	12	12.08	6.06188	3.88825	1.55902	0.12338	4.91918	3.41830	1.43907	0.18399	2.02893	**0.04247**	2.02893	**0.04247**
4473509	24	51	20.41	6.23480	3.02107	2.06377	**0.04264**	0.13949	1.14814	0.12150	0.90381	1.51889	0.12879	2.02718	**0.04264**
1945397	66	9	9.00	8.94714	4.33622	2.06335	**0.04269**	0.77865	9.37038	0.08310	0.93648	1.48950	0.13636	2.02677	**0.04269**
4453501	27	48	37.50	-6.24747	3.02889	-2.06263	**0.04276**	0.53813	0.61427	0.87604	0.38566	-0.81923	0.41265	-2.02608	**0.04276**
571642	40	35	5.00	4.43445	2.96275	1.49674	0.13883	3.04217	2.16463	1.40540	0.16954	2.01797	**0.04359**	2.01797	**0.04359**
192244	58	17	5.65	2.98394	3.46416	0.86138	0.39189	6.29280	2.87626	2.18785	**0.04614**	2.01548	**0.04385**	2.01548	**0.04385**
4338990	54	21	11.48	3.12418	3.21045	0.97313	0.33375	-4.52447	2.08919	-2.16566	**0.04401**	-0.74062	0.45893	-2.01398	**0.04401**
4469167	64	11	1.64	8.20917	4.00959	2.04738	**0.04427**	-2.31965	11.48733	-0.20193	0.84501	1.28413	0.19910	2.01153	**0.04427**
1136387	67	8	15.25	-4.62636	4.67216	-0.99020	0.32539	-2.71062	1.13746	-2.38304	0.06293	-2.01040	**0.04439**	-2.01040	**0.04439**
4480176	69	6	7.67	7.87826	5.26231	1.49711	0.13874	10.32855	5.82403	1.77344	0.17426	2.00758	**0.04469**	2.00758	**0.04469**
216111	47	28	4.11	-6.06107	2.96640	-2.04324	**0.04469**	0.78375	1.77941	0.44046	0.66339	-1.11181	0.26622	-2.00757	**0.04469**
589329	67	8	1.63	4.77580	4.66156	1.02451	0.30903	8.16097	3.53207	2.31053	0.06886	2.00578	**0.04488**	2.00578	**0.04488**
352529	59	16	5.94	-4.12452	3.60804	-1.14315	0.25676	-3.43329	1.87119	-1.83482	0.08951	-2.00256	**0.04522**	-2.00256	**0.04522**

Table S4c. OTUs associated with High-density lipoprotein (HDL) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
327209	67	8	3.00	8.80193	4.59939	1.91372	0.05963	8.58688	8.19939	1.04726	0.34294	2.00244	**0.04524**	2.00244	**0.04524**
2058521	60	15	3.13	-4.81946	3.57931	-1.34648	0.18237	5.28382	2.36585	2.23337	**0.04534**	0.47238	0.63666	2.00153	**0.04534**
180523	65	10	1.20	8.44456	4.14709	2.03626	**0.04540**	0.07714	22.53905	0.00342	0.99736	1.41719	0.15643	2.00090	**0.04540**
4413347	71	4	2.00	2.15577	6.51441	0.33092	0.74166	25.95507	1.85530	13.98967	**0.04543**	1.64778	0.09940	2.00066	**0.04543**
3141094	37	38	3.21	-3.65062	2.86679	-1.27342	0.20697	-2.61438	1.62843	-1.60546	0.11738	-1.99958	**0.04555**	-1.99958	**0.04555**
4427290	43	32	17.88	-5.83634	2.86984	-2.03369	**0.04567**	-0.53660	0.86273	-0.62198	0.53881	-1.84770	0.06465	-1.99844	**0.04567**
576400	69	6	2.67	10.62105	5.22295	2.03354	**0.04568**	-10.45265	31.45136	-0.33234	0.76150	1.19839	0.23077	1.99829	**0.04568**
196597	70	5	2.40	11.53299	5.67213	2.03327	**0.04571**	-37.42343	51.70579	-0.72378	0.54441	0.98421	0.32501	1.99804	**0.04571**
553080	28	47	17.00	1.81997	3.00434	0.60578	0.54656	2.66477	1.29666	2.05510	**0.04584**	1.83835	0.06601	1.99691	**0.04584**
111771	68	7	2.29	1.41162	4.98264	0.28331	0.77775	6.60811	2.30920	2.86164	**0.04585**	1.61149	0.10707	1.99674	**0.04585**
287790	54	21	33.05	3.12276	3.21565	0.97111	0.33474	3.20585	1.61853	1.98071	0.06311	1.99615	**0.04592**	1.99615	**0.04592**
192983	44	31	4.39	4.66682	2.96916	1.57176	0.12039	3.14849	2.43449	1.29329	0.20648	1.99153	**0.04642**	1.99153	**0.04642**
308386	51	24	81.96	3.55190	3.10337	1.14453	0.25619	2.44311	1.38897	1.75894	0.09315	1.99011	**0.04658**	1.99011	**0.04658**
310380	70	5	4.00	11.69708	5.77964	2.02384	**0.04670**	51.85262	71.09571	0.72934	0.54165	1.83802	0.06606	1.98903	**0.04670**
215097	70	5	17.80	2.22721	5.87566	0.37906	0.70576	-4.73434	1.06205	-4.45772	**0.04682**	-1.13872	0.25482	-1.98794	**0.04682**
265871	7	68	161.65	9.81761	4.85603	2.02174	**0.04692**	-0.50177	0.70003	-0.71678	0.47608	0.90113	0.36752	1.98701	**0.04692**
183030	65	10	4.90	8.38265	4.15012	2.01986	**0.04712**	-1.63515	2.42654	-0.67386	0.52202	0.95105	0.34158	1.98522	**0.04712**
656881	54	21	8.67	-0.97245	3.29060	-0.29552	0.76844	2.38835	1.12297	2.12681	**0.04752**	1.19304	0.23285	1.98162	**0.04752**
1061772	62	13	2.46	-1.60995	3.82460	-0.42095	0.67505	8.09224	3.58647	2.25632	**0.04767**	1.10387	0.26965	1.98034	**0.04767**
3887769	55	20	1.95	-1.89495	3.30108	-0.57404	0.56773	-4.32207	2.02493	-2.13443	**0.04767**	-1.80435	0.07118	-1.98034	**0.04767**
197890	60	15	4.20	-7.12737	3.54066	-2.01301	**0.04785**	-1.24207	2.20561	-0.56314	0.58371	-1.78660	0.07400	-1.97866	**0.04785**
2831841	54	21	3.67	-6.32698	3.14688	-2.01056	**0.04812**	0.61574	1.12027	0.54963	0.58933	-1.01577	0.30974	-1.97632	**0.04812**
209845	63	12	5.67	-2.28564	4.02686	-0.56760	0.57207	-4.14822	1.82028	-2.27889	**0.04865**	-1.79367	0.07287	-1.97164	**0.04865**
302333	63	12	2.50	-1.09751	3.96222	-0.27699	0.78258	7.15892	3.14145	2.27886	**0.04865**	1.19901	0.23052	1.97162	**0.04865**
110192	60	15	20.60	7.08739	3.53528	2.00476	**0.04875**	-1.42531	2.67173	-0.53348	0.60344	1.02624	0.30478	1.97078	**0.04875**
180421	62	13	3.85	-7.57263	3.77831	-2.00423	**0.04881**	-0.67186	3.63115	-0.18503	0.85691	-1.52069	0.12834	-1.97027	**0.04881**

[a] Statistics associated to the two part model for association analysis between the trait and OTUs adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe represented by an OTU with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the OTU for the subjects where that OTU is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per OTU-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S4d. OTUs associated with Low-density lipoprotein (LDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
4342104	59	16	5.38	-0.88834	5.95581	-0.14916	0.88185	-10.98124	1.92748	-5.69721	**0.00007**	-2.90906	**0.00363**	-3.96541	**0.00007**
178064	41	34	11.62	0.80299	4.75263	0.16896	0.86630	-8.93219	2.02240	-4.41663	**0.00011**	-2.61038	**0.00904**	-3.85998	**0.00011**
740158	66	9	3.44	26.12882	6.60018	3.95880	**0.00017**	-2.83192	5.21451	-0.54308	0.60664	2.28951	**0.02205**	3.75273	**0.00017**
187946	70	5	1.40	21.37459	9.20398	2.32232	**0.02305**	14.78806	0.79246	18.66102	**0.00286**	3.71594	**0.00020**	3.71594	**0.00020**
324015	70	5	2.40	-33.43605	8.70734	-3.83999	**0.00026**	-32.34373	25.44928	-1.27091	0.33158	-3.26755	**0.00108**	-3.65008	**0.00026**
187517	61	14	14.14	21.06695	5.54269	3.80086	**0.00030**	3.09742	4.55291	0.68031	0.51037	3.02243	**0.00251**	3.61610	**0.00030**
311947	52	23	38.52	17.28304	4.71044	3.66909	**0.00046**	-1.26185	2.79722	-0.45111	0.65676	2.16137	**0.03067**	3.50103	**0.00046**
194374	70	5	2.00	-21.57047	9.17216	-2.35173	**0.02143**	-39.12257	4.58833	-8.52654	**0.01348**	-3.37382	**0.00074**	-3.37382	**0.00074**
851733	59	16	70.75	13.90437	5.54136	2.50920	**0.01435**	3.86261	1.49837	2.57787	**0.02295**	3.33940	**0.00084**	3.33940	**0.00084**
4484075	38	37	4.03	15.18892	4.39600	3.45517	**0.00093**	-4.38594	2.97841	-1.47258	0.15006	1.32430	0.18540	3.31216	**0.00093**
315982	71	4	2.25	33.30952	9.80708	3.39648	**0.00111**	-13.56585	11.28947	-1.20164	0.44186	1.76129	0.07819	3.25990	**0.00111**
4416614	30	45	21.09	5.63321	4.78478	1.17732	0.24295	-6.17669	1.77187	-3.48597	**0.00116**	-1.47104	0.14128	-3.24802	**0.00116**
4388068	69	6	18.83	24.17466	8.25996	2.92673	**0.00458**	6.07410	2.35206	2.58246	0.08161	3.23611	**0.00121**	3.23611	**0.00121**
3946926	69	6	3.17	27.29196	8.19991	3.32832	**0.00138**	10.96050	10.29749	1.06439	0.36523	2.90228	**0.00370**	3.19899	**0.00138**
329798	65	10	5.80	21.11079	6.50161	3.24701	**0.00177**	-0.38698	4.84459	-0.07988	0.93857	2.15591	**0.03109**	3.12599	**0.00177**
275237	42	33	229.64	10.86722	4.59273	2.36618	**0.02067**	2.38419	1.12840	2.11289	**0.04304**	3.06696	**0.00216**	3.06696	**0.00216**
4398588	63	12	4.00	19.20976	6.06397	3.16785	**0.00225**	-3.45409	5.47678	-0.63068	0.54393	1.73079	0.08349	3.05459	**0.00225**
522582	61	14	27.86	17.89411	5.69572	3.14168	**0.00244**	-0.76225	3.16630	-0.24074	0.81419	1.97698	**0.04804**	3.03091	**0.00244**
303320	70	5	2.40	26.02100	9.16116	2.84036	**0.00586**	7.45560	3.14542	2.37031	0.14124	2.98893	**0.00280**	2.98893	**0.00280**
190453	60	15	17.07	11.63798	5.88280	1.97831	0.05172	5.53893	2.13016	2.60024	**0.02322**	2.98068	**0.00288**	2.98068	**0.00288**
196957	22	53	9.45	-9.00398	5.09603	-1.76686	0.08149	-4.76128	1.87110	-2.54464	**0.01407**	-2.96808	**0.00300**	-2.96808	**0.00300**
188262	71	4	1.00	30.99613	10.29887	3.00966	**0.00360**	0.00000	0.00000	0.00000	1.00000	2.05834	**0.03956**	2.91093	**0.00360**
290284	69	6	3.50	13.77370	8.60691	1.60031	0.11391	38.07767	7.02055	5.42374	**0.01230**	2.88807	**0.00388**	2.88807	**0.00388**
3907189	67	8	1.88	21.43065	7.26824	2.94853	**0.00430**	-16.40488	10.31099	-1.59101	0.17248	1.05416	0.29181	2.85508	**0.00430**
180421	62	13	3.85	-17.56717	5.99120	-2.93216	**0.00451**	1.18999	6.79918	0.17502	0.86456	-1.88763	0.05908	-2.84009	**0.00451**
3325758	28	47	17.45	4.14986	4.86838	0.85241	0.39681	-4.58239	1.53114	-2.99279	**0.00452**	-1.40858	0.15896	-2.83935	**0.00452**
157424	48	27	265.70	-7.77339	4.86883	-1.59656	0.11474	3.18072	1.01550	3.13216	**0.00452**	0.89228	0.37224	2.83911	**0.00452**
182647	63	12	29.67	-17.84602	6.11716	-2.91737	**0.00471**	0.03623	2.51661	0.01439	0.98883	-1.98876	**0.04673**	-2.82653	**0.00471**
166689	69	6	4.00	23.98735	8.27182	2.89989	**0.00495**	-5.87308	11.00322	-0.53376	0.63052	1.64721	0.09951	2.81050	**0.00495**
4309301	13	62	21.26	17.11552	5.92146	2.89042	**0.00508**	-2.11228	1.39001	-1.51961	0.13395	0.92143	0.35682	2.80181	**0.00508**
4483570	71	4	5.25	-28.91487	10.00572	-2.88984	**0.00509**	-4.38204	13.78066	-0.31798	0.80400	-2.15628	**0.03106**	-2.80127	**0.00509**
198754	70	5	2.80	-25.07785	9.01543	-2.78166	**0.00690**	-15.66293	8.53552	-1.83503	0.20793	-2.80081	**0.00510**	-2.80081	**0.00510**
192462	20	55	44.05	14.55947	5.07011	2.87163	**0.00536**	-0.22045	1.35137	-0.16313	0.87105	1.85419	0.06371	2.78455	**0.00536**
232900	67	8	5.38	-20.84384	7.26226	-2.87016	**0.00538**	5.22908	2.95984	1.76668	0.13753	-0.91794	0.35865	-2.78320	**0.00538**
184248	62	13	4.38	-3.51732	6.24363	-0.56334	0.57495	10.89764	3.08131	3.53670	**0.00539**	1.57133	0.11611	2.78297	**0.00539**
41229	70	5	19.20	-16.47291	9.29383	-1.77246	0.08055	-6.43039	1.11462	-5.76914	**0.02876**	-2.78199	**0.00540**	-2.78199	**0.00540**
4288931	60	15	21.40	16.26011	5.67422	2.86561	**0.00545**	-0.67602	3.49311	-0.19353	0.84978	1.83114	0.06708	2.77902	**0.00545**
3141342	60	15	4.33	2.64516	5.98503	0.44196	0.65984	-16.05184	4.77594	-3.36098	**0.00566**	-1.64508	0.09995	-2.76663	**0.00566**
1599042	63	12	21.33	17.40825	6.13678	2.83671	**0.00592**	4.26915	4.76930	0.89513	0.39403	2.54895	**0.01080**	2.75243	**0.00592**
4296763	70	5	102.80	-5.82722	9.48350	-0.61446	0.54085	-3.40194	0.26343	-12.91382	**0.00594**	-2.37760	**0.01743**	-2.75091	**0.00594**
1679707	62	13	3.23	-16.79015	5.94245	-2.82546	**0.00611**	7.62029	4.87507	1.56311	0.14909	-0.91875	0.35822	-2.74207	**0.00611**
186913	70	5	2.00	26.30931	9.31248	2.82517	**0.00611**	1.66314	15.78070	0.10539	0.92568	2.00470	**0.04500**	2.74180	**0.00611**
730906	53	22	2.91	-14.18083	5.04214	-2.81246	**0.00633**	-4.25170	4.17520	-1.01832	0.32132	-2.63174	**0.00849**	-2.73009	**0.00633**
750071	55	20	247.60	11.58759	5.19352	2.23116	**0.02879**	3.06767	1.74494	1.75804	0.09673	2.72047	**0.00652**	2.72047	**0.00652**
302333	63	12	2.50	-2.15856	6.46778	-0.33374	0.73955	-17.75142	5.07464	-3.49807	**0.00674**	-2.15079	**0.03149**	-2.70923	**0.00674**
180552	60	15	4.27	-8.88813	5.83295	-1.52378	0.13194	-12.93252	4.87103	-2.65499	**0.02098**	-2.69742	**0.00699**	-2.69742	**0.00699**
2331530	71	4	1.50	9.36121	10.48980	0.89241	0.37515	-37.54324	0.41251	-91.01093	**0.00699**	-1.28002	0.20054	-2.69710	**0.00699**
825808	43	32	39.38	-12.63515	4.55329	-2.77495	**0.00703**	-1.24808	1.63706	-0.76239	0.45198	-2.43781	**0.01478**	-2.69547	**0.00703**
191792	19	56	14.52	10.20674	5.30641	1.92347	0.05837	3.19653	1.63250	1.95806	0.05550	2.69255	**0.00709**	2.69255	**0.00709**
161007	68	7	1.43	-21.40135	7.75189	-2.76079	**0.00731**	-15.68725	15.65006	-1.00238	0.37288	-2.52683	**0.01151**	-2.68239	**0.00731**
4425368	68	7	19.71	-10.65979	8.06752	-1.32132	0.19058	-6.59063	1.54941	-4.25363	**0.01312**	-2.67949	**0.00737**	-2.67949	**0.00737**
564320	66	9	5.78	13.24153	7.11481	1.86112	0.06681	18.85883	7.77264	2.42631	0.05142	2.67351	**0.00751**	2.67351	**0.00751**
315223	62	13	6.62	-1.40114	6.25449	-0.22402	0.82338	-10.39660	3.11633	-3.33617	**0.00754**	-2.04725	**0.04063**	-2.67204	**0.00754**
347243	71	4	2.25	27.77277	10.15788	2.73411	**0.00787**	-12.63501	20.75306	-0.60883	0.65184	1.56023	0.11870	2.65771	**0.00787**
184174	31	44	9.09	-9.62928	4.68442	-2.05560	**0.04345**	-3.47876	1.97568	-1.76079	0.08573	-2.64297	**0.00822**	-2.64297	**0.00822**
4383922	69	6	2.00	-22.64861	8.34203	-2.71500	**0.00829**	-7.50364	9.39308	-0.79885	0.48277	-2.36305	**0.01813**	-2.64001	**0.00829**
4454531	48	27	12.30	-12.68433	4.72714	-2.68330	**0.00904**	1.69457	2.74605	0.61709	0.54299	-1.41585	0.15682	-2.61061	**0.00904**
289734	19	56	17.04	-7.82875	5.36297	-1.45978	0.14870	-3.73840	1.62514	-2.30035	**0.02539**	-2.60180	**0.00927**	-2.60180	**0.00927**
192912	68	7	4.00	-18.95798	7.82235	-2.42356	**0.01788**	-8.24358	5.24131	-1.57281	0.19087	-2.59935	**0.00934**	-2.59935	**0.00934**
194320	44	31	3.90	12.14700	4.58861	2.64721	**0.00996**	-1.18671	3.41837	-0.34716	0.73107	1.57924	0.11428	2.57709	**0.00996**
185861	66	9	2.33	18.45330	6.97165	2.64691	**0.00997**	13.86493	18.01223	0.76975	0.47066	2.33219	**0.01969**	2.57681	**0.00997**
197540	69	6	4.33	19.67821	8.46956	2.32340	**0.02299**	21.18513	11.80659	1.79435	0.17064	2.57657	**0.00998**	2.57657	**0.00998**
177758	64	11	4.82	-8.96680	6.60513	-1.35755	0.17885	-7.31402	2.56764	-2.84854	**0.02153**	-2.57593	**0.01000**	-2.57593	**0.01000**
114400	71	4	3.25	26.66706	10.08524	2.64417	**0.01005**	-16.62069	7.57721	-2.19351	0.27231	1.04404	0.29646	2.57426	**0.01005**
4459708	71	4	2.00	-20.99983	10.42537	-2.01430	**0.04771**	28.81613	0.46022	62.61387	**0.01017**	0.41734	0.67643	2.57011	**0.01017**
320786	67	8	3.38	-16.15355	7.43030	-2.17401	**0.03299**	-5.80914	3.25490	-1.78474	0.13437	-2.56627	**0.01028**	-2.56627	**0.01028**
194670	26	49	7.55	-4.07028	5.01197	-0.81211	0.41940	-5.08079	1.89914	-2.67532	**0.01031**	-2.38496	**0.01708**	-2.56539	**0.01031**
4333897	63	12	2.67	-16.20971	6.16842	-2.62786	**0.01049**	4.87649	6.01408	0.81084	0.43837	-1.26158	0.20710	-2.55909	**0.01049**
389371	60	15	4.07	-14.91224	5.67564	-2.62741	**0.01051**	9.81908	3.50484	2.80158	**0.01600**	-0.10585	0.91570	-2.55868	**0.01051**
187883	64	11	2.45	-13.47873	6.56141	-2.05424	**0.04358**	-10.57509	5.91339	-1.78833	0.11152	-2.55227	**0.01070**	-2.55227	**0.01070**
997267	70	5	3.00	24.18485	9.27333	2.60800	**0.01107**	0.13760	5.63260	0.02443	0.98273	1.81179	0.07002	2.54060	**0.01107**
293883	58	17	13.82	14.08985	5.40557	2.60655	**0.01111**	-2.47945	3.26659	-0.75903	0.46042	1.27357	0.20282	2.53925	**0.01111**
158649	71	4	2.25	-26.30537	10.11268	-2.60123	**0.01127**	11.03237	0.80464	13.71088	**0.04635**	-0.38332	0.70148	-2.53429	**0.01127**
4294457	64	11	4.64	13.49883	6.64306	2.03202	**0.04584**	8.82598	5.00543	1.76328	0.11587	2.52381	**0.01161**	2.52381	**0.01161**
197698	55	20	8.15	13.26671	5.13087	2.58566	**0.01174**	-5.06352	2.72049	-1.86125	0.08009	0.54421	0.58630	2.51978	**0.01174**
3014082	67	8	3.88	-19.31087	7.49072	-2.57797	**0.01198**	-0.92865	5.57384	-0.16661	0.87421	-1.88863	0.05894	-2.51261	**0.01198**
849535	65	10	315.80	17.17246	6.66786	2.57541	**0.01207**	-1.42239	2.63755	-0.53929	0.60641	1.41069	0.15834	2.51022	**0.01207**

Table S4d. OTUs associated with Low-density lipoprotein (LDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Presen	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
177222	43	32	5.00	-11.78192	4.58412	-2.57016	**0.01223**	0.31382	2.61503	0.12001	0.90531	-1.68741	0.09152	-2.50532	**0.01223**
175922	69	6	2.67	21.46556	8.36072	2.56743	**0.01232**	8.09196	6.61350	1.22355	0.30846	2.48989	**0.01278**	2.50277	**0.01232**
511378	69	6	4.67	-21.44740	8.36077	-2.56524	**0.01239**	3.27548	7.31088	0.44803	0.68451	-1.48098	0.13861	-2.50073	**0.01239**
4307484	39	36	3.86	3.00200	4.72340	0.63556	0.52708	-8.35818	3.17152	-2.63539	**0.01270**	-1.31485	0.18856	-2.49195	**0.01270**
186687	60	15	2.60	-14.51348	5.68532	-2.55280	**0.01281**	-1.54854	5.88471	-0.26315	0.79690	-1.94205	0.05213	-2.48911	**0.01281**
178478	34	41	4.98	1.27133	4.80131	0.26479	0.79193	-5.56927	2.15322	-2.58649	**0.01365**	-1.55741	0.11937	-2.46631	**0.01365**
760544	63	12	4.67	-8.76776	6.53087	-1.34251	0.18365	-9.28375	3.63697	-2.55261	**0.03106**	-2.46489	**0.01371**	-2.46489	**0.01371**
360636	32	43	29.30	1.27715	4.79847	0.26616	0.79088	-3.37064	1.31305	-2.56703	**0.01410**	-1.54817	0.12158	-2.45462	**0.01410**
197499	33	42	8.17	11.60486	4.61760	2.51318	**0.01420**	-0.34621	2.85142	-0.12142	0.90399	1.64858	0.09923	2.45207	**0.01420**
193968	17	58	13.78	7.28338	5.58655	1.30374	0.19648	-3.71631	1.47181	-2.52500	**0.01448**	-0.81558	0.41474	-2.44505	**0.01448**
300235	60	15	1.60	-14.21473	5.67404	-2.50522	**0.01450**	4.46741	7.88620	0.56648	0.58151	-1.33886	0.18062	-2.44462	**0.01450**
656881	54	21	8.67	-12.87293	5.15750	-2.49596	**0.01485**	0.11916	2.16502	0.05504	0.95671	-1.68410	0.09216	-2.43595	**0.01485**
198909	49	26	7.35	0.79076	5.06270	0.15619	0.87632	-7.34490	2.79113	-2.63151	**0.01492**	-1.61132	0.10711	-2.43439	**0.01492**
294791	69	6	2.67	1.62642	8.73296	0.18624	0.85278	-21.00166	4.16585	-5.04139	**0.01505**	-1.58791	0.11231	-2.43122	**0.01505**
4435309	70	5	7.80	22.83528	9.17929	2.48770	**0.01517**	4.16809	8.92702	0.46691	0.68649	2.00240	**0.04524**	2.42821	**0.01517**
782953	15	60	127.50	-9.95535	5.82218	-1.70990	0.09159	-1.73077	0.97519	-1.77480	0.08127	-2.42568	**0.01528**	-2.42568	**0.01528**
3826120	71	4	1.75	-18.05304	10.32667	-1.74820	0.08469	-8.13602	1.14236	-7.12211	0.08881	-2.42242	**0.01542**	-2.42242	**0.01542**
291644	63	12	3.08	-5.17380	6.47949	-0.79849	0.42721	19.54576	6.56809	2.97587	**0.01555**	1.14923	0.25046	2.41923	**0.01555**
19611	55	20	3.95	12.76410	5.15883	2.47422	**0.01571**	1.20255	4.00213	0.30048	0.76746	1.91717	0.05522	2.41558	**0.01571**
4418787	65	10	7.20	-13.66774	6.78117	-2.01554	**0.04758**	-6.32340	3.92928	-1.60930	0.15159	-2.41480	**0.01574**	-2.41480	**0.01574**
332588	58	17	2.88	-13.42256	5.42941	-2.47220	**0.01579**	10.09564	5.91530	1.70670	0.10995	-0.57648	0.56429	-2.41368	**0.01579**
2046330	67	8	5.88	18.17297	7.36124	2.46874	**0.01593**	-2.27283	12.74650	-0.17831	0.86548	1.58465	0.11305	2.41044	**0.01593**
177062	65	10	3.70	6.87323	6.91951	0.99331	0.32388	-19.62657	6.21263	-3.15914	**0.01595**	-1.00664	0.31411	-2.41012	**0.01595**
1105984	40	35	97.49	-9.09981	4.65098	-1.95653	0.05428	-1.55809	1.03099	-1.51126	0.14053	-2.40302	**0.01626**	-2.40302	**0.01626**
4429981	61	14	38.07	14.32305	5.83648	2.45406	**0.01655**	-6.29013	3.48664	-1.80407	0.09864	0.52694	0.59824	2.39666	**0.01655**
4469032	70	5	5.80	18.38960	9.25001	1.98806	0.05061	13.27955	5.85506	2.26805	0.15144	2.39656	**0.01655**	2.39656	**0.01655**
537219	63	12	1.92	10.49846	6.33782	1.65648	0.10198	16.85988	8.54719	1.97256	0.08001	2.39424	**0.01665**	2.39424	**0.01665**
208739	32	43	3.77	1.64480	4.81147	0.34185	0.73346	-5.40268	2.17100	-2.48856	**0.01709**	-1.44552	0.14831	-2.38480	**0.01709**
189235	68	7	8.29	19.11760	7.84773	2.43607	**0.01732**	-6.71625	7.03295	-0.95497	0.39367	1.07961	0.28032	2.37977	**0.01732**
184342	68	7	1.86	-19.09065	7.86798	-2.42637	**0.01776**	5.55774	9.70181	0.57286	0.59741	-1.30287	0.19262	-2.37067	**0.01776**
4311621	67	8	2.50	17.91572	7.40311	2.42003	**0.01804**	0.96770	9.36737	0.10331	0.92174	1.74157	0.08158	2.36470	**0.01804**
1110312	66	9	8.44	0.42777	7.31895	0.05845	0.95355	-12.45536	3.86404	-3.22340	**0.01806**	-1.63069	0.10296	-2.36439	**0.01806**
171559	61	14	1.57	-14.12991	5.85675	-2.41258	**0.01839**	10.99410	8.69877	1.26387	0.23240	-0.82273	0.41066	-2.35770	**0.01839**
180341	70	5	1.60	-21.99111	9.15306	-2.40260	**0.01886**	-3.00282	8.34702	-0.35975	0.75347	-1.88258	0.05976	-2.34831	**0.01886**
186732	18	57	11.37	1.10321	5.56063	0.19840	0.84329	-4.23834	1.75178	-2.41945	**0.01894**	-1.51957	0.12862	-2.34668	**0.01894**
230479	70	5	4.00	17.46260	9.27159	1.88345	0.06368	12.67720	5.39774	2.34861	0.14332	2.34616	**0.01897**	2.34616	**0.01897**
180121	71	4	3.25	24.43042	10.20386	2.39423	**0.01926**	-7.59061	44.31301	-0.17130	0.89200	1.55893	0.11901	2.34044	**0.01926**
3195500	46	29	5.17	-11.18393	4.67708	-2.39122	**0.01941**	5.33190	3.06658	1.73871	0.09392	-0.46847	0.63945	-2.33761	**0.01941**
179744	56	19	16.32	12.51322	5.23732	2.38924	**0.01950**	0.14947	2.90370	0.05148	0.95958	1.68745	0.09152	2.33574	**0.01950**
4419459	55	20	16.10	12.29083	5.16602	2.37917	**0.02000**	1.05787	2.88320	0.36691	0.71821	1.90007	0.05742	2.32626	**0.02000**
337161	62	13	9.46	9.33447	6.15498	1.51657	0.13375	8.03199	4.01682	1.99959	0.07344	2.32607	**0.02001**	2.32607	**0.02001**
187388	62	13	2.62	-1.22387	6.25571	-0.19564	0.84544	9.18356	3.32662	2.76062	**0.02011**	1.50570	0.13214	2.32432	**0.02011**
1602805	48	27	5.70	-10.27774	4.78211	-2.14920	**0.03498**	-4.21752	3.50291	-1.20400	0.24033	-2.32126	**0.02027**	-2.32126	**0.02027**
196061	68	7	5.57	-19.34828	8.16781	-2.36885	**0.02053**	-3.14524	7.53506	-0.41741	0.69781	-1.91260	0.05580	-2.31654	**0.02053**
193053	18	57	11.40	11.32337	5.37773	2.10560	**0.03873**	2.26632	1.86034	1.21823	0.22843	2.31329	**0.02071**	2.31329	**0.02071**
2280825	68	7	3.14	18.63227	7.88003	2.36449	**0.02075**	15.36177	15.38578	0.99844	0.37457	2.26301	**0.02364**	2.31243	**0.02075**
301578	61	14	14.29	-12.39692	6.07513	-2.04060	**0.04496**	-3.63350	2.73609	-1.32799	0.21108	-2.30209	**0.02133**	-2.30209	**0.02133**
2423305	57	18	7.61	12.64728	5.37668	2.35225	**0.02140**	2.61544	2.65562	0.98487	0.34031	2.30124	**0.02138**	2.30124	**0.02138**
158855	67	8	11.63	4.08597	7.65094	0.53405	0.59495	8.79437	2.66272	3.30277	**0.02141**	2.00277	**0.04520**	2.30068	**0.02141**
4377149	49	26	11.12	11.23167	4.79301	2.34334	**0.02188**	-1.45942	2.29166	-0.63684	0.53052	1.17753	0.23898	2.29249	**0.02188**
174831	43	32	34.69	10.82683	4.62079	2.34307	**0.02189**	-0.32078	1.78948	-0.17926	0.85898	1.49522	0.13486	2.29224	**0.02189**
187126	57	18	3.22	-12.51394	5.34099	-2.34300	**0.02190**	4.39269	3.42318	1.28322	0.21889	-0.75143	0.45240	-2.29217	**0.02190**
198190	69	6	1.50	-11.92948	8.60899	-1.38570	0.17012	38.48179	8.76711	4.38933	**0.02190**	0.65067	0.51526	2.29201	**0.02190**
190991	63	12	1.92	-14.59073	6.23319	-2.34081	**0.02202**	5.66536	6.46491	0.87632	0.40364	-1.02881	0.30357	-2.29011	**0.02202**
4254313	71	4	1031.50	23.95371	10.24013	2.33920	**0.02210**	0.33957	1.04991	0.32343	0.80086	1.79663	0.07239	2.28858	**0.02210**
4354103	66	9	34.22	16.39973	7.02284	2.33520	**0.02232**	-0.38447	4.18372	-0.09190	0.92977	1.55329	0.12035	2.28481	**0.02232**
183579	57	18	3.67	-12.58890	5.39147	-2.33497	**0.02234**	-0.66967	3.11961	-0.21467	0.83292	-1.76462	0.07763	-2.28459	**0.02234**
197760	70	5	2.00	2.20635	9.55392	0.23094	0.81802	-48.64536	7.50353	-6.48299	**0.02298**	-1.44514	0.14842	-2.27383	**0.02298**
688701	64	11	3.27	-3.34517	6.67787	-0.50093	0.61795	16.57325	5.91239	2.80314	**0.02309**	1.25387	0.20989	2.27201	**0.02309**
544996	65	10	5.00	5.82627	7.04130	0.82744	0.41072	-6.94180	2.39636	-2.89681	**0.02309**	-1.02481	0.30545	-2.27193	**0.02309**
1508541	40	35	4.03	-10.61321	4.57547	-2.31959	**0.02320**	-0.42923	3.12458	-0.13737	0.89160	-1.70155	0.08884	-2.27008	**0.02320**
4366834	61	14	113.14	9.63545	5.99526	1.60718	0.11239	4.13712	2.34583	1.76361	0.10552	2.26713	**0.02338**	2.26713	**0.02338**
199430	38	37	3.59	-10.60373	4.58387	-2.31327	**0.02357**	0.10751	2.40041	0.04479	0.96454	-1.56953	0.11652	-2.26411	**0.02357**
113827	68	7	2.71	18.46711	7.99844	2.30884	**0.02383**	-17.26034	9.68532	-1.78211	0.14932	0.57839	0.56300	2.25992	**0.02383**
307113	63	12	1.67	-14.38819	6.23492	-2.30768	**0.02389**	-3.72089	6.65580	-0.55904	0.58977	-1.97848	**0.04787**	-2.25883	**0.02389**
4045882	63	12	1.67	-14.38819	6.23492	-2.30768	**0.02389**	-3.72089	6.65580	-0.55904	0.58977	-1.97848	**0.04787**	-2.25883	**0.02389**
311718	71	4	3.25	23.49230	10.20105	2.30293	**0.02417**	-17.34392	3.53177	-4.91083	0.12789	0.51750	0.60481	2.25434	**0.02417**
192046	52	23	4.78	-1.68772	5.12868	-0.32908	0.74305	-9.01784	3.71423	-2.42791	**0.02475**	-1.81948	0.06884	-2.24532	**0.02475**
562410	69	6	1.50	-11.49070	8.65233	-1.32805	0.18836	-18.92804	6.57153	-2.88031	0.06351	-2.24226	**0.02494**	-2.24226	**0.02494**
3887769	55	20	1.95	-11.94135	5.21566	-2.28952	**0.02498**	9.33788	5.86436	1.59231	0.12974	-0.51373	0.60744	-2.24166	**0.02498**
186122	70	5	3.40	-11.73568	9.40720	-1.24752	0.21625	11.03317	1.78173	6.19238	**0.02510**	0.70943	0.47806	2.23985	**0.02510**
3709990	63	12	3.08	-7.09836	6.54274	-1.08492	0.28158	-12.41207	5.06068	-2.45265	**0.03660**	-2.23942	**0.02513**	-2.23942	**0.02513**
298451	70	5	26.80	18.49607	9.23481	2.00286	**0.04896**	18.12237	10.68748	1.69566	0.23204	2.23735	**0.02526**	2.23735	**0.02526**
72820	26	49	33.04	-7.75038	4.98050	-1.55615	0.12406	-2.18259	1.31743	-1.65670	0.10439	-2.23581	**0.02536**	-2.23581	**0.02536**
176077	66	9	2.67	16.04993	7.03326	2.28200	**0.02545**	-1.78713	9.95912	-0.17945	0.86349	1.45850	0.14470	2.23456	**0.02545**
268730	62	13	120.54	9.29059	6.15902	1.50845	0.13581	3.94175	2.14170	1.84048	0.09552	2.23340	**0.02552**	2.23340	**0.02552**
301253	40	35	35.94	10.44357	4.58086	2.27983	**0.02558**	0.10031	1.90424	0.05268	0.95832	1.61557	0.10619	2.23250	**0.02558**

Table S4d. OTUs associated with Low-density lipoprotein (LDL) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Presen	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
232222	70	5	6.60	20.89464	9.19952	2.27127	**0.02612**	7.18856	5.99562	1.19897	0.35333	2.22920	**0.02580**	2.22920	**0.02580**
1566691	69	6	2.33	-19.33250	8.50209	-2.27385	**0.02596**	-3.41588	12.62621	-0.27054	0.80429	-1.74984	0.08015	-2.22684	**0.02596**
663500	55	20	69.10	11.36734	5.18094	2.19407	**0.03146**	2.02326	1.97203	1.02598	0.31928	2.22538	**0.02606**	2.22538	**0.02606**
515299	70	5	14.60	-1.68632	9.48650	-0.17776	0.85941	-14.28656	2.35783	-6.05921	**0.02617**	-1.69759	0.08958	-2.22364	**0.02617**
182289	34	41	10.37	-8.72602	4.64148	-1.88001	0.06415	-2.34816	1.79101	-1.31109	0.19770	-2.21979	**0.02643**	-2.21979	**0.02643**
3465233	56	19	7.47	-0.38312	5.45073	-0.07029	0.94416	-8.06888	3.30135	-2.44411	**0.02648**	-1.61864	0.10552	-2.21906	**0.02648**
2341726	69	6	2.00	-8.85261	8.76251	-1.01028	0.31574	-9.69267	2.60847	-3.71584	**0.03390**	-2.20932	**0.02715**	-2.20932	**0.02715**
288651	26	49	32.88	0.12993	4.98313	0.02607	0.97927	-2.87694	1.26169	-2.28023	**0.02728**	-1.54259	0.12293	-2.20754	**0.02728**
4338990	54	21	11.48	8.27520	5.18534	1.59589	0.11489	3.85943	2.39106	1.61411	0.12390	2.20277	**0.02761**	2.20277	**0.02761**
197426	70	5	4.80	-14.65043	9.38497	-1.56105	0.12290	-13.52442	5.08152	-2.66149	0.11692	-2.19949	**0.02784**	-2.19949	**0.02784**
181918	69	6	2.50	0.59898	8.73785	0.06855	0.94554	20.29125	5.07391	3.99914	**0.02802**	1.60178	0.10920	2.19695	**0.02802**
4358921	59	16	1.69	-12.52384	5.59247	-2.23941	**0.02822**	-5.51719	6.44680	-0.85580	0.40760	-2.13712	**0.03259**	-2.19423	**0.02822**
180825	71	4	2.25	-20.13912	10.26693	-1.96155	0.05368	-22.44619	8.93889	-2.51107	0.24127	-2.19289	**0.02832**	-2.19289	**0.02832**
4020046	64	11	7.64	-14.54489	6.50600	-2.23561	**0.02848**	7.58041	4.36869	1.73517	0.12093	-0.45237	0.65100	-2.19063	**0.02848**
509709	68	7	1.71	8.88624	8.08137	1.09960	0.27517	-39.01595	11.63859	-3.35229	**0.02851**	-0.77710	0.43710	-2.19022	**0.02851**
229088	71	4	7.25	19.52727	10.27896	1.89973	0.06147	12.75541	4.63564	2.75160	0.22192	2.18604	**0.02881**	2.18604	**0.02881**
4425571	30	45	21.82	-9.56951	4.74944	-2.01487	**0.04765**	-1.66457	1.48188	-1.12328	0.26770	-2.18413	**0.02895**	-2.18413	**0.02895**
310247	70	5	2.00	-20.61426	9.26096	-2.22593	**0.02915**	35.96538	37.50569	0.95893	0.43878	-0.99505	0.31971	-2.18146	**0.02915**
4472130	40	35	5.14	10.21205	4.59527	2.22230	**0.02941**	1.62555	3.72024	0.43695	0.66508	1.84620	0.06486	2.17801	**0.02941**
518820	70	5	10.40	20.63366	9.31245	2.21571	**0.02987**	-24.44225	125.55012	-0.19468	0.86363	1.41421	0.15730	2.17176	**0.02987**
312311	68	7	2.57	-7.30610	8.10506	-0.90142	0.37037	-34.84946	10.57684	-3.29488	**0.03008**	-2.16718	**0.03022**	-2.16907	**0.03008**
185864	56	19	4.95	1.73437	5.48959	0.31594	0.75296	-8.71835	3.69074	-2.36223	**0.03117**	-1.30117	0.19320	-2.15487	**0.03117**
4379449	68	7	31.43	-0.87960	8.20209	-0.10724	0.91490	-5.75344	1.76883	-3.25267	**0.03130**	-1.59816	0.11001	-2.15328	**0.03130**
581201	48	27	4.33	10.64822	4.85181	2.19469	**0.03141**	3.34470	4.66840	0.71646	0.48062	2.02028	**0.04335**	2.15181	**0.03141**
4483045	67	8	1.50	-15.04771	7.49127	-2.00870	**0.04832**	-19.46028	16.31954	-1.19245	0.28658	-2.14974	**0.03158**	-2.14974	**0.03158**
199293	8	67	15.46	-11.36520	7.57708	-1.49995	0.13800	-2.18053	1.38343	-1.57618	0.11992	-2.14848	**0.03168**	-2.14848	**0.03168**
4433737	62	13	7.54	-2.12816	6.28482	-0.33862	0.73588	8.33726	3.34656	2.49130	**0.03192**	1.27853	0.20106	2.14542	**0.03192**
195436	37	38	4.76	-10.07244	4.61203	-2.18395	**0.03222**	3.99175	2.31523	1.72413	0.09351	-0.32841	0.74260	-2.14162	**0.03222**
591439	69	6	2.67	15.08804	8.59941	1.75454	0.08359	21.18671	12.71306	1.66653	0.19420	2.14145	**0.03224**	2.14145	**0.03224**
184238	31	44	10.70	10.21115	4.69852	2.17327	**0.03305**	-2.18242	2.04100	-1.06929	0.29120	0.76082	0.44676	2.13147	**0.03305**
168439	44	31	33.58	-0.15940	5.00842	-0.03183	0.97470	-3.48171	1.55291	-2.24205	**0.03306**	-1.52950	0.12614	-2.13132	**0.03306**
4438999	61	14	3.93	4.97028	6.04554	0.82214	0.41371	-9.33750	3.83606	-2.43414	**0.03317**	-0.92822	0.35330	-2.13008	**0.03317**
180107	61	14	3.93	4.97028	6.04554	0.82214	0.41371	-9.33750	3.83606	-2.43414	**0.03317**	-0.92822	0.35330	-2.13008	**0.03317**
191148	71	4	2.50	22.15507	10.20323	2.17138	**0.03320**	4.27399	27.50356	0.15540	0.90186	1.59310	0.11114	2.12967	**0.03320**
367813	65	10	18.50	11.84985	6.82684	1.73577	0.08688	7.25809	5.05213	1.43664	0.19397	2.12910	**0.03325**	2.12910	**0.03325**
299407	55	20	11.20	6.94225	5.28890	1.31261	0.19348	5.07175	2.79515	1.81448	0.08729	2.12854	**0.03329**	2.12854	**0.03329**
158423	60	15	79.60	0.38358	5.92762	0.06471	0.94858	4.82657	2.01114	2.39992	**0.03352**	1.54873	0.12145	2.12575	**0.03352**
4443846	46	29	6.10	-8.72608	4.82393	-1.80891	0.07464	-3.18156	2.53804	-1.25355	0.22116	-2.12565	**0.03353**	-2.12565	**0.03353**
113654	67	8	2.88	-9.90391	7.57691	-1.30712	0.19533	-34.62786	16.31350	-2.12265	0.08721	-2.12504	**0.03358**	-2.12504	**0.03358**
4315788	68	7	10.43	10.71832	8.06020	1.32978	0.18779	14.39060	6.58315	2.18597	0.09411	2.11512	**0.03442**	2.11512	**0.03442**
183604	65	10	3.50	-14.57335	6.76876	-2.15303	**0.03467**	1.47711	3.14414	0.46980	0.65278	-1.17544	0.23982	-2.11223	**0.03467**
175844	49	26	104.65	-10.37505	4.83180	-2.14724	**0.03514**	0.87230	1.48705	0.58660	0.56319	-1.08089	0.27975	-2.10672	**0.03514**
180462	68	7	2.29	-14.92898	7.95301	-1.87715	0.06455	-16.28100	12.36019	-1.31721	0.25815	-2.10656	**0.03516**	-2.10656	**0.03516**
187179	61	14	9.86	-12.80080	5.96275	-2.14679	**0.03518**	-0.82618	3.10108	-0.26642	0.79485	-1.67324	0.09428	-2.10629	**0.03518**
192741	49	26	10.42	10.37239	4.83213	2.14654	**0.03520**	-2.51507	2.28985	-1.09836	0.28341	0.73071	0.46496	2.10606	**0.03520**
3507744	68	7	4.57	-16.90750	7.89232	-2.14227	**0.03555**	5.87076	3.98831	1.47199	0.21500	-0.60957	0.54215	-2.10199	**0.03555**
535955	64	11	2.36	8.92343	6.61762	1.34843	0.18175	13.25453	7.19462	1.84228	0.10268	2.09825	**0.03588**	2.09825	**0.03588**
209760	65	10	2.20	-3.62594	6.96750	-0.52041	0.60438	8.34548	3.22090	2.59104	**0.03589**	1.11726	0.26388	2.09817	**0.03589**
192438	52	23	12.48	-7.77997	5.10533	-1.52389	0.13192	-3.28416	2.16076	-1.51991	0.14419	-2.09796	**0.03591**	-2.09796	**0.03591**
189271	68	7	1.29	1.91240	8.13619	0.23505	0.81484	23.58218	7.59865	3.10347	**0.03610**	1.64755	0.09944	2.09581	**0.03610**
319275	44	31	5.61	-5.46129	4.80729	-1.13604	0.25971	-5.30709	2.77666	-1.91132	0.06625	-2.09574	**0.03611**	-2.09574	**0.03611**
195252	67	8	4.00	15.85947	7.43458	2.13320	**0.03632**	-2.74168	7.13861	-0.38406	0.71672	1.22366	0.22108	2.09336	**0.03632**
187386	53	22	3.86	-2.14958	5.19411	-0.41385	0.68021	-6.75550	3.00246	-2.24999	**0.03649**	-1.77028	0.07668	-2.09138	**0.03649**
365385	56	19	7.63	-5.55062	5.40387	-1.02716	0.30779	-6.81965	3.27177	-2.08439	0.05351	-2.08643	**0.03694**	-2.08643	**0.03694**
4381555	62	13	3.92	-9.91791	6.30598	-1.57278	0.12015	-6.57244	4.36275	-1.50649	0.16286	-2.08571	**0.03700**	-2.08571	**0.03700**
197179	69	6	3.83	18.01847	8.47897	2.12508	**0.03701**	-18.11228	6.58765	-2.74943	0.07078	0.19708	0.84376	2.08562	**0.03701**
1986406	71	4	1.75	21.71645	10.22529	2.12380	**0.03712**	-20.90929	24.96693	-0.83748	0.55616	1.05773	0.29018	2.08441	**0.03712**
183454	67	8	15.75	14.75480	7.52768	1.96007	0.05386	5.33735	4.70240	1.13503	0.30783	2.08439	**0.03712**	2.08439	**0.03712**
539647	60	15	19.27	-0.16128	5.91782	-0.02725	0.97833	5.84996	2.50299	2.33719	**0.03758**	1.45118	0.14673	2.07944	**0.03758**
48084	35	40	36.20	9.74876	4.60219	2.11829	**0.03760**	-0.86021	1.41023	-0.60998	0.54560	1.04284	0.29702	2.07916	**0.03760**
182483	60	15	53.40	12.27166	5.79618	2.11720	**0.03770**	-1.60375	2.43407	-0.65888	0.52242	1.01717	0.30907	2.07812	**0.03770**
199761	25	50	9.80	-10.30836	4.88136	-2.11178	**0.03818**	-0.72255	1.93611	-0.37320	0.71068	-1.72810	0.08397	-2.07296	**0.03818**
182577	43	32	3.63	-6.77656	4.71760	-1.43644	0.15521	-5.00074	3.22103	-1.55253	0.13138	-2.07186	**0.03828**	-2.07186	**0.03828**
362765	46	29	4.90	-9.61136	4.76152	-2.01855	**0.04726**	-2.26776	2.35572	-0.96266	0.34459	-2.07119	**0.03834**	-2.07119	**0.03834**
197696	68	7	3.71	5.27779	8.16177	0.64665	0.51992	11.32303	3.73058	3.03519	**0.03858**	1.91773	0.05515	2.06860	**0.03858**
297385	68	7	2.57	-14.52409	8.04696	-1.80492	0.07527	-15.44160	11.54314	-1.33773	0.25198	-2.06785	**0.03865**	-2.06785	**0.03865**
4465124	40	35	31.57	6.66319	4.69373	1.41959	0.16004	2.98890	1.92300	1.55429	0.12995	2.06421	**0.03900**	2.06421	**0.03900**
158310	68	7	25.43	-7.81188	8.14482	-0.95912	0.34071	10.44594	3.45736	3.02137	**0.03911**	0.78507	0.43242	2.06302	**0.03911**
348304	53	22	3.86	-11.33853	5.40726	-2.09691	**0.03951**	6.18374	3.84062	1.61009	0.12387	-0.36773	0.71308	-2.05879	**0.03951**
186352	29	46	8.22	0.02397	4.94492	0.00485	0.99615	-4.98215	2.34806	-2.12182	**0.03965**	-1.45134	0.14669	-2.05733	**0.03965**
19080	62	13	64.00	7.65886	6.32742	1.21042	0.23008	3.25862	1.72014	1.89440	0.08743	2.05718	**0.03967**	2.05718	**0.03967**
617098	64	11	4.18	7.30232	6.64044	1.09967	0.27514	10.58742	5.04728	2.09765	0.06919	2.05661	**0.03972**	2.05661	**0.03972**
195081	48	27	6.33	-0.20218	4.93443	-0.04097	0.96743	-6.90726	3.18160	-2.17100	**0.04005**	-1.48076	0.13867	-2.05328	**0.04005**
192231	70	5	1.00	19.26371	9.21656	2.09012	**0.04014**	0.00000	0.00000	0.00000	1.00000	1.45120	0.14672	2.05231	**0.04014**
193191	40	35	34.80	-4.24936	4.72293	-0.89973	0.37126	-3.10459	1.48659	-2.08840	**0.04480**	-2.05103	**0.04026**	-2.05103	**0.04026**
189356	67	8	2.50	7.81426	7.74838	1.00850	0.31659	-11.10934	4.05008	-2.74299	**0.04065**	-0.73937	0.45968	-2.04711	**0.04065**
196724	70	5	1.60	-0.75162	9.55705	-0.07865	0.93753	-5.47871	1.14132	-4.80031	**0.04076**	-1.50212	0.13307	-2.04594	**0.04076**

Table S4d. OTUs associated with Low-density lipoprotein (LDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Presen	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
4462107	69	6	2.67	-17.63967	8.47461	-2.08147	**0.04095**	17.23641	10.41733	1.65459	0.19658	-0.53225	0.59455	-2.04407	**0.04095**
4434579	62	13	83.77	-9.46807	6.16375	-1.53609	0.12890	4.41660	1.88322	2.34525	**0.04097**	0.37149	0.71027	2.04382	**0.04097**
941096	61	14	3.00	-3.40332	6.09694	-0.55820	0.57844	10.13533	4.38219	2.31285	**0.04109**	1.05142	0.29307	2.04260	**0.04109**
1046997	70	5	2.20	-19.13633	9.21668	-2.07627	**0.04144**	41.96582	16.81099	2.49633	0.12992	-0.37102	0.71063	-2.03911	**0.04144**
3138798	31	44	219.93	-9.67748	4.66854	-2.07291	**0.04176**	-0.69154	0.90844	-0.76123	0.45088	-1.97273	**0.04853**	-2.03590	**0.04176**
588471	64	11	315.64	5.73387	6.66689	0.86005	0.39261	2.83990	1.18346	2.39965	**0.04320**	2.03410	**0.04194**	2.03410	**0.04194**
113542	45	30	17.70	9.72484	4.69996	2.06913	**0.04212**	-1.62757	2.09181	-0.77807	0.44329	0.89495	0.37081	2.03229	**0.04212**
180216	56	19	47.32	-7.48430	5.46507	-1.36948	0.17511	-2.90041	1.81801	-1.59537	0.13019	-2.02893	**0.04247**	-2.02893	**0.04247**
359445	67	8	4.13	15.43281	7.48200	2.06266	**0.04275**	2.11211	5.64371	0.37424	0.72357	1.68278	0.09242	2.02611	**0.04275**
163494	59	16	3.44	-11.63567	5.64195	-2.06235	**0.04278**	4.36595	6.43363	0.67861	0.50928	-0.96581	0.33414	-2.02582	**0.04278**
4458227	66	9	16.11	9.60854	7.19460	1.33552	0.18591	-9.83031	3.84348	-2.55766	**0.04304**	-0.49534	0.62036	-2.02328	**0.04304**
174862	70	5	2.80	-14.51379	9.40316	-1.54350	0.12709	-96.79624	48.16774	-2.00957	0.18221	-2.02208	**0.04317**	-2.02208	**0.04317**
3318103	59	16	2.38	-0.27603	5.78411	-0.04772	0.96207	13.99893	6.25365	2.23852	**0.04332**	1.39519	0.16296	2.02066	**0.04332**
211720	60	15	46.73	3.75318	5.92501	0.63345	0.52845	4.25210	1.88265	2.25857	**0.04332**	1.87451	0.06086	2.02058	**0.04332**
4451906	44	31	4.68	5.46640	4.83881	1.12970	0.26235	5.69919	3.16867	1.79861	0.08287	2.01887	**0.04350**	2.01887	**0.04350**
182911	39	36	7.17	-7.17050	4.66477	-1.53716	0.12864	-2.93806	2.16330	-1.35814	0.18363	-2.01465	**0.04394**	-2.01465	**0.04394**
197970	27	48	8.15	-9.90085	4.83607	-2.04729	**0.04428**	0.71590	1.91403	0.37403	0.71014	-1.15949	0.24625	-2.01144	**0.04428**
793109	68	7	13.57	13.97993	8.17689	1.70969	0.09163	6.13411	4.56736	1.34303	0.25040	2.00552	**0.04491**	2.00552	**0.04491**
188851	43	32	293.06	6.86774	4.79102	1.43346	0.15605	1.65990	1.14291	1.45235	0.15714	2.00340	**0.04513**	2.00340	**0.04513**
197539	69	6	4.50	12.68150	8.59460	1.47552	0.14443	12.39950	6.89900	1.79729	0.17014	2.00198	**0.04529**	2.00198	**0.04529**
350832	71	4	7.25	14.44295	10.39355	1.38961	0.16893	32.94819	7.67109	4.29511	0.14563	2.00168	**0.04532**	2.00168	**0.04532**
4357713	55	20	2.90	8.45181	5.25865	1.60722	0.11238	5.49913	4.26651	1.28891	0.21469	1.99993	**0.04551**	1.99993	**0.04551**
194735	71	4	2.25	20.85830	10.24940	2.03507	**0.04553**	30.14229	93.03498	0.32399	0.80054	1.59270	0.11123	1.99976	**0.04553**
4395096	48	27	4.19	-3.56369	4.91390	-0.72523	0.47066	6.01772	2.85405	2.10849	**0.04561**	0.90336	0.36633	1.99894	**0.04561**
36647	71	4	1.00	-20.82529	10.24151	-2.03342	**0.04570**	0.00000	0.00000	0.00000	1.00000	-1.41293	0.15768	-1.99818	**0.04570**
174571	64	11	2.82	-13.26388	6.53973	-2.02820	**0.04624**	-0.40650	7.51616	-0.05408	0.95819	-1.44647	0.14805	-1.99319	**0.04624**
4449055	60	15	2.53	-8.73611	5.83936	-1.49607	0.13900	-7.90382	5.57877	-1.41677	0.18198	-1.98992	**0.04660**	-1.98992	**0.04660**
841907	52	23	37.48	2.36605	5.14556	0.45982	0.64703	-4.66353	2.20072	-2.11909	**0.04679**	-1.08207	0.27922	-1.98817	**0.04679**
4436046	62	13	15.46	12.31058	6.08737	2.02232	**0.04686**	1.91411	3.65093	0.52428	0.61151	1.76458	0.07763	1.98757	**0.04686**
303326	71	4	2.25	20.79149	10.28947	2.02066	**0.04704**	-33.59815	22.26129	-1.50926	0.37253	0.77374	0.43908	1.98598	**0.04704**
2438203	43	32	7.66	-6.19707	4.73546	-1.30865	0.19481	-3.68890	2.37273	-1.55471	0.13086	-1.98498	**0.04715**	-1.98498	**0.04715**
4397092	57	18	5.94	9.43213	5.61595	1.67953	0.09738	5.07835	4.24984	1.19495	0.25066	1.98444	**0.04721**	1.98444	**0.04721**
182269	71	4	3.25	-9.42730	10.60116	-0.88927	0.37682	-15.60806	1.34625	-11.59377	0.05477	-1.98303	**0.04736**	-1.98303	**0.04736**
214036	39	36	11.44	-9.30230	4.61972	-2.01361	**0.04779**	0.65922	2.23043	0.29556	0.76942	-1.19226	0.23316	-1.97924	**0.04779**
157162	70	5	3.00	0.10857	9.48663	0.01144	0.99090	-5.79899	1.31705	-4.40302	**0.04791**	-1.39074	0.16431	-1.97820	**0.04791**
3544699	68	7	4.43	-15.92721	7.91541	-2.01218	**0.04794**	13.71178	11.27838	1.21576	0.29092	-0.65179	0.51454	-1.97787	**0.04794**
179267	45	30	6.03	-9.45764	4.70356	-2.01074	**0.04810**	-2.07545	2.67559	-0.77570	0.44467	-1.93806	0.05262	-1.97650	**0.04810**
191153	11	64	130.27	4.84967	6.67443	0.72660	0.46982	2.46685	1.22384	2.01566	**0.04825**	1.90774	0.05642	1.97519	**0.04825**
368236	67	8	2.13	-6.36010	7.63890	-0.83259	0.40783	-23.84230	9.24825	-2.57804	**0.04955**	-1.97392	**0.04839**	-1.97392	**0.04839**
43950	67	8	2.38	7.86195	7.69359	1.02188	0.31026	16.16711	7.23981	2.23309	0.07587	1.97272	**0.04853**	1.97272	**0.04853**
4379646	67	8	1.75	-14.97473	7.46609	-2.00570	**0.04865**	39.62625	27.01240	1.46696	0.20230	-0.49261	0.62229	-1.97167	**0.04865**
4111715	50	25	5.60	-7.61745	5.06027	-1.50534	0.13661	-3.26704	2.43879	-1.33961	0.19405	-1.97087	**0.04874**	-1.97087	**0.04874**
193672	43	32	6.75	9.31388	4.66087	1.99832	**0.04946**	-2.13200	2.68440	-0.79422	0.43352	0.83539	0.40350	1.96461	**0.04946**
185222	71	4	1.25	6.00101	10.50966	0.57100	0.56978	-25.09735	1.96561	-12.76820	**0.04976**	-0.98546	0.32440	-1.96204	**0.04976**
157327	31	44	5.41	-9.35015	4.68836	-1.99433	**0.04990**	1.08507	2.47003	0.43930	0.66275	-1.07811	0.28098	-1.96079	**0.04990**

[a] Statistics associated to the two part model for association analysis between the trait and OTUs adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe represented by an OTU with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the OTU for the subjects where that OTU is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per OTU-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S4e. OTUs associated with Total cholesterol (TC) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
207340	71	4	2.00	-1.97281	12.83521	-0.15370	0.87827	16.00000	0.00000	743.20468	**0.00000**	5.77705	**0.00000**	8.32315	**0.00000**
324015	70	5	2.40	-48.08228	10.17661	-4.72478	**0.00001**	-9.58050	30.00679	-0.31928	0.77978	-3.30522	**0.00095**	-4.39467	**0.00001**
389371	60	15	4.07	-28.67954	6.40380	-4.47852	**0.00003**	9.47758	4.06824	2.32965	**0.03809**	-1.49783	0.13418	-4.19209	**0.00003**
4342104	59	16	5.38	-9.60323	7.17084	-1.33921	0.18472	-15.22414	2.62694	-5.79538	**0.00006**	-3.76929	**0.00016**	-4.00420	**0.00006**
198754	70	5	2.80	-41.14252	10.49688	-3.91950	**0.00020**	-19.94406	7.10899	-2.80547	0.10704	-3.76924	**0.00016**	-3.76924	**0.00016**
3115852	65	10	2.20	25.95953	7.92211	3.27685	**0.00162**	16.36467	6.66430	2.45557	**0.04375**	3.65525	**0.00026**	3.65525	**0.00026**
361507	66	9	143.67	30.22423	8.12948	3.71785	**0.00039**	1.24020	1.94140	0.63882	0.54654	2.93215	**0.00337**	3.54373	**0.00039**
184248	62	13	4.38	-9.54302	7.54261	-1.26522	0.20987	15.20222	3.21518	4.72826	**0.00081**	1.48260	0.13818	3.35062	**0.00081**
180421	62	13	3.85	-24.58014	7.16158	-3.43222	**0.00100**	-1.87036	9.24480	-0.20231	0.84373	-2.46701	**0.01362**	-3.29175	**0.00100**
4347159	35	40	67.45	3.59414	5.77001	0.62290	0.53532	-5.15779	1.46392	-3.52328	**0.00115**	-1.85993	0.06290	-3.25025	**0.00115**
539647	60	15	19.27	-0.77631	7.21163	-0.10765	0.91458	7.98557	2.00165	3.98950	**0.00180**	2.13185	**0.03302**	3.12216	**0.00180**
760544	63	12	4.67	-21.43375	7.65218	-2.80100	**0.00654**	-12.16997	6.65957	-1.82744	0.10090	-3.08298	**0.00205**	-3.08298	**0.00205**
348304	53	22	3.86	-20.19467	6.35729	-3.17661	**0.00219**	9.33380	4.16605	2.24045	**0.03721**	-0.69228	0.48876	-3.06251	**0.00219**
194374	70	5	2.00	-19.76244	11.36347	-1.73912	0.08629	-62.98963	7.35475	-8.56448	**0.01336**	-2.96230	**0.00305**	-2.96230	**0.00305**
157424	48	27	265.70	-7.05658	5.98033	-1.17996	0.24190	3.60379	1.12006	3.21749	**0.00368**	1.22612	0.22015	2.90425	**0.00368**
196957	22	53	9.45	-9.89474	6.23576	-1.58677	0.11695	-6.68415	2.53652	-2.63517	**0.01117**	-2.90275	**0.00370**	-2.90275	**0.00370**
3236435	37	38	40.37	-3.70670	5.78750	-0.64047	0.52390	6.23049	2.04063	3.05322	**0.00431**	1.56798	0.11689	2.85480	**0.00431**
193654	59	16	4.25	-19.60743	6.70559	-2.92404	**0.00462**	-4.16101	6.95905	-0.59793	0.56016	-2.41495	**0.01574**	-2.83265	**0.00462**
740158	66	9	3.44	24.50788	8.39309	2.92001	**0.00467**	-2.62862	5.90241	-0.44535	0.67169	1.70067	0.08900	2.82895	**0.00467**
311718	71	4	3.25	17.87391	12.70858	1.40644	0.16389	-8.77365	0.06714	-130.66925	**0.00487**	-1.00642	0.31421	-2.81538	**0.00487**
4296763	70	5	102.80	-13.56549	11.47719	-1.18195	0.24111	-6.21280	0.44219	-14.05010	**0.00503**	-2.81250	**0.00492**	-2.81250	**0.00492**
4383922	69	6	2.00	-29.13657	10.10697	-2.88282	**0.00519**	-12.01821	8.30465	-1.44717	0.24366	-2.80066	**0.00510**	-2.80066	**0.00510**
177792	32	43	6.26	-4.36808	5.83940	-0.74803	0.45688	7.83040	2.64419	2.96136	**0.00513**	1.45284	0.14627	2.79862	**0.00513**
1044419	70	5	155.80	-31.41750	10.95516	-2.86783	**0.00542**	-2.25102	3.68229	-0.61131	0.60322	-2.33404	**0.01959**	-2.78105	**0.00542**
4464173	25	50	6.98	-16.60061	5.79693	-2.86369	**0.00548**	0.33690	2.37784	0.14168	0.88794	-1.86417	0.06230	-2.77725	**0.00548**
216599	20	55	35.02	0.88005	6.57290	0.13389	0.89386	-5.07634	1.76883	-2.86988	**0.00592**	-1.85159	0.06408	-2.75196	**0.00592**
174943	70	5	4.60	31.14306	11.00374	2.83023	**0.00602**	2.40381	5.20527	0.46180	0.68959	2.22447	**0.02612**	2.74646	**0.00602**
4469032	70	5	5.80	18.66595	11.36758	1.64203	0.10494	15.58326	2.46071	6.33283	**0.02404**	2.74205	**0.00611**	2.74205	**0.00611**
3318103	59	16	2.38	-0.09158	7.04933	-0.01299	0.98967	17.71583	5.44881	3.25132	**0.00631**	1.92203	0.05460	2.73111	**0.00631**
515299	70	5	14.60	-16.04482	11.40830	-1.40642	0.16390	-17.75530	2.09920	-8.45813	**0.01369**	-2.72754	**0.00638**	-2.72754	**0.00638**
199710	50	25	5.20	-10.74935	6.00832	-1.78908	0.07781	-7.43506	3.36680	-2.20834	**0.03794**	-2.71459	**0.00664**	-2.71459	**0.00664**
3907189	67	8	1.88	24.88858	8.90699	2.79428	**0.00666**	-10.76851	10.95066	-0.98337	0.37059	1.28548	0.19863	2.71331	**0.00666**
297385	68	7	2.57	-20.03704	9.74438	-2.05627	**0.04338**	-22.54206	9.36102	-2.40808	0.07371	-2.69297	**0.00708**	-2.69297	**0.00708**
4309301	13	62	21.26	20.07851	7.24730	2.77048	**0.00712**	-0.68737	1.72327	-0.39887	0.69143	1.62240	0.10472	2.69134	**0.00712**
196061	68	7	5.57	-27.06290	9.83047	-2.75296	**0.00747**	-7.10729	9.18274	-0.77398	0.48214	-2.38861	**0.01691**	-2.67515	**0.00747**
2157225	65	10	2.40	5.90157	8.45699	0.69783	0.48753	20.39533	5.57924	3.65557	**0.00812**	2.36271	**0.01814**	2.64713	**0.00812**
300235	60	15	1.60	-18.65437	6.86683	-2.71659	**0.00825**	6.39341	11.25001	0.56830	0.58031	-1.47683	0.13972	-2.64149	**0.00825**
1952	7	68	202.57	-1.18978	10.56638	-0.11260	0.91066	3.72990	1.36942	2.72370	**0.00828**	1.78768	0.07383	2.64037	**0.00828**
177758	64	11	4.82	-9.97263	8.06702	-1.23622	0.22039	-16.64450	5.15971	-3.22586	**0.01213**	-2.64017	**0.00829**	-2.64017	**0.00829**
851733	59	16	70.75	10.34719	6.93609	1.49179	0.14012	3.78745	1.48194	2.55573	**0.02393**	2.64002	**0.00829**	2.64002	**0.00829**
357930	64	11	110.91	-8.61168	8.09150	-1.06429	0.29075	-2.59847	0.74745	-3.47643	**0.00836**	-2.61170	**0.00901**	-2.63704	**0.00836**
184525	65	10	1.70	-20.70228	8.15344	-2.53909	**0.01328**	-23.53975	17.28289	-1.36203	0.21539	-2.62702	**0.00861**	-2.62702	**0.00861**
174516	43	32	22.66	11.25842	5.81506	1.93608	0.05678	4.51292	2.40327	1.87783	0.07050	2.62597	**0.00864**	2.62597	**0.00864**
34789	63	12	3.83	-20.33586	7.53596	-2.69851	**0.00867**	-8.24459	8.98557	-0.91754	0.38279	-2.47310	**0.01339**	-2.62472	**0.00867**
193477	38	37	5.78	-13.59704	5.58404	-2.43498	**0.01737**	-4.25673	3.14968	-1.35148	0.18547	-2.61832	**0.00884**	-2.61832	**0.00884**
182797	69	6	2.83	-26.09489	10.39567	-2.51017	**0.01432**	-57.03296	35.94635	-1.58661	0.21079	-2.61675	**0.00888**	-2.61675	**0.00888**
197581	69	6	2.00	-2.60991	10.62638	-0.24561	0.80669	11.03465	1.82183	6.05692	**0.00903**	1.67318	0.09429	2.61094	**0.00903**
105287	64	11	3.09	-20.87598	7.93603	-2.63053	**0.01042**	-9.78927	8.15517	-1.20038	0.26433	-2.60060	**0.00931**	-2.60060	**0.00931**
349123	70	5	2.60	-29.52283	11.04806	-2.67222	**0.00931**	6.15478	20.66671	0.29781	0.79394	-1.65401	0.09813	-2.60033	**0.00931**
194654	58	17	3.06	-4.01863	6.94065	-0.57900	0.56440	-10.42257	3.46151	-3.01099	**0.00935**	-2.24539	**0.02474**	-2.59914	**0.00935**
315223	62	13	6.62	-6.95411	7.58096	-0.91731	0.36204	-15.52809	4.87290	-3.18662	**0.00971**	-2.47309	**0.01339**	-2.58600	**0.00971**
4479443	65	10	2.90	8.68126	8.42458	1.03047	0.30624	19.09898	5.43480	3.51420	**0.00980**	2.54970	**0.01078**	2.58267	**0.00980**
182289	34	41	10.37	-10.07184	5.67096	-1.77604	0.07995	-4.25545	2.19417	-1.93944	0.05990	-2.56857	**0.01021**	-2.56857	**0.01021**
179267	45	30	6.03	-14.79331	5.62719	-2.62890	**0.01047**	-2.12136	3.17941	-0.66722	0.51029	-2.27578	**0.02286**	-2.56006	**0.01047**
195436	37	38	4.76	-14.56108	5.54448	-2.62623	**0.01054**	3.81049	2.75232	1.38446	0.17498	-0.84938	0.39567	-2.55758	**0.01054**
2046330	67	8	5.88	23.42965	8.92596	2.62489	**0.01058**	0.76831	6.65277	0.11549	0.91255	1.88525	0.05940	2.55633	**0.01058**
519880	70	5	3.80	-29.37026	11.20750	-2.62059	**0.01070**	9.04333	50.96898	0.17743	0.87552	-1.69399	0.09027	-2.55233	**0.01070**
320786	67	8	3.38	-23.40178	8.93187	-2.62003	**0.01072**	4.41092	4.92304	0.89598	0.41132	-1.22346	0.22115	-2.55181	**0.01072**
158625	70	5	2.80	-29.03759	11.08341	-2.61991	**0.01072**	-14.53078	15.66249	-0.92774	0.45148	-2.33674	**0.01945**	-2.55170	**0.01072**
591118	71	4	3.25	-4.44682	12.88000	-0.34525	0.73091	-32.64721	0.56302	-57.98563	**0.01098**	-2.04164	**0.04119**	-2.54340	**0.01098**
4379646	67	8	1.75	-23.28756	8.93797	-2.60547	**0.01114**	57.52187	41.02106	1.40225	0.21977	-0.92709	0.35388	-2.53824	**0.01114**
197179	69	6	3.83	26.47442	10.18549	2.59923	**0.01133**	-5.49181	6.26266	-0.87691	0.44509	1.25073	0.21103	2.53243	**0.01133**
562410	69	6	1.50	-16.97317	10.48403	-1.61896	0.10983	-21.25801	6.65907	-3.19234	**0.04962**	-2.51883	**0.01177**	-2.51883	**0.01177**
3946926	69	6	3.17	26.43685	10.27261	2.57353	**0.01213**	2.51636	14.91550	0.16871	0.87676	1.88341	0.05965	2.50846	**0.01213**
187517	61	14	14.14	18.11422	7.08718	2.55591	**0.01270**	1.63510	4.48943	0.36421	0.72261	2.01313	**0.04410**	2.49202	**0.01270**
197696	68	7	3.71	1.59393	9.97400	0.15981	0.87348	16.72011	3.89366	4.29418	**0.01270**	1.87468	0.06084	2.49196	**0.01270**
4318284	69	6	28.83	7.15038	10.60342	0.67435	0.50225	-7.90968	1.48529	-5.32533	**0.01294**	-1.28305	0.19947	-2.48546	**0.01294**
4457268	67	8	4.25	10.25587	9.33144	1.09907	0.27540	17.21897	4.76907	3.61055	**0.01537**	2.48493	**0.01296**	2.48493	**0.01296**
183598	70	5	2.60	-26.16156	11.14316	-2.34777	**0.02164**	-13.96977	8.30400	-1.68229	0.23454	-2.46456	**0.01372**	-2.46456	**0.01372**
194488	71	4	2.50	-31.33784	12.42411	-2.52234	**0.01387**	-39.29670	53.09788	-0.74008	0.59440	-2.11645	**0.03431**	-2.46064	**0.01387**
4405104	39	36	18.58	-9.66556	5.65975	-1.70777	0.09199	-4.07045	2.19556	-1.85395	0.07270	-2.46052	**0.01387**	-2.46052	**0.01387**
165257	71	4	4.75	1.57965	12.86550	0.12278	0.90262	-9.79677	0.21849	-44.83877	**0.01420**	-1.64751	0.09945	-2.45227	**0.01420**
113654	67	8	2.88	-17.78722	9.10487	-1.95359	0.05463	-32.65153	17.61453	-1.85367	0.12297	-2.44956	**0.01430**	-2.44956	**0.01430**
193053	18	57	11.40	16.12775	6.47972	2.48896	**0.01512**	0.34524	2.22496	0.15517	0.87727	1.82704	0.06769	2.42939	**0.01512**
189647	63	12	3.33	-18.87538	7.59093	-2.48657	**0.01522**	6.55902	6.42973	1.02011	0.33431	-1.03357	0.30134	-2.42715	**0.01522**
4369434	69	6	4.00	-4.39919	10.66674	-0.41242	0.68126	-24.43354	4.91368	-4.97255	**0.01563**	-1.99989	**0.04551**	-2.41753	**0.01563**
189356	67	8	2.50	7.63049	9.46697	0.80601	0.42289	-19.53090	5.43857	-3.59118	**0.01569**	-1.14174	0.25356	-2.41608	**0.01569**

Supplemental Information

Table S4e. OTUs associated with Total cholesterol (TC) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
178478	34	41	4.98	-9.75791	5.74026	-1.69991	0.09346	-4.65850	2.61399	-1.78214	0.08272	-2.41302	**0.01582**	-2.41302	**0.01582**
192676	69	6	3.83	-1.19380	10.74543	-0.11110	0.91185	-18.54316	3.75226	-4.94186	**0.01589**	-1.78337	0.07453	-2.41135	**0.01589**
337161	62	13	9.46	-1.49092	7.61805	-0.19571	0.84539	10.84224	3.74320	2.89652	**0.01593**	1.56663	0.11720	2.41055	**0.01593**
179201	52	23	5.70	-14.85216	6.02522	-2.46500	**0.01609**	-3.88865	4.59863	-0.84561	0.40777	-2.28731	**0.02218**	-2.40693	**0.01609**
2949328	63	12	3.42	0.17037	7.87047	0.02165	0.98279	16.75681	5.68582	2.94712	**0.01630**	1.71386	0.08655	2.40220	**0.01630**
808794	71	4	3.25	-8.64693	12.80201	-0.67544	0.50156	-30.73374	0.78940	-38.93293	**0.01635**	-2.17300	**0.02978**	-2.40105	**0.01635**
315982	71	4	2.25	30.30829	12.36861	2.45042	**0.01670**	5.55113	16.90290	0.32841	0.79799	1.87326	0.06103	2.39325	**0.01670**
181452	66	9	3.00	-20.92043	8.56771	-2.44178	**0.01707**	5.20614	14.34388	0.36295	0.72908	-1.44164	0.14940	-2.38513	**0.01707**
191928	65	10	1.80	-19.92159	8.17091	-2.43811	**0.01723**	1.37879	9.79716	0.14073	0.89204	-1.58814	0.11225	-2.38169	**0.01723**
925131	69	6	76.17	-24.89420	10.21799	-2.43631	**0.01731**	6.67263	6.50091	1.02641	0.38022	-1.06244	0.28804	-2.38000	**0.01731**
188262	71	4	1.00	31.16998	12.80096	2.43497	**0.01737**	0.00000	0.00000	0.00000	1.00000	1.68203	0.09256	2.37874	**0.01737**
191874	48	27	3.07	-4.74986	6.03417	-0.78716	0.43377	-12.60701	4.95984	-2.54182	**0.01790**	-2.22767	**0.02590**	-2.36765	**0.01790**
341730	67	8	2.50	-19.24887	9.07267	-2.12163	**0.03731**	-16.89469	11.63935	-1.45151	0.20635	-2.36599	**0.01798**	-2.36599	**0.01798**
175761	58	17	2.41	-0.98717	6.90710	-0.14292	0.88675	-20.60406	7.71999	-2.66892	**0.01834**	-1.76856	0.07697	-2.35871	**0.01834**
310633	71	4	10.00	-16.15987	12.75873	-1.26657	0.20939	-14.71017	0.87027	-16.90301	**0.03762**	-2.35765	**0.01839**	-2.35765	**0.01839**
4094866	57	18	5.89	2.88451	6.74686	0.42753	0.67027	-10.35846	3.92012	-2.64238	**0.01847**	-1.36491	0.17228	-2.35605	**0.01847**
158423	60	15	79.60	-7.56135	7.16915	-1.05471	0.29509	5.86865	2.15747	2.72015	**0.01860**	0.92375	0.35562	2.35340	**0.01860**
190913	30	45	9.82	-0.54321	6.14181	-0.08844	0.92977	5.70763	2.33971	2.43946	**0.01901**	1.59603	0.11048	2.34526	**0.01901**
329798	65	10	5.80	19.58336	8.16403	2.39874	**0.01904**	4.03780	5.81663	0.69418	0.50994	2.12387	**0.03368**	2.34468	**0.01904**
730906	53	22	2.91	-14.87998	6.23168	-2.38780	**0.01958**	-3.79768	4.93909	-0.76890	0.45140	-2.18316	**0.02902**	-2.33438	**0.01958**
176108	64	11	2.18	-18.89827	7.92605	-2.38432	**0.01975**	15.00937	12.48698	1.20200	0.26373	-0.85807	0.39085	-2.33111	**0.01975**
187196	71	4	2.25	-29.40984	12.35899	-2.37963	**0.01998**	0.83969	16.15505	0.05198	0.96694	-1.61592	0.10611	-2.32670	**0.01998**
178151	71	4	3.25	-18.91848	12.64449	-1.49618	0.13898	-8.37890	0.93065	-9.00329	0.07042	-2.32554	**0.02004**	-2.32554	**0.02004**
851323	47	28	57.61	-10.69397	5.84376	-1.82998	0.07139	-3.58044	2.33293	-1.53474	0.13741	-2.32531	**0.02006**	-2.32531	**0.02006**
197988	42	33	14.00	-13.28355	5.59736	-2.37318	**0.02031**	0.26346	2.46296	0.10697	0.91552	-1.56592	0.11737	-2.32062	**0.02031**
182033	56	19	3.68	-10.33662	6.77714	-1.52522	0.13159	-10.26423	5.41752	-1.89464	0.07636	-2.31937	**0.02037**	-2.31937	**0.02037**
288651	26	49	32.88	-3.49789	6.05907	-0.57730	0.56554	-3.63627	1.51606	-2.39849	**0.02057**	-2.04377	**0.04098**	-2.31570	**0.02057**
176077	66	9	2.67	20.21362	8.55052	2.36402	**0.02078**	0.02050	8.06401	0.00254	0.99805	1.63655	0.10172	2.31199	**0.02078**
3141342	60	15	4.33	4.69473	7.28299	0.64462	0.52122	-17.84493	6.70793	-2.66027	**0.02078**	-1.18121	0.23752	-2.31194	**0.02078**
553080	28	47	17.00	-13.61482	5.77471	-2.35766	**0.02111**	2.65357	2.38546	1.11240	0.27201	-0.85387	0.39318	-2.30600	**0.02111**
4311621	67	8	2.50	21.26075	9.04133	2.35151	**0.02144**	-7.55005	5.96474	-1.26578	0.26137	0.83228	0.40525	2.30019	**0.02144**
4438999	61	14	3.93	0.51318	7.40209	0.06933	0.94492	-14.33736	5.35490	-2.67743	**0.02151**	-1.57676	0.11485	-2.29897	**0.02151**
180107	61	14	3.93	0.51318	7.40209	0.06933	0.94492	-14.33736	5.35490	-2.67743	**0.02151**	-1.57676	0.11485	-2.29897	**0.02151**
180312	51	24	4.92	-14.01917	5.97179	-2.34757	**0.02165**	-0.93061	4.63320	-0.20086	0.84274	-1.76413	0.07771	-2.29648	**0.02165**
3709990	63	12	3.08	-10.95802	7.93429	-1.38110	0.17152	-14.01236	6.54866	-2.13973	0.06105	-2.29137	**0.02194**	-2.29137	**0.02194**
302333	63	12	2.50	-8.23645	7.82857	-1.05210	0.29627	-24.60621	9.48717	-2.59363	**0.02904**	-2.28212	**0.02248**	-2.28212	**0.02248**
303320	70	5	2.40	26.46978	11.35304	2.33151	**0.02253**	-1.87917	6.32334	-0.29718	0.79435	1.42883	0.15305	2.28133	**0.02253**
2996838	46	29	9.14	-5.81087	5.96127	-0.97477	0.33294	-5.04090	2.10231	-2.39779	**0.02397**	-2.28103	**0.02255**	-2.28103	**0.02255**
182577	43	32	3.63	-10.88096	5.68850	-1.91280	0.05975	-5.11298	3.72207	-1.37369	0.18006	-2.27914	**0.02266**	-2.27914	**0.02266**
4462107	69	6	2.67	5.11967	10.61726	0.48220	0.63112	-38.83900	9.02298	-4.30445	**0.02308**	-1.26715	0.20510	-2.27216	**0.02308**
197970	27	48	8.15	-13.52053	5.84982	-2.31127	**0.02368**	-0.51752	2.45194	-0.21107	0.83379	-1.74802	0.08046	-2.26222	**0.02368**
189478	66	9	1.44	-9.63102	8.81755	-1.09226	0.27836	-25.35312	9.30843	-2.72367	**0.03447**	-2.26167	**0.02372**	-2.26167	**0.02372**
176947	69	6	3.83	-22.05129	10.38990	-2.12238	**0.03725**	-8.58882	6.27766	-1.36816	0.26473	-2.26157	**0.02372**	-2.26157	**0.02372**
2774254	69	6	13.50	-19.75752	10.37282	-1.90474	0.06081	-11.84969	6.94798	-1.70549	0.18665	-2.25952	**0.02385**	-2.25952	**0.02385**
4060501	46	29	13.69	-0.25794	5.95116	-0.04334	0.96555	-7.63167	3.19898	-2.38566	**0.02462**	-1.61961	0.10532	-2.24728	**0.02462**
186687	60	15	2.60	-16.02744	6.98471	-2.29465	**0.02467**	-4.00832	5.98827	-0.66936	0.51594	-2.04788	**0.04057**	-2.24651	**0.02467**
180352	70	5	4.00	-25.62911	11.17757	-2.29291	**0.02478**	-7.47438	13.30091	-0.56194	0.63073	-1.92727	0.05395	-2.24487	**0.02478**
4306262	33	42	261.55	-8.97558	5.71544	-1.57041	0.12070	-2.40477	1.45076	-1.65759	0.10542	-2.24219	**0.02495**	-2.24219	**0.02495**
294791	69	6	2.67	-4.76407	10.63079	-0.44814	0.65540	-26.37107	6.31319	-4.17714	**0.02499**	-1.90058	0.05736	-2.24155	**0.02499**
4398588	63	12	4.00	17.40154	7.61729	2.28448	**0.02529**	-3.42502	6.29547	-0.54405	0.59963	1.21054	0.22607	2.23690	**0.02529**
322835	38	37	7.84	-12.75940	5.58732	-2.28363	**0.02535**	4.54343	3.07070	1.47961	0.14819	-0.55870	0.57636	-2.23610	**0.02535**
125270	61	14	3.50	4.83975	7.42466	0.65185	0.51657	-7.48035	2.89493	-2.58395	**0.02541**	-1.12180	0.26195	-2.23510	**0.02541**
1679707	62	13	3.23	-16.80224	7.37182	-2.27925	**0.02562**	6.08068	5.86905	1.03606	0.32458	-0.88166	0.37796	-2.23195	**0.02562**
3609545	45	30	6.00	-13.10806	5.76529	-2.27362	**0.02597**	-0.73576	3.90986	-0.18818	0.85214	-1.70626	0.08796	-2.22662	**0.02597**
305318	37	38	6.50	-12.69619	5.58487	-2.27332	**0.02599**	3.92794	2.67778	1.46686	0.15134	-0.55970	0.57569	-2.22634	**0.02599**
4483570	71	4	5.25	-26.81850	12.48823	-2.14750	**0.03512**	-16.22480	8.19172	-1.98064	0.29765	-2.22629	**0.02599**	-2.22629	**0.02599**
3138798	31	44	219.93	-9.75340	5.74307	-1.69829	0.09377	-1.70766	1.13815	-1.50039	0.14118	-2.22545	**0.02605**	-2.22545	**0.02605**
337538	67	8	1.63	-10.21361	9.29994	-1.09824	0.27576	-15.46566	5.63557	-2.74430	**0.04058**	-2.21866	**0.02651**	-2.21866	**0.02651**
189450	66	9	2.44	-19.58847	8.65648	-2.26287	**0.02666**	-4.69062	7.99431	-0.58674	0.57877	-1.95984	0.05001	-2.21645	**0.02666**
193534	68	7	2.43	5.45579	9.89320	0.55147	0.58302	31.33216	9.18755	3.41029	**0.02702**	1.95176	0.05097	2.21122	**0.02702**
346253	69	6	2.67	-13.78278	10.62288	-1.29746	0.19861	-28.64492	10.13936	-2.82512	0.06646	-2.20677	**0.02733**	-2.20677	**0.02733**
189007	67	8	2.00	-12.47547	9.24722	-1.34910	0.18153	-18.99121	8.47508	-2.24083	0.07514	-2.20312	**0.02759**	-2.20312	**0.02759**
174818	20	55	10.71	4.87704	6.50307	0.74996	0.45572	-4.89853	2.16583	-2.26173	**0.02792**	-1.02705	0.30440	-2.19838	**0.02792**
175922	69	6	2.67	23.03253	10.29373	2.23753	**0.02835**	3.60844	14.18708	0.25435	0.81567	1.71514	0.08632	2.19245	**0.02835**
4442459	63	12	4.58	-17.02474	7.61727	-2.23502	**0.02852**	4.32539	5.06654	0.85372	0.41541	-0.97273	0.33069	-2.19007	**0.02852**
4347652	70	5	2.20	-25.13730	11.32236	-2.22015	**0.02956**	-16.47632	15.92920	-1.03435	0.40965	-2.12165	**0.03387**	-2.17597	**0.02956**
4429981	61	14	38.07	10.86885	7.29292	1.49033	0.14051	-8.66154	3.46908	-2.49678	**0.02968**	-0.49532	0.62038	-2.17439	**0.02968**
4103317	17	58	56.57	14.76989	6.66558	2.21584	**0.02986**	-0.33087	1.45545	-0.22733	0.82101	1.37578	0.16889	2.17189	**0.02986**
187126	57	18	3.22	-14.46392	6.53409	-2.21361	**0.03002**	7.24551	3.76510	1.92439	0.07349	-0.26869	0.78817	-2.16977	**0.03002**
552235	68	7	4.57	-21.10516	9.59864	-2.19877	**0.03111**	-0.91157	8.35234	-0.10914	0.91835	-1.59679	0.11031	-2.15568	**0.03111**
297266	67	8	7.38	-11.19396	9.25280	-1.20979	0.23032	7.97933	2.69040	2.96585	**0.03131**	0.67430	0.50012	2.15315	**0.03131**
180552	60	15	4.27	-9.69567	7.13150	-1.35956	0.17821	-12.93708	7.00468	-1.84692	0.08955	-2.15246	**0.03136**	-2.15246	**0.03136**
157631	71	4	11.25	-27.34829	12.46407	-2.19417	**0.03145**	11.09732	10.97248	1.01138	0.49640	-1.04026	0.29822	-2.15132	**0.03145**
307113	63	12	1.67	-16.66465	7.62580	-2.18530	**0.03212**	-6.04089	11.28364	-0.53537	0.60537	-1.88061	0.06002	-2.14290	**0.03212**
4045882	63	12	1.67	-16.66465	7.62580	-2.18530	**0.03212**	-6.04089	11.28364	-0.53537	0.60537	-1.88061	0.06002	-2.14290	**0.03212**
275237	42	33	229.64	10.93765	5.66604	1.93039	0.05750	1.64449	1.43445	1.14642	0.26068	2.13850	**0.03248**	2.13850	**0.03248**
1607319	64	11	1.27	-11.67123	8.26564	-1.41202	0.16225	-10.63693	5.80475	-1.83245	0.10424	-2.13698	**0.03260**	-2.13698	**0.03260**

Table S4e. OTUs associated with Total cholesterol (TC) at FDR < 0.05 level [a] (Cont.)

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
OTU	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
181756	70	5	4.20	-11.92971	11.48241	-1.03896	0.30230	-13.00165	2.90890	-4.46961	**0.04659**	-2.13656	**0.03263**	-2.13656	**0.03263**
4411875	67	8	2.75	-0.24781	9.36333	-0.02647	0.97896	14.19238	4.84527	2.92912	**0.03267**	1.49185	0.13574	2.13616	**0.03267**
842193	51	24	70.96	-13.17286	6.06049	-2.17356	**0.03303**	1.00164	2.28998	0.43740	0.66628	-1.20243	0.22920	-2.13175	**0.03303**
180468	12	63	15.57	-4.74880	7.84952	-0.60498	0.54709	4.65638	2.13631	2.17964	**0.03322**	1.07996	0.28016	2.12942	**0.03322**
4414420	71	4	1.75	5.86402	12.85548	0.45615	0.64966	15.23606	0.81136	18.77853	**0.03387**	1.82141	0.06854	2.12162	**0.03387**
4020046	64	11	7.64	-6.76508	8.16074	-0.82898	0.40986	14.05703	5.50812	2.55206	**0.03407**	0.91581	0.35977	2.11930	**0.03407**
272886	71	4	2.25	-14.01511	12.74402	-1.09974	0.27511	45.97852	2.48434	18.50736	**0.03436**	0.72435	0.46885	2.11577	**0.03436**
196139	69	6	2.33	1.18886	10.73568	0.11074	0.91213	67.78870	18.35715	3.69277	**0.03445**	1.57339	0.11563	2.11476	**0.03445**
258099	64	11	4.64	-17.08436	7.93939	-2.15185	**0.03476**	1.02887	6.38459	0.16115	0.87597	-1.38241	0.16685	-2.11110	**0.03476**
4381555	62	13	3.92	-11.30755	7.70170	-1.46819	0.14641	-7.00063	4.20287	-1.66568	0.12675	-2.10675	**0.03514**	-2.10675	**0.03514**
4393532	60	15	5.53	-15.04464	7.00762	-2.14690	**0.03517**	-0.64027	4.78001	-0.13395	0.89567	-1.58217	0.11361	-2.10639	**0.03517**
194320	44	31	3.90	12.18770	5.67925	2.14600	**0.03524**	-3.09034	4.04773	-0.76348	0.45157	0.95653	0.33881	2.10554	**0.03524**
187569	64	11	2.36	-16.91932	7.90854	-2.13937	**0.03580**	5.96508	6.78705	0.87889	0.40508	-0.89566	0.37043	-2.09923	**0.03580**
4466707	63	12	14.75	-13.78011	7.77887	-1.77148	0.08071	-5.95251	4.55424	-1.30703	0.22361	-2.09555	**0.03612**	-2.09555	**0.03612**
2438203	43	32	7.66	-8.15308	5.75984	-1.41550	0.16123	-4.55476	2.83458	-1.60685	0.11892	-2.09322	**0.03633**	-2.09322	**0.03633**
558839	70	5	1.80	2.79406	11.55793	0.24174	0.80967	16.82027	3.29738	5.10110	**0.03635**	1.65030	0.09888	2.09301	**0.03635**
189292	64	11	6.00	-16.88374	7.93858	-2.12680	**0.03686**	2.58791	5.68822	0.45496	0.66123	-1.16605	0.24359	-2.08726	**0.03686**
178713	63	12	34.50	0.15867	7.87168	0.02016	0.98397	-7.21391	2.95424	-2.44188	**0.03725**	-1.45871	0.14464	-2.08302	**0.03725**
183576	66	9	3.56	-14.18300	8.73528	-1.62365	0.10882	-10.67633	7.06943	-1.51021	0.18173	-2.07815	**0.03770**	-2.07815	**0.03770**
183604	65	10	3.50	-17.47602	8.25768	-2.11634	**0.03777**	2.44284	4.76861	0.51227	0.62422	-1.12248	0.26166	-2.07730	**0.03777**
192912	68	7	4.00	-16.15085	9.73008	-1.65989	0.10129	-9.38830	6.06632	-1.54761	0.19663	-2.07173	**0.03829**	-2.07173	**0.03829**
157162	70	5	3.00	1.88571	11.55943	0.16313	0.87087	-13.34827	2.69467	-4.95358	**0.03842**	-1.34901	0.17733	-2.07035	**0.03842**
556126	63	12	4.17	6.00632	7.83474	0.76663	0.44581	13.77797	5.70122	2.41667	**0.03882**	2.00004	**0.04550**	2.06607	**0.03882**
182911	39	36	7.17	-7.27268	5.71367	-1.27286	0.20716	-4.67100	2.76136	-1.69156	0.10015	-2.05451	**0.03993**	-2.05451	**0.03993**
2497335	29	46	20.37	4.54098	5.89824	0.76989	0.44389	3.92621	1.85492	2.11664	**0.04012**	1.99277	**0.04629**	2.05255	**0.04012**
535955	64	11	2.36	1.39975	8.16458	0.17144	0.86436	15.94772	6.52342	2.44469	**0.04027**	1.57106	0.11617	2.05098	**0.04027**
4381430	38	37	3.92	-11.74236	5.62697	-2.08680	**0.04045**	-0.21595	2.98657	-0.07231	0.94278	-1.49972	0.13369	-2.04915	**0.04045**
178331	70	5	3.80	-24.02767	11.53625	-2.08280	**0.04082**	29.46669	27.34630	1.07754	0.39394	-0.84346	0.39897	-2.04533	**0.04082**
184342	68	7	1.86	-20.17123	9.68574	-2.08257	**0.04084**	26.44693	12.40327	2.13225	0.09995	-0.28287	0.77728	-2.04511	**0.04084**
208739	32	43	3.77	-1.25038	5.86674	-0.21313	0.83183	-5.20414	2.46283	-2.11308	**0.04088**	-1.59600	0.11049	-2.04472	**0.04088**
179384	68	7	1.86	20.03844	9.63435	2.07990	**0.04110**	-2.33202	9.92321	-0.23501	0.82574	1.28863	0.19753	2.04256	**0.04110**
185607	60	15	2.07	-14.56056	7.00931	-2.07732	**0.04134**	10.93694	9.99470	1.09427	0.29531	-0.70256	0.48233	-2.04010	**0.04134**
173965	60	15	2.40	-14.53509	7.00583	-2.07471	**0.04159**	6.87750	7.48061	0.91938	0.37601	-0.81483	0.41517	-2.03762	**0.04159**
187883	64	11	2.45	-12.67475	8.09080	-1.56656	0.12160	-10.71882	7.34360	-1.45962	0.18252	-2.03726	**0.04162**	-2.03726	**0.04162**
189384	71	4	1.00	26.45629	12.78350	2.06956	**0.04208**	0.00000	0.00000	0.00000	1.00000	1.43734	0.15062	2.03270	**0.04208**
4425512	71	4	3.50	-1.33977	12.86587	-0.10413	0.91735	-20.25496	1.34915	-15.01311	**0.04234**	-1.50891	0.13132	-2.03015	**0.04234**
4388068	69	6	18.83	14.29651	10.51443	1.35970	0.17817	4.30248	2.05826	2.09035	0.12773	2.02906	**0.04245**	2.02906	**0.04245**
4326573	70	5	11.00	21.09246	11.31825	1.86358	0.06646	11.41105	8.27877	1.37835	0.30203	2.02756	**0.04261**	2.02756	**0.04261**
573061	69	6	3.50	4.13811	10.63443	0.38912	0.69833	50.63745	14.95752	3.38542	**0.04292**	1.70559	0.08808	2.02449	**0.04292**
110192	60	15	20.60	-5.24261	7.20199	-0.72794	0.46901	-7.44671	3.29346	-2.26106	**0.04313**	-1.94211	0.05212	-2.02247	**0.04313**
178799	69	6	4.00	-2.04063	10.64219	-0.19175	0.84848	-7.03765	2.09034	-3.36675	**0.04352**	-1.56256	0.11816	-2.01873	**0.04352**
177024	70	5	9.40	-3.92276	11.62042	-0.33757	0.73667	-23.33428	5.03988	-4.62993	**0.04362**	-1.66452	0.09601	-2.01772	**0.04362**
4337970	57	18	10.00	0.08319	6.75276	0.01232	0.99020	-9.14735	4.15568	-2.20117	**0.04380**	-1.41686	0.15652	-2.01602	**0.04380**
4473509	24	51	20.41	-2.59374	6.17925	-0.41975	0.67592	4.22386	2.04320	2.06728	**0.04412**	1.12775	0.25942	2.01292	**0.04412**
16054	56	19	19.47	-6.28479	6.59098	-0.95354	0.34350	-4.17527	2.04062	-2.04608	0.05755	-2.01270	**0.04415**	-2.01270	**0.04415**
134671	68	7	4.43	-7.67474	9.87713	-0.77702	0.43969	-12.37322	4.27745	-2.89267	**0.04444**	-1.96759	**0.04912**	-2.00988	**0.04444**
663500	55	20	69.10	7.53020	6.46113	1.16546	0.24768	4.05092	2.27884	1.77762	0.09336	2.00390	**0.04508**	2.00390	**0.04508**
4364405	41	34	10.06	3.01978	5.90734	0.51119	0.61078	-7.13128	3.41718	-2.08689	**0.04521**	-1.05621	0.29087	-2.00266	**0.04521**
4354103	66	9	34.22	17.57991	8.63193	2.03661	**0.04537**	-0.37822	2.63523	-0.14352	0.89057	1.31781	0.18757	2.00123	**0.04537**
340960	69	6	20.83	11.35225	10.57778	1.07322	0.28676	8.46282	3.20694	2.63891	0.07773	2.00060	**0.04544**	2.00060	**0.04544**
110060	70	5	2.00	-23.16109	11.38075	-2.03511	**0.04552**	30.28886	31.91999	0.94890	0.44283	-0.87142	0.38353	-1.99980	**0.04552**
4370025	59	16	7.63	-7.86759	7.16301	-1.09836	0.27571	-6.38082	3.39897	-1.87728	0.08310	-1.99616	**0.04592**	-1.99616	**0.04592**
4294457	64	11	4.64	4.62176	8.30712	0.55636	0.57969	11.05265	4.68512	2.35910	**0.04602**	1.80243	0.07148	1.99518	**0.04602**
33112	70	5	3.60	-4.08595	11.90918	-0.34309	0.73253	18.01051	4.00461	4.49745	**0.04605**	1.16896	0.24242	1.99493	**0.04605**
367946	69	6	7.50	3.32992	10.94231	0.30432	0.76176	-24.09126	7.32276	-3.28992	**0.04608**	-1.19606	0.23167	-1.99465	**0.04608**
193968	17	58	13.78	3.02549	6.87911	0.43981	0.66139	-3.93056	1.92907	-2.03755	**0.04642**	-1.09855	0.27196	-1.99158	**0.04642**
4458227	66	9	16.11	-5.55195	8.85201	-0.62720	0.53251	-11.58563	4.63551	-2.49932	**0.04657**	-1.84863	0.06451	-1.99018	**0.04657**
4358921	59	16	1.69	-13.86867	6.85693	-2.02258	**0.04683**	1.15544	9.29572	0.12430	0.90298	-1.31940	0.18703	-1.98782	**0.04683**
4318177	67	8	268.38	-18.39584	9.10755	-2.01984	**0.04712**	0.85841	0.90988	0.94343	0.38878	-0.79434	0.42700	-1.98520	**0.04712**
365717	62	13	3.69	-15.05745	7.46295	-2.01763	**0.04736**	5.30989	5.49118	0.96699	0.35636	-0.75006	0.45322	-1.98308	**0.04736**
367814	54	21	2.24	-12.67384	6.28268	-2.01727	**0.04740**	13.76642	6.70365	2.05357	0.05484	-0.04426	0.96470	-1.98274	**0.04740**
146554	20	55	91.85	4.17387	6.51845	0.64032	0.52400	-3.50296	1.72831	-2.02681	**0.04782**	-0.94876	0.34274	-1.97894	**0.04782**
175520	69	6	2.33	-21.19602	10.53273	-2.01240	**0.04792**	6.09738	11.16824	0.54596	0.62308	-1.05117	0.29318	-1.97808	**0.04792**
183579	57	18	3.67	-10.18264	6.70845	-1.51788	0.13342	8.83997	4.10929	2.15122	**0.04816**	0.33600	0.73687	1.97592	**0.04816**
182653	70	5	8.80	-22.82301	11.35417	-2.01010	**0.04817**	11.78824	3.10277	3.79927	0.06282	-0.08162	0.93495	-1.97588	**0.04817**
177520	44	31	6.06	-11.48136	5.71953	-2.00740	**0.04846**	3.50370	3.56412	0.98305	0.33400	-0.71221	0.47634	-1.97330	**0.04846**
192127	38	37	4.57	-11.25158	5.61487	-2.00389	**0.04884**	1.35493	3.04026	0.44566	0.65867	-1.08059	0.27988	-1.96994	**0.04884**
326482	30	45	534.71	11.48164	5.73014	2.00373	**0.04886**	-0.23410	0.83908	-0.27899	0.78162	1.19684	0.23137	1.96979	**0.04886**
192015	64	11	2.09	-12.80557	8.07239	-1.58634	0.11704	14.61068	6.32559	2.30977	**0.04971**	0.27943	0.77991	1.96248	**0.04971**

[a] Statistics associated to the two part model for association analysis between the trait and OTUs adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe represented by an OTU with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the OTU for the subjects where that OTU is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per OTU-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Supplemental Information

S5 Table. Taxonomies associated with Body mass index (a); triglycerides (b); HDL, high-density lipoprotein (c); LDL, low-density lipoprotein (d); and TC, total cholesterol (e) at FDR < 0.05 level.

Table S5a. Associated taxonomies with Body mass index (BMI) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
Taxonomie	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	*t* value	*P* value	Estimate	s.e.	*t* value	*P* value	Meta z value	*Meta P* value	*z*	*P value*
k Bacteria p Proteobacteria c Gammaproteobacteria o Cardiobacteriales f Cardiobacteriaceae g Cardiobacterium	71	4	1.75	-4.24195	2.37290	-1.78766	0.07804	-5.52718	0.11621	-47.56072	**0.01338**	-2.99501	**0.00274**	-2.99501	**0.00274**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f Rikenellaceae g Alistipes	16	59	53.17	-3.67338	1.25130	-2.93565	**0.00447**	-0.10021	0.26910	-0.37238	0.71102	-2.27248	**0.02306**	-2.84328	**0.00447**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f [Paraprevotellaceae] g Paraprevotella	21	54	130.56	3.25642	1.12945	2.88319	**0.00519**	0.03801	0.34127	0.11138	0.91175	2.05485	**0.03989**	2.79517	**0.00519**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Clostridiaceae g Clostridium	5	70	129.89	2.61207	2.15636	1.21133	0.22973	0.72489	0.27335	2.65187	**0.00998**	2.67109	**0.00756**	2.67109	**0.00756**
k Bacteria p Bacteroidetes c__Bacteroidia o Bacteroidales f Prevotellaceae g_	71	4	2.25	-0.27906	2.41506	-0.11555	0.90833	-2.24329	0.02838	-79.03470	**0.00805**	-1.95510	0.05057	-2.64978	**0.00805**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f Prevotellaceae g Prevotella	1	74	1607.95	0.00000	0.00000	0.00000	1.00000	0.33719	0.12479	2.70211	**0.00861**	1.85762	0.06322	2.62708	**0.00861**
k Archaea p__Euryarchaeota c Methanobacteria o Methanobacteriales f Methanobacteriaceae g Methanobrevibacter	49	26	31.38	-0.44387	1.13651	-0.39056	0.69728	-1.12528	0.39786	-2.82833	**0.00953**	-2.10823	**0.03501**	-2.59250	**0.00953**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g Citrobacter	66	9	9.33	4.18009	1.57318	2.65710	**0.00970**	0.09168	1.37712	0.06657	0.94909	1.87393	0.06094	2.58628	**0.00970**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Clostridiaceae g_	6	69	38.58	-0.13521	1.98847	-0.06800	0.94598	0.70845	0.26806	2.64287	**0.01026**	1.76725	0.07719	2.56703	**0.01026**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Acidaminococcus	38	37	331.81	2.70483	1.06660	2.53594	**0.01338**	0.23528	0.22186	1.06049	0.29640	2.48728	**0.01287**	2.48728	**0.01287**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f [Odoribacteraceae] g Odoribacter	3	72	46.57	0.82753	2.78051	0.29762	0.76685	-0.75739	0.29845	-2.53775	**0.01342**	-1.53863	0.12390	-2.47244	**0.01342**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g Enterobacter	66	9	2.67	2.75462	1.63425	1.68555	0.09621	2.65084	1.19708	2.21443	0.06872	2.46338	**0.01376**	2.46338	**0.01376**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g [Eubacterium]	27	48	66.77	-0.07356	1.11868	-0.06575	0.94776	0.47227	0.18553	2.54549	**0.01441**	1.68389	0.09220	2.44691	**0.01441**
k Bacteria p Cyanobacteria c Chloroplast o Streptophyta f g_	51	24	10.63	2.78753	1.11435	2.50149	**0.01464**	0.48019	0.63407	0.75731	0.45728	2.25176	**0.02434**	2.44113	**0.01464**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Megamonas	61	14	178.79	0.30809	1.37636	0.22384	0.82352	0.85183	0.29729	2.86532	**0.01537**	1.87141	0.06129	2.42355	**0.01537**
k Bacteria p Cyanobacteria c 4C0d-2 o YS2 f g_	49	26	26.58	-1.16649	1.11858	-1.04283	0.30051	-0.97098	0.40491	-2.39800	**0.02499**	-2.31708	**0.02050**	-2.31708	**0.02050**
k Bacteria p Firmicutes c Bacilli o Turicibacterales f Turicibacteraceae g__Turicibacter	59	16	13.88	1.10087	1.30605	0.84290	0.40208	1.12218	0.43689	2.56858	**0.02336**	2.19590	**0.02810**	2.26755	**0.02336**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Christensenellaceae g__Christensenella	64	11	4.45	-3.23294	1.46582	-2.20556	**0.03061**	-1.04834	1.23857	-0.84640	0.42192	-2.09673	**0.03602**	-2.16213	**0.03061**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Veillonella	14	61	66.15	-0.20052	1.39277	-0.14398	0.88592	0.53177	0.24093	2.20713	**0.03128**	1.42134	0.15522	2.15355	**0.03128**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Lactobacillaceae g_	70	5	29.00	-1.21748	2.14394	-0.56787	0.57189	-0.77258	0.14000	-5.51839	**0.03130**	-1.92224	0.05458	-2.15319	**0.03130**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Lactobacillaceae g Lactobacillus	29	46	161.57	2.24965	1.07070	2.10110	**0.03913**	0.02393	0.27279	0.08772	0.93051	1.52027	0.12844	2.06278	**0.03913**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g_	6	69	305.32	0.24939	2.08736	0.11947	0.90523	0.36601	0.17720	2.06558	**0.04280**	1.51655	0.12938	2.02567	**0.04280**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g Pantoea	32	43	269.12	1.37740	1.07161	1.28535	0.20279	0.37846	0.24448	1.54802	0.12949	1.97265	**0.04854**	1.97265	**0.04854**

[a] Statistics associated to the two part model for association analysis between the trait and microbes adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the microbe for the subjects where that microbe is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S5b. Associated taxonomies with Triglycerides at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
Taxonomie	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	t value	P value	Estimate	s.e.	t value	P value	Meta z value	Meta P value	*z*	*P value*
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Dialister	17	58	268.71	-75.53458	22.52918	-3.35274	**0.00128**	-0.42802	2.06444	-0.20733	0.83652	-2.42339	**0.01538**	-3.22084	**0.00128**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f g_	67	8	5.63	101.77620	30.59668	3.32638	**0.00139**	-82.60462	58.64759	-1.40849	0.21803	1.38978	0.16459	3.19725	**0.00139**
k Bacteria p Fusobacteria c Fusobacteriia o Fusobacteriales f Leptotrichiaceae g Leptotrichia	69	6	2.67	33.97669	37.24747	0.91219	0.36471	-58.45030	9.29023	-6.29159	**0.00811**	-1.23109	0.21829	-2.64744	**0.00811**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Lachnospiraceae g Oribacterium	59	16	2.88	62.41962	23.64636	2.63971	**0.01017**	16.93305	52.48570	0.32262	0.75211	2.04069	**0.04128**	2.57012	**0.01017**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Lachnospiraceae g Lachnospira	0	75	288.77	0.00000	0.00000	0.00000	1.00000	-9.43567	3.79208	-2.48826	**0.01515**	-1.71737	0.08591	-2.42873	**0.01515**
k Bacteria p Firmicutes c Clostridia o Clostridiales f [Mogibacteriaceae] g Mogibacterium	67	8	2.13	29.00754	32.78823	0.88469	0.37927	-66.69096	20.12746	-3.31343	**0.02116**	-1.00824	0.31334	-2.30511	**0.02116**
k Bacteria p TM7 c TM7-3 o CW040 f g_	25	50	155.90	-40.58598	21.04268	-1.92875	0.05770	-3.95866	3.02501	-1.30864	0.19702	-2.25427	**0.02418**	-2.25427	**0.02418**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Phascolarctobacterium	12	63	301.17	13.04554	27.79236	0.46939	0.64021	8.48866	3.75927	2.25806	**0.02759**	1.88829	0.05899	2.20304	**0.02759**
k Bacteria p Firmicutes c Bacilli o Gemellales f Gemellaceae g_	36	39	7.87	40.40409	19.77700	2.04298	**0.04472**	12.83030	11.63355	1.10287	0.27740	2.18743	**0.02871**	2.18743	**0.02871**
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g Atopobium	52	23	5.39	47.54758	21.39399	2.22247	**0.02939**	-18.08059	20.05529	-0.90154	0.37803	0.91687	0.35921	2.17818	**0.02939**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g__Coprobacillus	60	15	4.93	54.46843	24.72433	2.20303	**0.03079**	-18.83968	31.35802	-0.60079	0.55915	1.11414	0.26522	2.15973	**0.03079**
k Bacteria p Firmicutes c Clostridia o Clostridiales f [Tissierellaceae] g WAL_1855D	65	10	4.00	63.21708	28.94208	2.18426	**0.03220**	-49.29793	61.30630	-0.80413	0.44777	0.97777	0.32819	2.14191	**0.03220**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Lactobacillaceae g Lactobacillus	29	46	161.57	-39.88948	20.35123	-1.96005	0.05386	-3.66895	3.38111	-1.08513	0.28391	-2.12099	**0.03392**	-2.12099	**0.03392**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Leuconostocaceae g Weissella	61	14	7.00	15.93906	26.21858	0.60793	0.54515	12.11592	5.09175	2.37952	**0.03653**	1.90636	0.05660	2.09096	**0.03653**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Ruminococcaceae g Ruminococcus	0	75	308.87	0.00000	0.00000	0.00000	1.00000	-12.14391	5.72785	-2.12015	**0.03744**	-1.47144	0.14117	-2.08093	**0.03744**
k Bacteria p Proteobacteria c Epsilonproteobacteria o Campylobacterales f Campylobacteraceae g Campylobacter	62	13	3.92	53.26154	26.04491	2.04499	**0.04451**	-103.67951	45.88597	-2.25950	**0.04741**	0.01881	0.98499	2.00924	**0.04451**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g Catenibacterium	48	27	61.48	-41.98303	20.62267	-2.03577	**0.04545**	-0.50362	4.92345	-0.10229	0.91938	-1.48609	0.13725	-2.00043	**0.04545**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g__Erwinia	49	26	13.69	-6.62846	21.29569	-0.31126	0.75650	11.25878	5.36355	2.09913	**0.04698**	1.18539	0.23586	1.98647	**0.04698**

[a] Statistics associated to the two part model for association analysis between the trait and microbes adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the microbe for the subjects where that microbe is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S5c. Associated taxonomies with High-density lipoprotein (HDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
Taxonomie	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	t value	P value	Estimate	s.e.	t value	P value	Meta z value	Meta P value	*z*	*P value*
k Bacteria p Tenericutes c Mollicutes o Anaeroplasmatales f Anaeroplasmataceae g_	71	4	687.50	7.70278	6.47966	1.18876	0.23844	-10.39396	0.01165	-892.05595	**0.00071**	-1.55944	0.11889	-3.38428	**0.00071**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Lachnospiraceae g Oribacterium	59	16	2.88	1.71378	3.53338	0.48503	0.62913	-19.03891	5.11684	-3.72084	**0.00257**	-1.79074	0.07334	-3.01544	**0.00257**
k Bacteria p Firmicutes c Bacilli o Bacillales f Staphylococcaceae g Staphylococcus	71	4	2.75	18.30074	6.17725	2.96260	**0.00413**	-8.84490	15.41236	-0.57388	0.66832	1.72499	0.08453	2.86795	**0.00413**
k Bacteria p Proteobacteria c Betaproteobacteria o Burkholderiales f Oxalobacteraceae g Oxalobacter	42	33	9.12	6.31233	2.82518	2.23431	**0.02857**	4.51120	2.97952	1.51407	0.14047	2.59043	**0.00959**	2.59043	**0.00959**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g Catenibacterium	48	27	61.48	7.56556	2.89679	2.61170	**0.01096**	1.05257	1.54711	0.68035	0.50279	2.27275	**0.02304**	2.54405	**0.01096**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g_	17	58	36.62	-8.19344	3.32906	-2.46119	**0.01625**	-0.03991	0.52314	-0.07630	0.93946	-1.75313	0.07958	-2.40335	**0.01625**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f Rikenellaceae g Alistipes	16	59	53.17	-0.59856	3.58254	-0.16708	0.86778	-1.87315	0.80143	-2.33727	**0.02303**	-1.72498	0.08453	-2.27301	**0.02303**
k Bacteria p Firmicutes c Bacilli o Gemellales f Gemellaceae g_	36	39	7.87	-5.03986	2.84586	-1.77094	0.08080	-1.58707	1.07656	-1.47421	0.14912	-2.25475	**0.02415**	-2.25475	**0.02415**
k Bacteria p Proteobacteria c Gammaproteobacteria o Cardiobacteriales f Cardiobacteriaceae g Cardiobacterium	71	4	1.75	9.78147	6.46054	1.51403	0.13440	21.38645	3.19541	6.69286	0.09442	2.24119	**0.02501**	2.24119	**0.02501**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f Bacteroidaceae g Bacteroides	0	75	4087.83	0.00000	0.00000	0.00000	1.00000	-2.47105	1.10913	-2.22791	**0.02901**	-1.54385	0.12262	-2.18334	**0.02901**
k Bacteria p Actinobacteria c Actinobacteria o Bifidobacteriales f Bifidobacteriaceae g_	68	7	5.00	10.66025	4.83020	2.20700	**0.03050**	-12.34710	4.71863	-2.61667	0.05901	0.19470	0.84563	2.16350	**0.03050**
k Bacteria p Proteobacteria c Gammaproteobacteria o Pseudomonadales f Moraxellaceae g Acinetobacter	64	11	2.45	8.08179	3.98966	2.02568	**0.04650**	10.40523	9.35575	1.11217	0.29836	2.14305	**0.03211**	2.14305	**0.03211**
k Bacteria p Actinobacteria c Actinobacteria o Bifidobacteriales f Bifidobacteriaceae g Alloscardovia	67	8	15.25	-4.62636	4.67216	-0.99020	0.32539	-2.71062	1.13746	-2.38304	0.06293	-2.01040	**0.04439**	-2.01040	**0.04439**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g Escherichia	54	21	8.67	-0.97245	3.29060	-0.29552	0.76844	2.38835	1.12297	2.12681	**0.04752**	1.19304	0.23285	1.98162	**0.04752**
k Bacteria p Firmicutes c Bacilli o Bacillales f Planococcaceae g_	62	13	2.46	-1.60995	3.82460	-0.42095	0.67505	8.09224	3.58647	2.25632	**0.04767**	1.10387	0.26965	1.98034	**0.04767**
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g Atopobium	52	23	5.39	-6.18905	3.07532	-2.01249	**0.04791**	-0.12069	1.53345	-0.07870	0.93805	-1.45373	0.14602	-1.97817	**0.04791**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Dehalobacteriaceae g Dehalobacterium	48	27	6.04	3.98383	2.98866	1.33298	0.18674	3.92576	2.59107	1.51511	0.14280	1.96979	**0.04886**	1.96979	**0.04886**

[a] Statistics associated to the two part model for association analysis between the trait and microbes adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the microbe for the subjects where that microbe is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S5d. Associated taxonomies with Low-density lipoprotein (HDL) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
Taxonomie	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	t value	P value	Estimate	s.e.	t value	P value	Meta z value	Meta P value	*z*	*P value*
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g_	23	52	14.87	0.79347	5.15124	0.15403	0.87801	5.00216	1.69927	2.94371	**0.00495**	2.09589	**0.03609**	2.81054	**0.00495**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g_	6	69	305.32	-22.70412	8.82754	-2.57196	**0.01218**	-1.08496	0.75551	-1.43606	0.15571	-2.77657	**0.00549**	-2.77657	**0.00549**
k Bacteria p Bacteroidetes c_Bacteroidia o Bacteroidales f Prevotellaceae g_	71	4	2.25	27.77277	10.15788	2.73411	**0.00787**	-12.63501	20.75306	-0.60883	0.65184	1.56023	0.11870	2.65771	**0.00787**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g Escherichia	54	21	8.67	-12.87293	5.15750	-2.49596	**0.01485**	0.11916	2.16502	0.05504	0.95671	-1.68410	0.09216	-2.43595	**0.01485**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f g_	67	8	5.63	17.84628	7.37449	2.42000	**0.01805**	5.25760	7.90853	0.66480	0.53561	2.11011	**0.03485**	2.36468	**0.01805**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Ruminococcaceae g Clostridium	71	4	1.25	-20.64467	10.40515	-1.98408	0.05106	-29.27143	12.88989	-2.27088	0.26407	-2.16926	**0.03006**	-2.16926	**0.03006**
k Bacteria p Firmicutes c Erysipelotrichi o Erysipelotrichales f Erysipelotrichaceae g_Clostridium	68	7	31.43	-0.87960	8.20209	-0.10724	0.91490	-5.75344	1.76883	-3.25267	**0.03130**	-1.59816	0.11001	-2.15328	**0.03130**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Clostridiaceae g SMB53	53	22	3.14	-0.05586	5.21953	-0.01070	0.99149	10.33790	4.45099	2.32261	**0.03146**	1.51361	0.13013	2.15123	**0.03146**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Leuconostocaceae g Weissella	61	14	7.00	8.17949	6.05740	1.35033	0.18114	8.24201	4.40721	1.87012	0.08830	2.15081	**0.03149**	2.15081	**0.03149**
k Bacteria p Proteobacteria c Gammaproteobacteria o Pseudomonadales f Pseudomonadaceae g_Pseudomonas	61	14	27.07	-5.70772	6.05318	-0.94293	0.34887	-4.01931	1.71127	-2.34873	**0.03857**	-2.12520	**0.03357**	-2.12520	**0.03357**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Veillonellaceae g Dialister	17	58	268.71	-0.24483	5.65247	-0.04331	0.96557	-1.57944	0.74002	-2.13433	**0.03729**	-1.50313	0.13280	-2.08259	**0.03729**
k Bacteria p Bacteroidetes c_Bacteroidia o Bacteroidales f g_	54	21	223.33	1.72619	5.27233	0.32741	0.74431	2.43587	1.09260	2.22942	**0.03876**	1.69200	0.09065	2.06670	**0.03876**
k Bacteria p Firmicutes c Clostridia o Clostridiales f [Tissierellaceae] g Peptoniphilus	68	7	7.14	-0.32745	8.21295	-0.03987	0.96831	-9.50340	3.25620	-2.91856	**0.04331**	-1.45698	0.14512	-2.02075	**0.04331**
k Bacteria p Proteobacteria c Gammaproteobacteria o Pasteurellales f Pasteurellaceae g_Actinobacillus	65	10	3.10	2.55452	7.11844	0.35886	0.72075	-13.90841	5.87645	-2.36680	**0.04984**	-1.13411	0.25675	-1.96134	**0.04984**

[a] Statistics associated to the two part model for association analysis between the trait and microbes adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the microbe for the subjects where that microbe is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

Table S5e. Associated taxonomies with Total cholesterol (TC) at FDR < 0.05 level [a]

	Summary of OTUs reads			Binary model				Quantitative model				Meta analysis		Final association	
Taxonomie	No. Absent	No. Present	mean counts in presents	Estimate	s.e.	t value	P value	Estimate	s.e.	t value	P value	Meta z value	Meta P value	*z*	*P value*
k Bacteria p Firmicutes c Clostridia o Clostridiales f Ruminococcaceae g__Anaerotruncus	35	40	5.48	-9.27950	5.74866	-1.61420	0.11086	-6.72385	2.77878	-2.41972	**0.02056**	-2.76497	**0.00569**	-2.76497	**0.00569**
k Bacteria p Proteobacteria c Gammaproteobacteria o Pasteurellales f Pasteurellaceae g_	63	12	4.42	-18.95427	7.59835	-2.49453	**0.01491**	1.12192	6.19121	0.18121	0.86022	-1.59700	0.11026	-2.43460	**0.01491**
k Bacteria p Firmicutes c Bacilli o Gemellales f Gemellaceae g_	36	39	7.87	4.76046	5.75766	0.82681	0.41108	-5.97766	2.53353	-2.35942	**0.02385**	-1.01652	0.30938	-2.25958	**0.02385**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Christensenellaceae g__Christensenella	64	11	4.45	-17.72210	7.88383	-2.24790	**0.02765**	9.16884	8.21083	1.11668	0.29655	-0.81912	0.41272	-2.20228	**0.02765**
k Bacteria p Bacteroidetes c__Bacteroidia o Bacteroidales f g_	54	21	223.33	-3.93402	6.41354	-0.61339	0.54155	2.98066	1.24762	2.38909	**0.02805**	1.12158	0.26204	2.19662	**0.02805**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Clostridiaceae g SMB53	53	22	3.14	-3.42593	6.34833	-0.53966	0.59110	10.51976	4.45741	2.36006	**0.02912**	1.16292	0.24486	2.18186	**0.02912**
k Bacteria p Proteobacteria c Gammaproteobacteria o Enterobacteriales f Enterobacteriaceae g_	6	69	305.32	-23.61437	10.89174	-2.16810	**0.03346**	0.39100	0.95821	0.40805	0.68456	-1.21644	0.22382	-2.12655	**0.03346**
k Bacteria p Proteobacteria c Deltaproteobacteria o Desulfovibrionales f Desulfovibrionaceae g__Desulfovibrio	28	47	205.96	6.29885	5.95658	1.05746	0.29384	2.52163	1.25830	2.00401	0.05125	2.12070	**0.03395**	2.12070	**0.03395**
k Bacteria p Bacteroidetes c Bacteroidia o Bacteroidales f [Paraprevotellaceae] g Paraprevotella	21	54	130.56	10.14829	6.31136	1.60794	0.11223	2.44311	1.70817	1.43025	0.15874	2.11961	**0.03404**	2.11961	**0.03404**
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g Eggerthella	60	15	5.53	-15.04464	7.00762	-2.14690	**0.03517**	-0.64027	4.78001	-0.13395	0.89567	-1.58217	0.11361	-2.10639	**0.03517**
k Bacteria p Firmicutes c Bacilli o Lactobacillales f Leuconostocaceae g Weissella	61	14	7.00	-0.06516	7.47518	-0.00872	0.99307	10.41354	4.43221	2.34952	**0.03852**	1.45706	0.14510	2.06928	**0.03852**
k Bacteria p Actinobacteria c Actinobacteria o Bifidobacteriales f Bifidobacteriaceae g Bifidobacterium	7	68	91.18	-2.49938	9.90983	-0.25221	0.80160	-2.91829	1.39534	-2.09145	**0.04040**	-1.62700	0.10374	-2.04964	**0.04040**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Ruminococcaceae g Ruminococcus	0	75	308.87	0.00000	0.00000	0.00000	1.00000	-3.38723	1.63083	-2.07700	**0.04137**	-1.44236	0.14920	-2.03980	**0.04137**
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g Adlercreutzia	60	15	3.47	-14.53509	7.00583	-2.07471	**0.04159**	5.99994	5.26070	1.14052	0.27632	-0.67104	0.50219	-2.03762	**0.04159**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Peptococcaceae g_	40	35	13.69	-3.96541	5.83952	-0.67906	0.49927	-5.88691	2.77386	-2.12228	**0.04165**	-1.91813	0.05509	-2.03701	**0.04165**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Ruminococcaceae g Clostridium	71	4	1.25	-20.34598	12.80038	-1.58948	0.11633	-36.28571	11.20860	-3.23731	0.19073	-2.03560	**0.04179**	-2.03560	**0.04179**
k Bacteria p Firmicutes c Clostridia o Clostridiales f Clostridiaceae g Sarcina	68	7	30.14	6.51830	9.90905	0.65781	0.51276	-10.72036	3.80527	-2.81724	**0.04796**	-0.93561	0.34948	-1.97770	**0.04796**
k Bacteria p Actinobacteria c Coriobacteriia o Coriobacteriales f Coriobacteriaceae g_	23	52	14.87	0.42868	6.27876	0.06827	0.94576	4.22936	2.10125	2.01278	**0.04965**	1.43614	0.15096	1.96297	**0.04965**

[a] Statistics associated to the two part model for association analysis between the trait and microbes adjusting for age and gender. The binomial analysis that tests for association of detecting a microbe with the trait, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The quantitative analysis tests for association between the trait and the abundance of the microbe for the subjects where that microbe is present, where s.e. represents the standard error of the estimate, *t* -value of a *t* test for the estimate and its associated probability (*P* value). The Meta analysis combine the effect of both binary and quantitative analysis, a meta *P* value was derived using an unweighted Z method. The final association *P* value per microbe-trait pair was assigned from the minimum of *P* values from the binary analysis, quantitative analysis, and meta-analysis.

IX. ANNEXES

FUNDACIÓN ESPAÑOLA DE ARTERIOSCLEROSIS
SOCIEDAD ESPAÑOLA DE ARTERIOSCLEROSIS

El **Comité Científico de la Sociedad Española de Arteriosclerosis**
y el **Comité Organizador del XXVI Congreso Nacional**
han decidido otorgar, por su calidad científica,

MENCIÓN ESPECIAL 2013 (15/23)
A LA COMUNICACIÓN PRESENTADA EN EL XXVI CONGRESO NACIONAL S.E.A. ZARAGOZA 2013

a la comunicación ORAL:

LA COMUNIDAD MICROBIANA INTESTINAL CAMBIA EN FUNCIÓN DEL NÚMERO DE CRITERIOS DE SÍNDROME METABÓLICO (SMET).

presentada por los autores:

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Fernando Civeira
Presidente S.E.A.

Miguel Pocovi
Presidente XXVI Congreso Nacional

XXVI Congreso Nacional de la Sociedad Española de Arteriosclerosis
Zaragoza 22, 23 y 24 de Mayo de 2013

Sociedad Española de Arteriosclerosis

El COMITÉ CIENTÍFICO DE LA SOCIEDAD ESPAÑOLA DE ARTERIOSCLEROSIS

Y

El COMITÉ ORGANIZADOR DEL XXVIII CONGRESO NACIONAL

han decidido otorgar por su calidad científica el

PREMIO MENCIÓN ESPECIAL 2015

a la comunicación presentada

EN EL XXVIII CONGRESO NACIONAL S.E.A.

LOGROÑO 2015

La comunidad microbiana intestinal en pacientes con síndrome metabólico es modificada por la dieta.

Presentada por los autores

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Juan F. Ascaso Gimilio
Presidente S.E.A.

Ángel Brea Hernando
Presidente XXVIII Congreso Nacional

El COMITÉ CIENTÍFICO DE LA SOCIEDAD ESPAÑOLA DE ARTERIOSCLEROSIS

Y

El COMITÉ ORGANIZADOR DEL XXIX CONGRESO NACIONAL

Han decidido otorgar por su calidad científica el

PREMIO MENCIÓN ESPECIAL 2016

A la comunicación Oral presentada

EN EL XXIX CONGRESO NACIONAL S.E.A.

GRANADA 2016

Mejora de la disbiosis intestinal en pacientes con Síndrome Metabólico tras dos modelos de dietas saludables: dieta baja en grasa y dieta Mediterránea. Estudio CORDIOPREV

Presentada por los autores

Carmen María Haro Mariscal; Ana León-acuña; Jose David Torres-peña; Ruth Blanco-rojo; Sonia Garcia-carpintero; Juan Criado; Antonio Camargo; Francisco Pérez-Jiménez

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Juan F. Ascaso Gimilio
Presidente S.E.A.

Granada, 20 de mayo de 2016