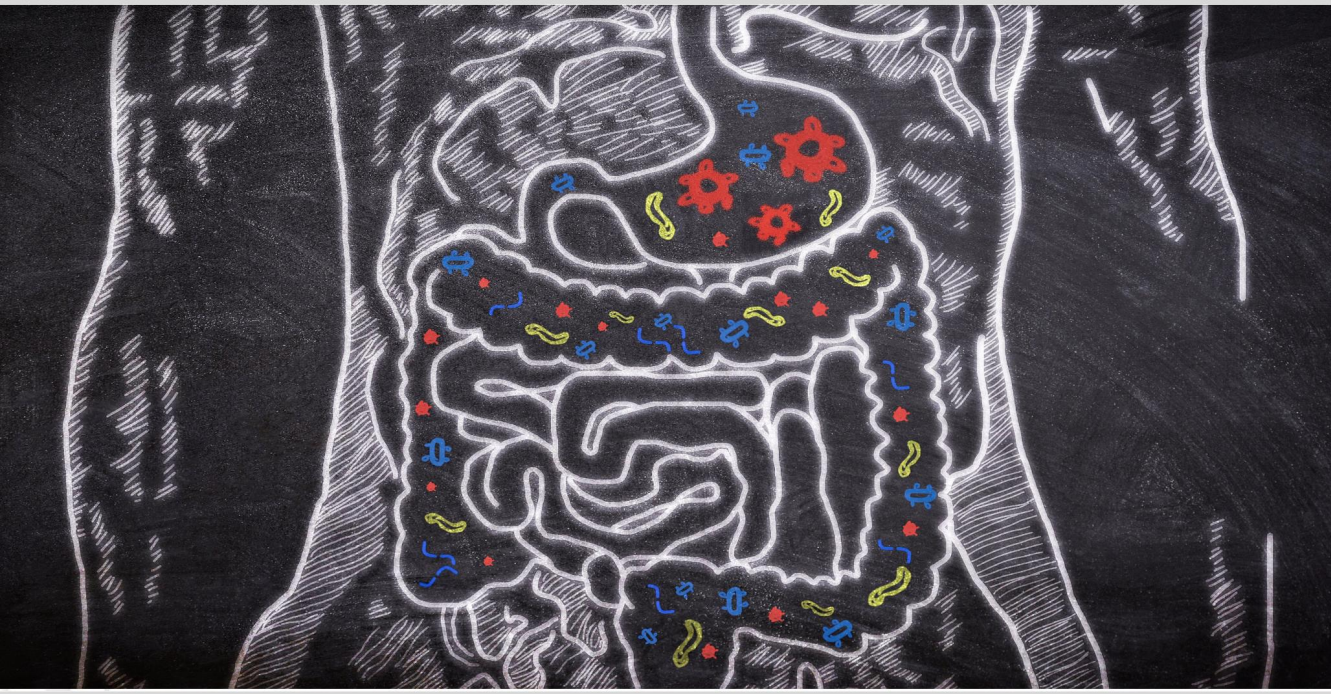
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME

Alessandro Atzeni

UNIVERSITAT ROVIRA i VIRGILI

Gut Microbiota Signatures Associated with Weight Loss and Insulin Resistance in an Elderly Mediterranean Population with Overweight/Obesity and Metabolic Syndrome

ALESSANDRO ATZENI



DOCTORAL THESIS
2022

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

Alessandro Atzeni

Gut Microbiota Signatures Associated with Weight Loss and Insulin Resistance in an Elderly Mediterranean Population with Overweight/Obesity and Metabolic Syndrome

DOCTORAL THESIS

Thesis supervised by:

Dr. Monica Bulló

&

Prof. Jordi Salas-Salvadó

UNIVERSITAT ROVIRA i VIRGILI

Unitat de Nutrició Humana

Departament de Bioquímica i Biotecnologia

Universitat Rovira i Virgili

2022

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA i VIRGILI

FAIG CONSTAR que aquest treball, titulat "Gut Microbiota Signatures Associated with Weight Loss and Insulin Resistance in an Elderly Mediterranean Population with Overweight/Obesity and Metabolic Syndrome", que presenta Alessandro Atzeni per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament Bioquímica i Biotecnologia d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "Gut Microbiota Signatures Associated with Weight Loss and Insulin Resistance in an Elderly Mediterranean Population with Overweight/Obesity and Metabolic Syndrome", que presenta Alessandro Atzeni para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento Bioquímica y Biotecnología de esta universidad.

I STATE that the present study, entitled "Gut Microbiota Signatures Associated with Weight Loss and Insulin Resistance in an Elderly Mediterranean Population with Overweight/Obesity and Metabolic Syndrome", presented by Alessandro Atzeni for the award of the degree of Doctor, has been carried out under my supervision at the Department Biochemistry and Biotechnology of this university.

Reus, 14th February 2022

El/s director/s de la tesi doctoral
El/los director/es de la tesis doctoral
Doctoral Thesis Supervisor/s

Monica Bulló, PhD

Jordi Salas-Salvadó, PhD, MD

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

A Francesca,

forever scientist

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

Acknowledgments

First of all, I would express my gratitude to my doctoral thesis supervisors Jordi and Monica, for the support, the assistance and precious advice during these 40 months, that was crucial to reach this objective. It has been a pleasure and a privilege to share with you this professional step of my life.

I would like to thank all the scientists, workers and participants of the PREDIMED-Plus study, which make possible to create such a huge and important scientific platform that has started years ago and follows nowadays producing a lot of research.

I would like to thank all my colleagues and ex colleagues of the human nutrition unit for the moments we shared and the professional and emotional support. I wish you all the best.

To all the Gut-Microbiota-Brain axis team of the APC Microbiome Cork, especially to Thomaz and John for giving me the opportunity to work together during my “virtual” secondment, considering the special conditions produced by the pandemic. Despite all we were able to successfully generate a valid piece of research, and most important you gave me the opportunity to learn and grow as a scientist. It was an honor to collaborate with you and I hope to have the opportunity to do it again in the future.

To the Martí i Franquès COFUND fellowship programme coordinators for the support in all the administrative and bureaucratic steps and for involving me in all the divulgation events, it was a great experience.

Non finirò mai di essere grato per il resto della mia vita ai miei genitori, per avermi fatto diventare la persona che sono ora. Senza di loro tutto questo non sarebbe mai potuto accadere.

Alla mia famiglia e ai miei amici, alla mia terra, dal quale mi separano solamente 900 km di mar Mediterraneo, ma che a volte sono sembrano anni luce.

En fin, por último y no por ello menos importante, a mi alma gemela, mejor compañera de vida, y futura mujer Ledi, por su amor incondicional y por haberme aguantado y por seguir aguantándome (espero aún por mucho tiempo). Te quiero.

Thank you.

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

Abstract

ENGLISH

Alterations in gut microbial composition and functionality may contribute to the development of obesity and insulin resistance. The identification of specific gut bacteria and metabolic pathways associated with obesity and related metabolic disorders represents a promising novel perspective to tailor personalized interventions.

The main objectives of this doctoral thesis, designed within the frame of the PREDIMED (PREvención con DIeta MEDiterránea)-Plus clinical trial, are: (i) Explore the association between fecal microbiota, body mass index and changes in total body weight after 12-month lifestyle intervention in elderly subjects (aged 55–75 years) with overweight/obesity and metabolic syndrome; (ii) explore the association between fecal microbiota and insulin resistance, estimated by HOMA-IR index, in non-diabetic subjects from the same cohort.

Results shown specific fecal microbiota taxonomic features associated with lower body mass index and higher weight loss at 12-month follow-up. Specific genera and related metabolic pathways were negatively associated with higher HOMA-IR index and linked with improved glucose homeostasis via increased amino acid breakdown and sulfate reduction. Besides, specific genera and related metabolic pathways were positively associated with HOMA-IR index and linked to increased saccharide degradation inducing abnormal butyrate production.

Our results support the identification of specific gut microbiota signatures to predict obesity treatments' success, but also to target potential bacterial candidates for the development of therapies for insulin resistance. These findings encourage further investigation especially in terms of deepen taxonomic exploration and metabolomics profiling.

CASTELLANO

Las alteraciones en la composición y la funcionalidad de la microbiota intestinal pueden contribuir al desarrollo de la obesidad y la resistencia a la insulina. La identificación de específicas bacterias intestinales y de vías metabólicas asociadas a la obesidad y a los trastornos metabólicos relacionados, representa una perspectiva novedosa y prometedora para adaptar las intervenciones personalizadas.

Los principales objetivos de esta tesis doctoral, diseñada en el marco del ensayo clínico PREDIMED (PREvención con DIeta MEDiterránea)-Plus, son: (i) Explorar la asociación entre la microbiota fecal, el índice de masa corporal y los cambios en el peso corporal total tras una intervención de 12 meses sobre el estilo de vida en sujetos de edad avanzada (entre 55 y 75 años) con sobrepeso/obesidad y síndrome metabólico; (ii) explorar la asociación entre la microbiota fecal y la resistencia a la insulina, estimada mediante el índice HOMA-IR, en sujetos no diabéticos de la misma cohorte.

Los resultados mostraron distintivos taxonómicos específicos de la microbiota fecal asociados con un menor índice de masa corporal y una mayor pérdida de peso a los 12 meses de seguimiento. Algunos géneros en concreto y las vías metabólicas relacionadas se asociaron negativamente con un mayor índice HOMA-IR y se vincularon con una mejor homeostasis de la glucosa a través de una mayor descomposición de aminoácidos y reducción de sulfatos. Además, otros géneros específicos y las vías metabólicas relacionadas se asociaron positivamente con el índice HOMA-IR y se relacionaron con una mayor degradación de los sacáridos que induce una producción anormal de butirato.

Estos resultados apoyan la identificación de distintivos específicos de la microbiota intestinal para predecir el éxito de los tratamientos contra la obesidad, pero también para seleccionar posibles candidatos bacterianos para el desarrollo de terapias contra la resistencia a la insulina. Estos resultados animan a seguir investigando, especialmente en lo que se refiere a la profundización de la exploración taxonómica y la elaboración de perfiles metabolómicos.

CATALÀ

Les alteracions en la composició i la funcionalitat de la microbiota intestinal poden contribuir al desenvolupament de l'obesitat i la resistència a la insulina. La identificació d'específiques bactèries intestinals i vies metabòliques associades a l'obesitat i als trastorns metabòlics relacionats, representa una perspectiva innovadora i prometedora per adaptar les intervencions personalitzades.

Els objectius principals d'aquesta tesi doctoral, dissenyada en el marc de l'assaig clínic PREDIMED (PREvenció amb DIeta MEDiterrània)-Plus són: (i) Explorar l'associació entre la microbiota fecal, l'índex de massa corporal i els canvis en el pes corporal total, després de 12 mesos d'intervenció en l'estil de vida de persones grans (entre 55 i 75 anys) amb sobrepès/obesitat i síndrome metabòlic; (ii) explorar l'associació entre la microbiota fecal i la resistència a la insulina, estimada per l'índex HOMA-IR, en subjectes no diabètics de la mateixa cohort.

Els resultats van mostrar distintius taxonòmics específics de microbiota fecal associats a un menor índex de massa corporal i una major pèrdua de pes als 12 mesos de seguiment. Alguns gèneres en concret i les vies metabòliques relacionades es van associar negativament amb un índex HOMA-IR més alt i es van relacionar amb una millor homeòstasi de la glucosa a través d'una major degradació dels aminoàcids i reducció de sulfats. A més, altres gèneres específics i les vies metabòliques relacionades es van associar positivament amb l'índex HOMA-IR i es van relacionar amb un augment de la degradació de sacàrids que indueix una producció anormal de butirat.

Els nostres resultats donen suport a la identificació de distintius específics de microbiota intestinal per predir l'èxit dels tractaments contra l'obesitat, però també per seleccionar possibles candidats bacterians per al desenvolupament de teràpies contra la resistència a la insulina. Aquests resultats fomenten el seguir investigant, especialment pel que fa a l'aprofundiment de l'exploració taxonòmica i l'elaboració de perfils metabolòmics.

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

Abbreviations

WHO	World Health Organization
BMI	Body mass index
CVD	Cardiovascular disease
T2D	Type 2 diabetes
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
SNS	Sympathetic nervous system
IR	Insulin receptor
PI3K	Phosphoinositide-3-kinase
Akt/PKB	Protein kinase B
GSK-3β	Glycogen synthase kinase-3β
IRS	Insulin receptor substrates
TNF-α	Tumor necrosis factor-α
IL-6	Interleukin-6
IL-1β	Interleukin-1
ER	Endoplasmic reticulum
ATH	Adipose tissue hypoxia

ADA	American Diabetes Association
FPG	Fasting plasma glucose
OGTT	Oral glucose tolerance test
NGSP	National Glycohemoglobin Standardization Program
DCCT	Diabetes Control and Complications Trial
GWAS	Genome wide association studies
SCFA	Short-chain fatty acid
LPS	Lipopolysaccharide
GF	Germ-free
HFD	High fat diet
Fiaf	Fasting-induced adipocyte factor
Pcg-1α	Peroxisomal proliferator-activated receptor coactivator
AMPK	Adenosine monophosphate activated protein kinase
WAT	White adipose tissue
TLR4	Toll-like receptor
GALT	Gut-associated lymphoid tissues
CLA	Conjugated linoleic acid
BCAA	Branched-chain amino acids

PAMP	Pathogen-associated molecular patterns
MyD88	Myeloid differentiation protein 88
MAL	Myd88 adapter-like protein
IRAK	IL-1 receptor associate kinase
TRAF6	TNF receptor associated factor
TAK1	Transforming growth factor B-associated kinase
JNK	c-Jun N-terminal kinase
IKK	I kappa B kinase
NF-κB	Nuclear factor kappa B
IκB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor
iNOS	Inducible nitric oxide synthase
DAMP	Damage-associated molecular patterns
GPR43	G-protein-coupled receptors 43
GPR41	G-protein-coupled receptors 41
Treg	regulatory T cells
GPBAR1	G protein-coupled bile acid receptor 1
GLP-1	glucagon-like peptide 1
MedDiet	Mediterranean diet

MetS	Metabolic syndrome
IG	Intervention group
CG	Control group
FMT	Fecal microbiota transplantation
MEDAS	Mediterranean Diet Adherence Screener
HOMA-IR	Homeostatic model assessment of insulin resistance
ANOVA	One-way analysis of variance
BAM	Binary Alignment Map
GMM	Gut metabolic module

Table of Contents

I. INTRODUCTION 1

1. OBESITY 3

1.1 Definition, epidemiology and public health burden 3

1.2 Pathophysiology and consequences of obesity 4

1.3 Management of obesity 6

2. OBESITY INDUCED INSULIN RESISTANCE AND TYPE 2 DIABETES 8

2.1 Glucose homeostasis and insulin resistance 8

2.1.1 Insulin signaling 8

2.1.2 Cellular and molecular mechanisms of insulin resistance 9

2.1.3 The link between obesity, insulin resistance and type 2 diabetes ... 10

2.2 Prediabetes and type 2 diabetes 12

3. ROLE OF GUT MICROBIOTA IN OBESITY AND INSULIN RESISTANCE 15

3.1 General characteristics of the human gut microbiota 15

3.2 The link between gut microbiota and obesity 16

3.3 The link between gut microbiota and insulin resistance 19

3.3.1 Molecular mechanisms of lipopolysaccharide-induced insulin resistance 19

3.3.2 The anti-inflammatory effects of short-chain fatty acids 21

3.3.3 The action of bile acids and branched-chain amino acids 21

3.4 Microbiota modulation to treat obesity and insulin resistance 22

3.4.1 The role of diet 22

3.4.2 Lifestyle interventions 25

3.4.3 Prebiotics and probiotics 26

3.4.4 Fecal microbiota transplantation 27

II. JUSTIFICATION 29

III. HYPOTHESIS 33

IV. OBJECTIVES **37**

V. METHODOLOGY **41**

1. STUDY PARTICIPANTS: THE PREDIMED-PLUS TRIAL 43

2. CLINICAL AND DIETARY ASSESSMENT 44

3. BLOOD SAMPLING AND BIOCHEMICAL ANALYSIS 45

4. STOOL SAMPLES COLLECTION, MICROBIAL DNA EXTRACTION AND 16S AMPLICON SEQUENCING 45

5. STATISTICAL ANALYSIS 46

5.1 Fecal microbiota analysis 46

5.2 Analysis of participants´ clinical characteristics 46

VI. RESULTS **49**

PUBLICATION 1 51

Atzeni A, Galié S, Muralidharan J, Babio N, Tinahones FJ, Vioque J, Corella D, Castañer O, Vidal J, Moreno-Indias I, *et al*. Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome. Microorganisms. 2021; 9 (2): 346.

PUBLICATION 2 73

Atzeni A, Bastiaanssen TFS, Cryan JF, Tinahones FJ, Vioque J, Corella D, Fitó M, Vidal J, Moreno-Indias I, Gómez Pérez AM, Torres-Collado L, Coltell O, Castañer O, Bulló M, Salas-Salvadó J. Taxonomic and Functional Fecal Microbiota Signatures Associated with Insulin Resistance in Non-Diabetic Subjects with Overweight/Obesity Within the Frame of the PREDIMED-Plus Study. Frontiers in Endocrinology. Accepted on March 21, 2022.

VII. DISCUSSION **103**

VIII. CONCLUSIONS **113**

IX. REFERENCES **117**

X. APPENDICES **139**

List of figures

Figure 1: Metabolic and non-metabolic comorbidities associated to obesity

Figure 2: Cellular and molecular insulin signaling pathway

Figure 3: Mechanisms linking obesity and insulin resistance

Figure 4: Cellular and molecular pathway of lipopolysaccharide inducing insulin resistance

Figure 5: Mechanism contributing to explain the link between gut microbiota and insulin resistance

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

I. INTRODUCTION

1. Obesity

1.1 Definition, epidemiology and public health burden

Obesity is a chronic multifactorial disease characterized by an excess of adipose tissue accumulation affecting health and often associated to the development of several related disorders [1]. According to the World Health Organization (WHO), a body mass index (BMI) ≥ 25 kg/m^2 is considered overweight, whereas a BMI $\geq$ 30 kg/m^2 is considered obese [2], however, the use of BMI as classification criteria has been criticized because does not completely reflect the total fat mass and its distribution [3].

In the last decades obesity has become a worldwide major public health problem that has rapidly increased up to reach alarming epidemic proportions in Western populations [4]. WHO estimated that worldwide, in 2016 more than 1.9 billion adults were overweight and over 650 millions of these were obese, respectively 39% and 13% of adult population aged 18 years and over. The worldwide prevalence of obesity nearly tripled between 1975 and 2016 [2]. The Global Burden of Disease group has estimated that elevated BMI was linked to 4 million deaths in 2015 [5]. The National Health and Nutrition Examination Survey conducted in the United States reported that obesity prevalence has reached almost 40% in 2015–2016 [6]. According on 2005 incidence figures it has been projected that by 2030 almost 40% of the world's population will be overweight and one in five people will be obese [7].

Obesity has an important negative impact on health and in reducing the quality of life, specifically contributing to the global incidence of various conditions linked with increased mortality such as cardiovascular diseases (CVD), certain types of cancer, type 2 diabetes (T2D), osteoarthritis, work disability and sleep apnea [8]. As documented by the Global Burden of Disease Study, obesity is indeed a major contributor to ill-health, disability and mortality worldwide [9].

The treatment of obesity and related conditions has also an additional load on healthcare system [10].

1.2 Pathophysiology and consequences of obesity

Obesity is driven by an imbalance between energy intake and energy expenditure [11]. The causes of this imbalance involve a complex interplay of genetic, behavioral, socioeconomic, and environmental factors [12]. Among the environmental factors, the easier and greater food availability, the increase of highly processed food consumption and sedentary lifestyle are considered the major causes of obesity, but culture and socialization also play an important role [13].

Adipose tissue accumulation evolves slowly over time as a consequence of a long-term positive energy balance. The accumulation of lipids, mainly triglycerides, occurs principally in the subcutaneous adipose tissue, and is accompanied by an increase in the volume of skeletal muscle, liver and other organs and tissues. There is an increase in macrophages and other immune cell in the visceral adipose tissue, due to tissue remodeling in response to adipocytes apoptosis. These immune cells secrete pro-inflammatory cytokines, contributing to metabolic disturbances associated with obesity [14]. Adipocytes and macrophages within the adipose tissue synthesize cell-signaling proteins, such as adipokines, which in case of excessive secretion, bring to low-grade systemic inflammation [15]. The hydrolysis of triglycerides within adipocytes brings to the release of free fatty acids in plasma, whereas lipid intermediates accumulate in non-adipose tissues causing lipotoxicity, with cellular dysfunction and apoptosis [15]. Lipids are also contained in liposomes, that can access into the cytoplasm of many cells, like hepatocytes, causing hepatic damage [16,17]. All these factors contribute to impaired insulin sensitivity, a condition that frequently characterizes individuals with overweight and obesity, and especially those with

central obesity [15] and that will be deepen discussed in the next sections of this thesis introduction.

The metabolic disturbances associated with obesity are one of the mechanisms underlying the dyslipidemia of obesity, a condition characterized by high levels of fasting plasma triglycerides and low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol; but also other disturbances such as insulin resistance and T2D and obesity related liver disease [14]. Moreover, obesity induces hypertension, a chronic medical condition in which the blood pressure is persistently increased. Mechanisms such as the activation of the sympathetic nervous system (SNS), increased intra-abdominal and intra-vascular fat, sodium retention leading to increase in renal reabsorption, and the renin-angiotensin system, seems to have important functions in the pathogenesis of obesity-related hypertension. However, the mechanism by which obesity directly causes hypertension is still under investigation [18]. The excessive omental and mesenteric fat accumulation could be also be responsible of mechanical and functional alterations associated to obesity such us osteoarthritis, restrictive respiratory insufficiency, sleep apnea syndrome, or cardiac insufficiency, among others. Figure 1 depicts all metabolic and non-metabolic comorbidities associated to obesity.

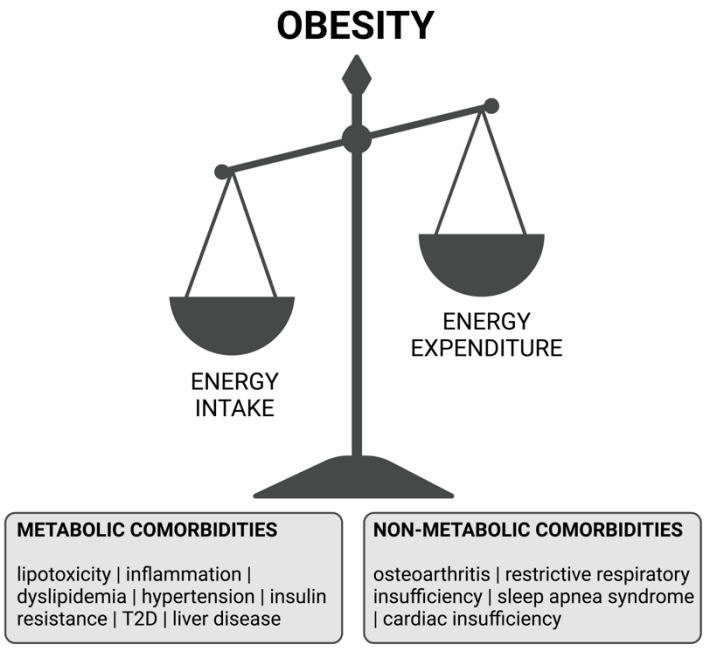


Figure 1: Metabolomic and non-metabolic comorbidities associated to obesity

1.3 Management of obesity

The type of treatments for obesity should be adjusted according with the severity of the pathology [19]. In any case, weight loss should be recommended to all patients with overweight, obesity and related comorbidities [20]. Lifestyle interventions are the first choice for obesity treatment due to safety and contained costs, and they may include dietary modifications, physical activity promotion, and behavioral support [21]. However, long-term effectiveness of lifestyle interventions remains challenging and needs continuous active participations of patients and regular contacts with specialist during one year or more of follow-up [20]. Pharmacological therapeutic approaches should be considered for adult patients with a BMI $\geq$ 30 kg/m^2 and BMI 27–29 kg/m^2 with weight-related comorbidities [20], and should be used in combination with lifestyle interventions in order to support long-term weight management, but also in those patients who did not achieve beneficial results from lifestyle modification approaches [22,23].

Antiobesity drugs aim to reduce caloric intake by improving satiety, helping patients to achieve better compliance with their diet plan, reducing fat absorption, or increasing energy expenditure. Again, the support of professionals about lifestyle changes and food selection in necessary in order to improve the effectiveness of the combination with pharmacotherapies and avoid weight regain [24]. Surgical treatments are more effective than other weight loss medical approaches and are used in case of severe obesity (BMI ≥ 40 kg/m^2) or obesity (BMI 35–39 kg/m^2) in presence of weight-related comorbidities. However, these procedures are more invasive and associated with greater risks [19,23]. Different surgical techniques may be adopted according to the rate of morbidity and mortality, the degree of weight loss desired, the weight loss maintenance, and the rate of comorbidities resolution over time [25]. In general, according to the type of surgery, weight loss is induced limiting the amount of food consumed by reducing the size of the stomach (restrictive surgeries) or limiting nutrients absorption bypassing portions of the small intestine (malabsorptive surgeries) [25].

2. Obesity induced insulin resistance and type 2 diabetes

2.1 Glucose homeostasis and insulin resistance

2.1.1 Insulin signaling

Insulin is a peptide hormone that plays an essential role in the process of glucose homeostasis inducing the access of glucose into muscle, the synthesis of glycogen in the liver and muscle, and fat deposition into adipocytes. In addition, insulin enhances the synthesis of proteins, promotes cell survival and growth, prevents of protein catabolism, and has anti-inflammatory effects [26]. All of the physiologic effects mentioned are produced by a series of downstream intracellular signals activated by insulin [27]. Figure 2 shows cellular and molecular insulin signaling pathway. When insulin binds the insulin receptor (IR), this is able to phosphorylate various substrates that can activate the enzyme phosphoinositide-3-kinase (PI3K). PI3K targets and activates the protein kinase B (Akt/PKB), that drives the above-mentioned metabolic actions of insulin. In addition, Akt/PKB targets glycogen synthase kinase-3β (GSK-3β), which is a negative regulator of glycogen synthase, the enzyme that catalyzes the synthesis of glycogen. GSK-3β also operates as a negative regulator of upstream insulin signaling attenuating the insulin response by inhibiting the phosphorylation of insulin receptor substrates (IRS). Increased GSK-3β activity decreases the synthesis of glycogen leading to upstream insulin signaling impairment [26].

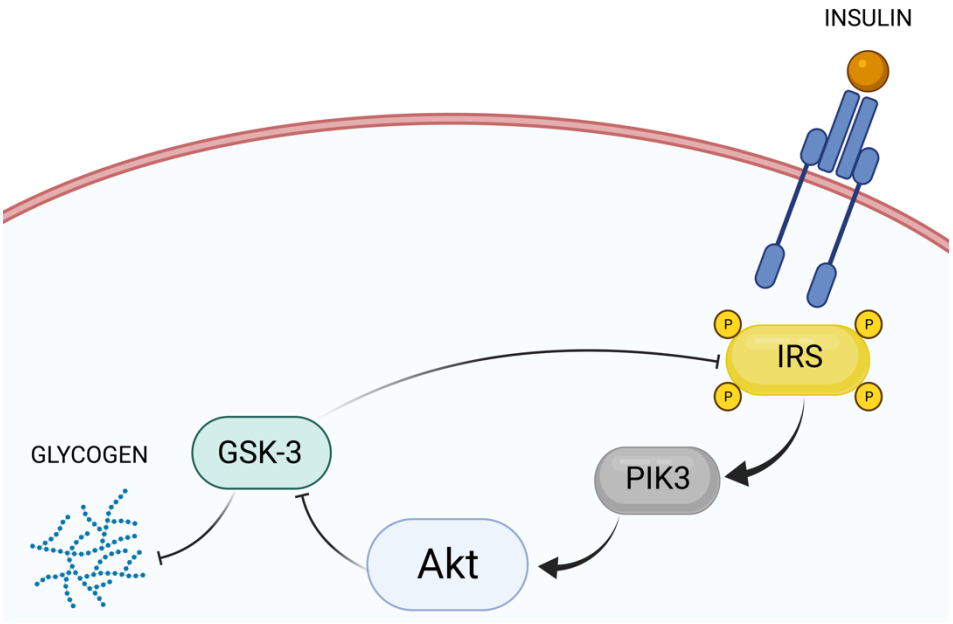


Figure 2: Cellular and molecular insulin signaling pathway

2.1.2 Cellular and molecular mechanisms of insulin resistance

Insulin resistance is a condition in which beta cells of the pancreatic islets secrete excessive levels of insulin in order to compensate normal levels of glucose in the blood, and is caused by a reduced sensitivity or responsiveness to insulin from peripheral tissues [28]. At intracellular level there is an impaired insulin signaling from the IR downstream to the final substrates involved in multiple functional mechanisms [29]. Several animal and human studies have consistently demonstrated that insulin resistance may be attributed to a reduced strength of insulin signaling via the IRS and PI3K pathway, resulting in a decrease of glucose uptake and utilization in insulin target tissues [29]. Among the potential mechanism involved it seem that serine phosphorylation of IRS proteins, in response to a number of intracellular serine kinases, can reduce the attraction to PI3K minimizing its activation, it means that an excessive phosphorylation of IRS proteins could disturb the insulin signaling downstream, causing insulin

resistance [29]. Potential triggering mechanisms such as mitochondrial dysfunction [30], hyperinsulinemia [31], or hyperglycemia [32] have also been described and confirmed by different studies. A second potential complementary molecular mechanism involved in the pathogenesis of insulin resistance could be attributed to an imbalance between PI3K subunits that occurs under certain circumstances, such as short-term overfeeding, gestational diabetes, steroid- and GH-induced insulin resistance, obesity and diabetes. However, it seems that a combination of the two mechanisms above mentioned is necessary in order to lead to insulin resistance [29].

2.1.3 The link between obesity, insulin resistance, and type 2 diabetes

Obesity has been identified as a major cause for the development of insulin resistance and hyperglycemia associated with T2D [26], as increased body fat and abdominal adiposity is an important risk factor for the development of T2D [28]. Mechanisms linking obesity and insulin resistance are summarized in Figure 3. Overstressed adipocytes suffer a process of apoptosis, activating an inflammatory response [33]. In addition, the infiltration of monocytes and activated macrophages in the adipose tissue promotes the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1β), from the visceral adipose tissue into the portal vein, a mechanism which have been directly implicated in the pathogenesis of insulin resistance [26]. One suggested mechanism of action of these inflammatory mediators is through serine phosphorylation of IRS [34]. Macrophage infiltration in the liver promotes the development of hepatic inflammation and steatosis [35].

Another mechanism associating obesity with insulin resistance and T2D seem to be linked with the accumulation of triglycerides in muscle and liver which might cause a decrease in mitochondrial function [36]. Excessive and ectopic lipid accumulation in adipocytes, liver, muscle, and outside may cause insulin resistance even without inflammatory responses through metabolically toxic

product formation. These lipids may activate signaling pathways that negatively impact insulin signal transduction like one or more of the protein kinase C proteins [37]. Further association comes from the assumption that adipocyte, which are involved in the secretion of many peptide hormones, such as leptin, retain the capacity to perform their local functions and systemic metabolism in different organs [37]. In addition, it has been observed that an increase in the level of adipokines such resistin induces insulin resistance through inhibition of glucose transport in vitro, and increases hepatic glucose production and fasting blood glucose concentrations in vivo [38].

Studies revealed that stress of the endoplasmic reticulum (ER) brings to unfolded protein response which are central factors for insulin resistance and development of T2D. Enhanced unfolded and/or misfolded proteins in the ER lumen may be caused by the depletion of calcium stores from the ER. The presence of a high level of misfolded proteins results in the activation of signaling pathways to restore homeostasis and may lead to insulin resistance [39]. Adipose tissue hypoxia (ATH) may stimulate cellular mechanisms for leptin elevation, mitochondrial dysfunction, macrophage infiltration, chronic inflammation, adiponectin reduction, ER stress, and adipocyte death in obese individuals. In addition, inhibition of adipogenesis and triglyceride synthesis by hypoxia may be a new mechanism for elevated fatty acids in the blood during obesity [40].

Hypothalamic IRs function inhibition results in impaired hepatic glucose metabolism and insulin resistance [37]. IRs, IRS-1 gene polymorphisms affect insulin signaling, but also polymorphisms of other genes associated with visceral obesity, such as uncoupling protein gene, β3 adrenergic receptor gene, and GLUT-4 gene [41].

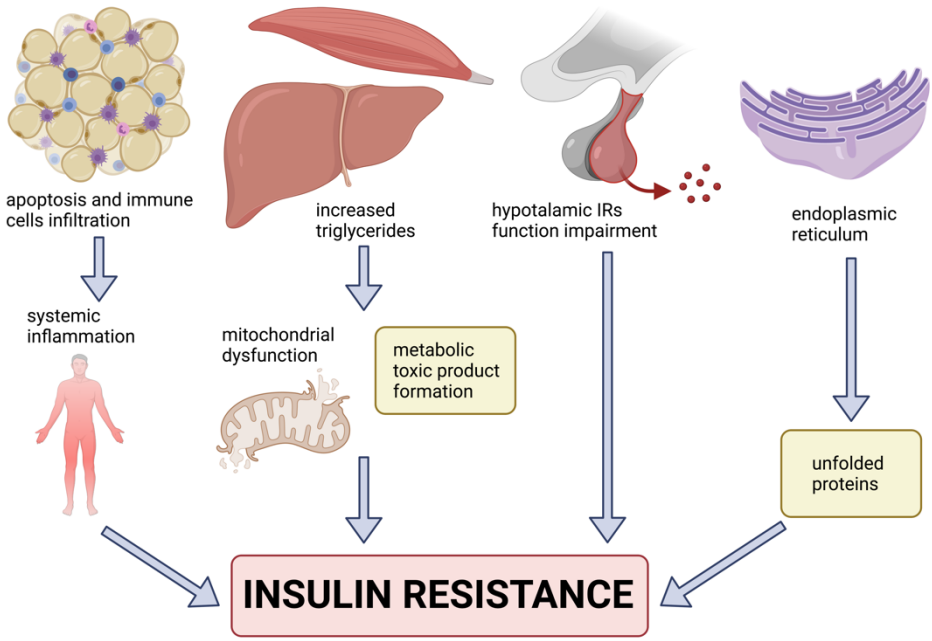


Figure 3: Mechanisms linking obesity and insulin resistance

2.2 Prediabetes and type 2 diabetes

T2D is a chronic metabolic disorder characterized by a progressive disruption of glucose metabolism and homeostasis [42]. According to the WHO, about 442 million people worldwide, around 9% of the adult population, have diabetes, and T2D accounts more than 90% of the total [43,44]. T2D is considered an important public health concern worldwide with a considerable impact on human health and quality of life, leading to significant morbidity and premature mortality [45]. People with T2D are at increased risk of many complications such as CVD, nephropathy, retinopathy, diabetic foot, cancer, cognitive decline, sleep apnea, mood disorders, bone metabolism impairment. Most of these T2D complications are mainly due to complex and interconnected mechanism such as hyperglycemia, insulin resistance, low-grade inflammation, accelerated atherogenesis [46].

The American Diabetes Association (ADA) "Standards of Medical Care in Diabetes–2021", described the criteria for the diagnosis of T2D: (i) Fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L) in at least two consecutive determinations, with fasting defined as no caloric intake for at least 8 h; (ii) Plasma glucose concentration 2 hours after oral glucose tolerance test (OGTT) (ingestion of 75 g of anhydrous glucose dissolved in water) ≥ 200 mg/dL (11.1 mmol/L); (iii) Glycated hemoglobin (HbA1c) ≥ 6.5% (48 mmol/mol), determined using the method certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized by Diabetes Control and Complications Trial (DCCT); (iv) Plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L) randomly determined in hyperglycemic patients [47].

T2D is usually preceded by a pre-diabetic stage defined by impaired fasting glucose and/or impaired glucose tolerance [48]. In other words, prediabetes is a condition characterized by intermediate hyperglycemia, impaired insulin sensitivity and reduced function of beta cell that in the 70% of cases induces T2D development [49].

Several genome wide association studies (GWAS) suggested that genetic predisposition is one of the major contributors for higher incidence of T2D. In addition, intrauterine growth restriction during the fetus development and external environmental factors (mainly obesogenic during the growth) may contribute to the development T2D [50]. Lifestyle and dietary habits also play a key role in the pathogenesis of T2D. The excessive consumption of saturated fats, refined sugars or carbohydrates of low glycemic index can lead to dyslipidemia and impaired insulin sensitivity [51].

Caloric restriction and physical activity activate cellular mechanisms that can improve patient's metabolic state and represent the main approach of the non-pharmacological treatment of T2D. In case that lifestyle changes do not produce adequate improvements, the use of antihyperglycemic drugs should be considered. Metformin is recommended for initial drug treatment, but in case this

drug is contraindicated, poorly tolerated, or inadequately effective, many therapeutic alternative drugs are available. In any case pharmacological treatment is individually oriented, depending on the patient's age, disease stage, body weight and comorbidities [52]. In the last decades a huge number of antidiabetic drugs have been developed to control diabetes through different mechanism of action.

3. The role of gut microbiota in obesity and insulin resistance

3.1 General characteristics of human gut microbiota

The adult human gut contains up to 100 trillion microorganisms, including mainly bacteria followed by a smaller proportion of archaea, viruses, fungi and protozoa. The size of the microbial population inhabiting the human gut exceed the number of eukaryotic cells in the human body, in fact, the number of genes in the human gut microbiota is more than 100-fold higher than the human genome itself [53,54]. In 2005, Bäckhed and colleagues defined the human gut microbiota as a complex collective of genomes (microbiome) that has co-evolved with the host over thousands of years creating a mutual relationship thanks to the capacity to communicate between each other [53]. An efficient intercommunication with the host permits the gut microbes to consume, store, and redistribute energy; to mediate physiologically important chemical transformations; and to maintain and repair itself through self- replication [53]. The gut microbiota clearly influences host's health of providing a series of benefits including, intestinal epithelium integrity maintenance [55], protection against pathogens [56], regulation of immune functions [57] and modulation of energy metabolism [58]. The increased energy intake from the digested food is linked with the production of metabolites and microbial products such as short-chain fatty acids (SCFAs), secondary bile acids, and lipopolysaccharide (LPS), which play a role of signal molecules, modulating appetite, gut motility, energy uptake and storage, and energy expenditure [59].

Gut microbial phyla Firmicutes and Bacteroidetes represent the 90% of the gut microbiota; *Clostridium* genera represents 95% of the Firmicutes phyla; Bacteroidetes phyla is mainly constituted by *Bacteroides* and *Prevotella* genus; Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* genus [60]. However, taxonomic variations

of the gut microbiota undergo among individuals but also individually and according to the gastro intestinal tract portion [60].

Human intestinal microbial colonization begins during delivery, follows postpartum, and increments from infancy to adulthood, mainly influenced by environmental and genetic factors [61]. The first colonizers of the neonatal intestinal tract are determined by the birth delivery method (natural or cesarean) [62] and subsequently by feeding method (breastfeeding or formula feeding) [63]. During the first periods of life the intestinal microbiota is also influenced by the use of prebiotics and antibiotics and by the introduction of complex dietary substrates during weaning [61], and reaches more stability after around 3 years of age when it becomes more similar to the adult intestinal microbiota [60]. During adulthood, the healthy gut microbiome is relatively stable, but the variability and complexity can certainly be driven by lifestyle choices.

Genetic and environmental factors can explain just the 30% of the interindividual variation of the human gut microbiota, whereas the remaining 70% is attributed to unpredictable variations driven by stochastic ecological processes [61]. The high degree of variability makes difficult to establish a specific profile of healthy gut microbiota, but with the advent of omics, parameters such as microbial diversity and richness, abundance of SCFAs producers, in addition with host parameters such as gut barrier function and immunity, can be considered in order to establish if the gut microbial environment is healthy or not [64].

3.2 The link between gut microbiota and obesity

In the early beginning of century pioneering animal studies were conducted in order to elucidate the gut microbiota profiles and linked mechanism associated with obesity. Turnbaugh and colleagues reported that genetically obese (*ob/ob*) mice have a significant reduction in relative abundance of Bacteroidetes and a proportional increase in Firmicutes and Archaea resulting in increased capacity to harvest energy from diet. Further investigation demonstrated that this trait is

transmissible: the introduction of *ob/ob* mice gut microbiota in germ free (GF) mice results in a significant greater increase in total body fat than introduction of lean mice gut microbiota [65]. Therefore, changes in gut microbial composition contribute to the development of obesity via a complex interplay between microbiome genetic factors, host genotype and lifestyle. Bäckhed and colleagues observed that transferring the gut microbiota from conventionally raised mice into GF mice recipients, induced a consistent increase in body fat content within two weeks [66]. This mechanism take place via bacterial absorption of monosaccharides from the gut lumen with downstream induction of de novo hepatic lipogenesis [66]. Few years later they observed that GF mice are protected against obesity development after high fat diet (HFD) by two complementary but independent mechanisms that result in increased fatty acid metabolism. Specifically, (i) elevated levels of fasting-induced adipose factor (Fiaf), a circulating lipoprotein whose expression is normally selectively suppressed in the gut epithelium by the microbiota, inducing expression of peroxisomal proliferator-activated receptor coactivator (Pcg-1α); (ii) increased skeletal muscle and liver levels of phosphorylated AMP-activated protein kinase (AMPK) [67].

Human research did not totally confirm what was observed in animal studies, but more information have been added helping to better understand the gut microbial profile associated with obesity [68]. Among different bacteria inhabiting the human intestine, Bacteroidetes and Firmicutes phyla are the dominant ones, and earlier human studies were focused in elucidate the relationship between Firmicutes-to-Bacteroidetes ratio and obesity [69]. Several studies suggest that an augmented Firmicutes-to-Bacteroidetes ratio is associated to increased low-grade inflammatory status and capability to harvest energy from food [70]. However, too many discordant results have been reported in literature, suggesting that Firmicutes-to-Bacteroidetes ratio can change depending on study population and/or sequencing method used, and deepen taxonomic identification is required

in order to better understand the relationship between obesity and intestinal bacteria [71]. Improvements in high-throughput DNA sequencing technologies helped to unlock information under this point of view [72]. A systematic review conducted by Crovesy *et al*. in 2019 aimed to describe the differences between the gut microbiota of individuals with obesity and lean individuals. According to their assessments obtained from 32 eligible studies, obesity is associated with higher Firmicutes-to-Bacteroidetes ratio, Firmicutes, *Fusobacteria*, *Proteobacteria*, *Mollicutes*, *Lactobacillus (reuteri)*, and lower *Verrucomicrobia* (*Akkermansia muciniphila*), *Faecalibacterium (prausnitzii)*, *Bacteroidetes*, *Methanobrevibacter smithii*, *Lactobacillus plantarum* and *paracasei*. Finally they suggest that gut microbiota of individuals with obesity differ from gut microbiota of lean individuals [68]. Several studies shown that low microbial diversity and richness is associated with worst outcome in terms of inflammation and adiposity [69]. Low bacterial richness and an obese phenotype is associated with a reduction in butyrate-producing bacteria, a reduction in hydrogen and methane production, an increase in mucus degradation and an increase in the potential to manage oxidative stress [73].

Microbes mediate white adipose tissue (WAT) inflammation by activating Toll-like receptor (TLR4) signaling and increasing serum LPS levels inducing endotoxemia [73,74]. Although there is a large heterogeneity in the available current knowledge, a common agreement is that obesity is characterized by gut dysbiosis. A review conducted by Gomes and colleagues, extensively describes the possible mechanisms linking obesity with gut dysbiosis: (i) Obese-related changes in the microbial composition can affect the intestinal barrier integrity promoted by the gut-associated lymphoid tissues (GALT), leading to the translocation of pathogenic bacteria; (ii) Translocation of bacterial LPS from gut lumen into systemic circulation, causing an innate immune response in liver and adipose tissue; (iii) Reduction of conjugated linoleic acid (CLA) producing bacteria, with consequent increase in energy extraction from diet, adipogenesis,

lipolysis and adipocyte apoptosis; (iv) Hyperactivation of the endocannabinoid system, expressed in tissues that control energy balance and regulates feeding behavior and metabolism; (v) Alteration of adiposity process; vi) Assimilation of cholesterol by bacterial cell wall, inhibition of hepatic cholesterol synthesis, redistribution of cholesterol from the plasma to the liver through the action of SCFAs and/or deconjugation of bile acids by hydrolysis; (vi) Alterations in hormones involved in food intake and satiety [75].

3.3 The link between gut microbiota and insulin resistance

The main mechanisms contributing to explain the link between gut microbiota and insulin resistance involve LPS, SCFAs, bile acids and branched-chain amino acids (BCAAs) (Figure 5).

3.3.1 Molecular mechanisms of lipopolysaccharide-induced insulin resistance

LPS binds TLR4, which is expressed in most cells and macrophages and recognizes pathogen-associated molecular patterns (PAMP). The interaction of LPS with TLR4 activates intracellular signaling pathways inducing inflammatory response and cytokine expression and secretion [76] (Figure 4). Increased level of LPS is directly associated with increased intestinal permeability, due reduced expression of proteins that compose tight junctions, leading to the translocation of LPS outside the gut lumen into the circulation and causing inflammation [77]. LPS binds and activates TLR4, which dimerizes and recruits downstream adaptor molecules such as myeloid differentiation protein 88 (MyD88) and Myd88 adapter-like protein (MAL). The molecular cascade that follows involves the activation of IL-1 receptor associate kinase (IRAK), TNF receptor associated factor (TRAF6), transforming growth factor B-associated kinase (TAK1), and serine-kinases c-Jun N-terminal kinase (JNK) and I kappa B kinase (IKK) complexes. The IKK complex converges at nuclear factor kappa B (NF-κB), which is maintained in the inactive state by nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB). This is in turn, degraded by

proteasomes, resulting in the translocation of NF-κB into the nucleus, activating the inflammatory response by increasing TNF-α and IL-6. The activation of JNK and IKK can induce IRSs serine phosphorylation, which has an important role in insulin resistance [77]. In addition, the molecular cascade activated by LPS-TLR4 bond leads to increased expression of inducible nitric oxide synthase (iNOS), provoking S-nitrosation/S-nitrosylation of IR and IRS in insulin sensitive tissues [78].

Different studies demonstrated that the inactivation of TLR4 in tissues and cells with relevant metabolic roles, such as muscle, adipose tissue, hepatic cells, protects from insulin resistance and systemic inflammation [79]. In addition, other TLRs, PAMPs and damage-associated molecular patterns (DAMPs), such as the inflammasome, may be involved in molecular mechanisms linking gut dysbiosis, inflammation and insulin resistance [80,81].

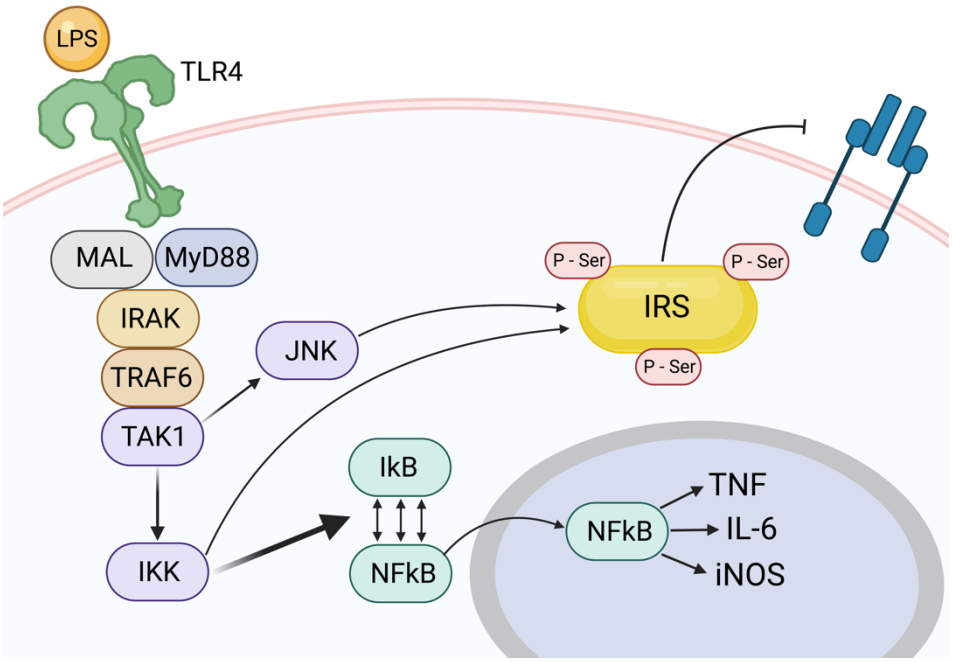


Figure 4: Cellular and molecular pathway of lipopolysaccharide inducing insulin resistance

3.3.2 The anti-inflammatory effects of short-chain fatty acids

The microbial community in the large intestine is responsible of the fermentation of non-digestible carbohydrates, an important fraction of dietary fiber, producing SCFAs that are absorbed across the colonic mucosa. Acetate, propionate and butyrate are the primary end-products, and their production have been estimated to recover 10% of total energy intake from the diet, also increasing the amount of dietary energy available to the host [82]. Modifications in the gut microbial composition and metabolism resulting from carbohydrate fermentation can have beneficial effects in host health [82]. Butyrate plays an important role in promoting the integrity of the large bowel and small intestinal barrier and providing energy to epithelial cells in the large intestine. In addition, it seems to play a role in immune response regulation. Some amount acetate is converted to butyrate by luminal bacteria, whereas part reaches adipose tissues where induces lipogenesis and part is oxidized by muscle. Propionate is sequestrated in the liver where it may induce gluconeogenesis [83]. SCFAs are able to bind G-protein-coupled receptors 43 and 41 (GPR43 and GPR41) in host immune cells and inhibit NF-κB activation, inducing anti-inflammatory effects [77]. The reduced secretion of proinflammatory cytokines and chemokines, diminish local macrophage infiltration. Butyrate and Propionate can induce the extrathymic generation and differentiation of anti-inflammatory regulatory T cells (Treg) and IL-10 production. An increase in Treg cells can reduce macrophage infiltration in adipose tissue, improving insulin resistance [84].

3.3.3 The action of bile acids and branched-chain amino acids

Bile acids are conjugated to glycine in the liver and then released in the circulation reaching the intestine, where they are targeted by the gut microbiota. Secondary bile acids act through G protein-coupled bile acid receptor 1 (GPBAR1) activating glucagon-like peptide 1 (GLP-1) secretion from L-cells in the intestine, and this secretion can protect against insulin resistance [85].

An increase in circulating BCAAs, such as leucine, isoleucine and valine, probably related to altered peripheral amino acid metabolism but also to an excessive supply from a dysbiotic gut microbiota, induce insulin resistance [77].

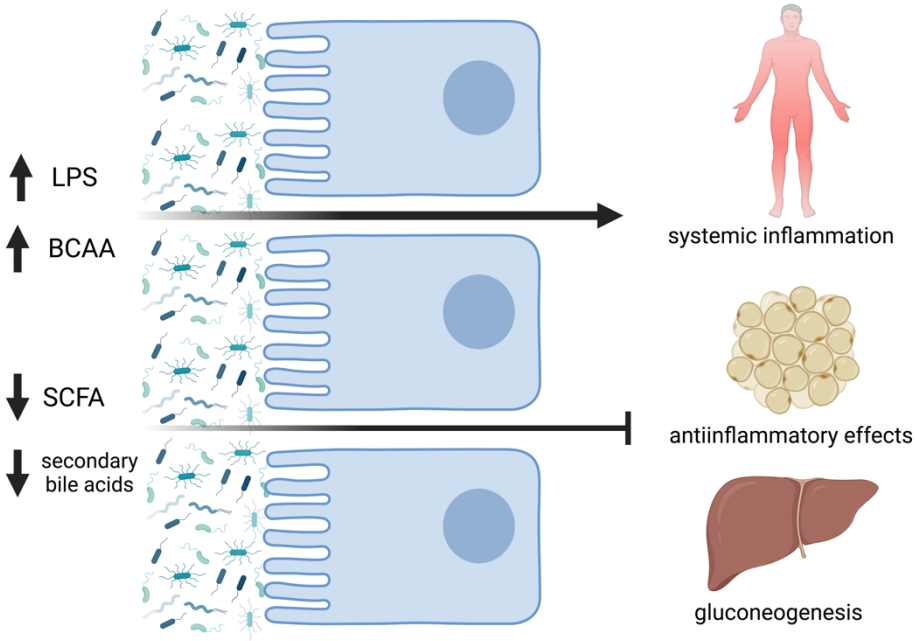


Figure 5: Mechanism contributing to explain the link between gut microbiota and insulin resistance. Gut dysbiosis increases levels of lipopolysaccharide and branched-chain amino acids affecting gut permeability and inducing systemic inflammation. Decreased levels of short-chain fatty acids and secondary bile acids inhibit anti-inflammatory effects and gluconeogenesis.

3.4 Microbiota modulation to treat obesity and insulin resistance

3.4.1. The role of diet

Is has been observed that the "westernization" of lifestyle and especially diet, is associated with the growing of several health issues [86], and that the modification of these habits represent a valid strategy to prevent or alleviate these issues [73]. Among all the possible lifestyle choices diet represents a dominant contributing factor to shape the gut microbial composition. Interindividual

variations are in part explained by the enterotypes [87]. The concept of enterotypes was introduced in 2011 by Arumugam *et al*., who defined three specific host-microbial symbiotic states driven by the dominant bacteria clusters: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). Each enterotype represents a functional association of several bacteria genera that characterizes and is conserved in each individual and defines how these genera are capable to generate energy from fermentable substrates in the colon. The nature of these enterotypes seems to be driven principally by diet [87]. The influence of diet on the intestinal microbiota composition has been widely demonstrated [88]. Different bacterial clusters have been observed by comparing the gut microbiota of children from rural Africa areas and children from Italy, exposed to completely different dietary patterns especially in terms of fiber intake [89]. Dietary carbohydrates intake from diet contribute to big variations in gut microbiota composition, as observed in a study showing that gut microbiome of African and South American people clustered together, correlating with large consumption of plant-based polysaccharides, but contrarily were different from North Americans mainly consuming low-fiber diet [90]. Wu and colleagues reported that the prevalence of *Bacteroides* and *Actinobacteria* was positively associated with a HFD and negatively associated with fiber intake, whereas *Firmicutes* and *Proteobacteria* show the opposite associations. High consumption of animal protein and saturated fats is associated with the *Bacteroides*-dominant enterotype, suggesting its prevalence in Western countries. In contrast, high consumption of carbohydrates and simple sugars is associated with the *Prevotella*-dominant enterotype, indicating a correlation with a carbohydrate based diet more typical of agrarian societies and vegetarians [91]. Diets rich in fiber increase the production of SCFAs by microbes, lowering the intestinal pH and preventing the growth of pathogenic bacteria [92]. On the other hand, western diet is considered to be responsible of intestinal dysbiosis by inducing the proliferation of certain species which can result in increased

intestinal LPS or by inducing the secretion of bile acids, causing metabolic endodotoxemia and low-grade inflammation.

Among different dietary patterns, Mediterranean diet (MedDiet) has been suggested as a valid therapeutic strategy for health promotion. The traditional MedDiet is characterized by high consumption of vegetables, fruits, nuts, legumes, and unprocessed cereals; low consumption of meat and meat products; low consumption of dairy products (with the exception of the long-preservable cheeses); moderate alcohol consumption in form of wine and, as a rule, during meals; use of olive oil as primary source of dietary fat for dressing and cooking [93,94]. Numerous studies and clinical trials have demonstrated the beneficial effects of MedDiet on human health by reducing the risk of all-cause mortality and multiple chronic diseases [95,96]. The PREDIMED (PREvención con DIeta MEDiterránea) randomized trial, designed to assess the efficacy of MedDiet supplemented with extra-virgin olive oil or nuts, shown a reduction in the incidence of major cardiovascular events and mortality compared to a low-fat diet [96].

Several studies aimed to assess MedDiet influence on gut microbiota [97,98]. A study conducted in a cohort of 153 individuals following omnivore, vegetarian or vegan diets, and stratified according to adherence to MedDiet, shown a significant association between high consumption of vegetables consistent with a MedDiet and increased levels of SCFAs, *Prevotella* and some fibre-degrading *Firmicutes* [99]. Accordingly, MedDiet score was associated with higher abundance of Bacteroidetes, *Prevotellaceae* and *Prevotella*, lower concentration of Firmicutes and *Lachnospiraceae* and higher concentrations of fecal SCFAs in a cohort of adults with non-declared pathology [100]. In Support to these evidences Garcia-Mantrana and colleagues observed that higher adherence to MedDiet was associated with lower ratio of Firmicutes-to-Bacteroidetes and higher fecal SCFAs levels in 27 healthy volunteers [101]. More recently, the results of the NU-AGE projects shown that the adherence to MedDiet was

positively associated with taxa linked with beneficial effects on frailty and negatively associated with inflammatory markers in 612 participants from different European countries [102]. An enrichment in *Lachnospiracea*e and *Ruminococcaceae* was observed in the fecal microbiota of adult participants with metabolic syndrome (MetS) exposed to MedDiet *versus* habitual non-MedDiet supplemented with nuts, highlighting the beneficial effects of MedDiet on cardiometabolic risk factors [103].

3.4.2 Lifestyle interventions

A recent observational study was conducted to assess the effects of weight loss lifestyle modifications in the composition, richness and predicted functional profiles of the gut microbiota in children and adolescents (aged 7–18 years) with obesity. Participants who lost weight after intervention shown a decrease of Bacteroidetes phylum, Bacteroidia class, Bacteroidales order, *Bacteroidaceae* family, and *Bacteroides* genus, along with increased proportions of Firmicutes phylum, Clostridia class, and Clostridiales order. Patients who gained weight after intervention shown decreased Firmicutes phylum, Clostridia class, Clostridiales order, *Lachnospiraceae* family, and *Eubacterium hallii* group genus, without a change in diversity after the intervention. The functional metabolic analysis shown that the "Nitrate Reduction VI" and "Aspartate Superpathway" pathways were predicted to increase significantly in case of weight loss [104]. Stanislawski and colleagues conducted an ongoing behavioral weight loss trial, comparing daily caloric restriction to intermittent fasting in adults (age 18–55) with overweight or obesity during three months of follow-up. The aim of the study was to determine whether gut microbial signatures assessed at either baseline or in at three months of follow-up were associated with early clinical outcomes. The overall microbial community structure and diversity increased significantly from baseline to three months, whereas in terms of bacterial relative abundance, there was an increase of genera *Parabacteroides*, *Alistipes*, and *Bacteroides,* and a decrease of genus *Subdoligranulum* and

Collinsella. Analysis by intervention group found increased relative abundance of *Akkermansia* in patients exposed to intermittent fasting [105]. The effect of a one-year intensive lifestyle weight loss intervention on the gut microbiota of elderly subjects (aged 55–75) with overweight/obesity and MetS was recently determined. The study conducted within the frame of the PREDIMED-Plus trial compared an intervention group (IG), in which participants were exposed to an energy-restricted MedDiet, behavioral support and physical activity, *versus* a control group (CG) in which participants underwent just an energy unrestricted MedDiet. Differential abundance analysis between the two groups shown a decrease in *Butyricicoccus*, *Haemophilus*, *Ruminiclostridium*, and *Eubacterium hallii* and an increase in *Coprobacter* and uncultured bacteria from Rhodospirales order in IG compared with CG group. Several other genera especially linked to the production of SCFAs, shifted in the same direction within both intervention groups after one year of follow-up, suggesting an overall effect of the MedDiet on gut microbiota composition and function associated to a better metabolic profile [106].

3.4.3 Prebiotics and probiotics

Prebiotics are nondigestible polysaccharides that promote the growth of beneficial intestinal bacteria. Oligofructose, inulin, fructooligosaccharides, galactooligosaccharides, and few types of pectin have been reported to be beneficial for obesity management. Other substances such as polyphenols and polyunsaturated fatty acids can be considered as prebiotic, since their beneficial effects on health via gut microbiota modulation have been demonstrated [71]. Different studies shown that prebiotics administration is associated with increased satiety hormones and reduced postprandial hyperglycemia and insulin secretion [69]. In addition prebiotics have a positive effect on serum lipids, body weight and serum inflammatory markers [71].

Probiotics are defined as live microorganism that can induce beneficial effects to the host when administered in appropriate doses [71]. There are several studies

showing an increase in metabolism, but barrier integrity and reduced inflammation associated with the use of probiotics. In addition, has been documented the action of probiotics reduces plasma lipids and pro-inflammatory genes and increase the production of SCFAs [69]. The use of some probiotics as therapy to attenuate T2D have been documented. It seems that the efficacy of these treatments is associated to a reduction of low-grade inflammation, via improved tight junction function and preventing metabolic endotoxemia [73]. The main targets for probiotic-mediated T2D therapy are the SCFAs, as these molecules interact with the host enteroendocrine system regulating the expression of gut hormones affecting gut permeability, satiety, gastric emptying and food intake [73]. In addition SCFAs are able to increase intestinal gluconeogenesis contributing to satiety [107]. Another proposed target of probiotics for the treatment of T2D is the endocannabinoid system, via upregulating the expression and correcting the localization of intestinal epithelial tight junction proteins [108].

Several studies have shown that the combination of prebiotics and probiotics have better efficacy on host health compared to when used individually, as prebiotic can stimulate the probiotic activity [71].

3.4.4 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) consists in the administration of a fecal suspension from a healthy donor into the gastrointestinal tract of a recipient in order to restore a healthy gut microbiota [69]. The use of FMT have been demonstrated to be an effective therapy for recurrent *Clostridium difficile* infection [109]. However, the therapeutic potential of FMT to treat obesity still needs to be clarified. Some animal studies shown that FMT from obese donors can increase adiposity and induce metabolic complications [110,111]. There are just few human studies evaluating the efficacy of FMT for the treatment of obesity. Among these some shown an improvement in insulin sensitivity in subjects with MetS after FMT from healthy donors, suggesting that the therapy

had short-term beneficial effects but not long-term changes of MetS were observed [112,113]. However, even if FMT is considered a promising strategy for the treatment of obesity and metabolic disorders, further studies are necessary in order to standardize the procedure and reduce safety risks for health. Most short-term risks are associated with the method of administration (endoscopic procedure) rather than FMT itself. Longer term risks are less well described, and several longitudinal follow-up studies do not suggest any cause of significant concern. Some rare cases documented adverse outcomes including the transmission of antibiotic resistant bacteria, and even death [114].

II. JUSTIFICATION

Overweight and obesity are considered a major worldwide public health problem which has rapidly increased up to reach global epidemic proportions. Obesity is a chronic multifactorial disease characterized by an excess of adipose tissue accumulation directly associated with several metabolic complications including insulin resistance, an important risk factor for the development of T2D.

Emerging evidences shown that alterations in intestinal microbial composition and functionality could play a pathological role in the development of obesity and insulin resistance. These alterations are the result of a complex interplay between microbiome genetic factors, host genotype and lifestyle. The main factors linking obesity-related gut microbial changes are characterized by an increased capacity to harvest energy from diet, altered adiposity and lipid metabolism, impaired feeding behavior, among several other mechanism linked. Gut microbiota alterations affect intestinal barrier integrity inducing endotoxemia with consequent low-grade systemic inflammation. The activation of intracellular inflammatory molecular pathways, together with a disproportion in the production of SCFAs and the action of bile acids and BCAAs, represent the main factors directly involved in the development of insulin resistance.

Lifestyle modifications based on weight loss dietary interventions as strategy to manage overweight, obesity and related metabolic disorder through gut microbiota modulation, have shown promising results. However, even if the association between gut microbes and weight dynamic has been widely explored, contrasting results were described, especially because the microbiota represents a complex environment with a lot of interindividual variability. The identification of specific bacteria and metabolic patterns associated with obesity and related metabolic disorders would offer a promising novel perspective to tailor personalized interventions. Accordingly, the studies enclosed in this doctoral thesis aimed to explore the association between fecal microbiota, weight loss and insulin resistance in the framework of a large study population of elderly subjects

with overweight/obesity and MetS exposed to a lifestyle intervention based on Mediterranean diet, physical activity and behavioral support.

Promising evidences already provided, the lack of consensus in the current knowledge, and the need of methodological improvements, are the main reasons that moves lots of research on this topic nowadays, including the studies enclosed in this doctoral thesis.

III. HYPOTHESIS

We hypothesized that specific fecal microbiota taxonomic signatures may be linked with body weight control in a Mediterranean population of elderly subjects with overweight/obesity and MetS exposed to a 12-month lifestyle intervention. Moreover, we hypothesized that specific fecal microbiota genera and metabolic pathways associated may be linked with insulin resistance in subjects without T2D from the same cohort.

IV. OBJECTIVES

Objective 1

Explore the association between fecal microbiota taxonomic signatures, BMI and changes in total body weight after 12-month lifestyle intervention in a Mediterranean population of elderly subjects with overweight/obesity and MetS.

Objective 2

Explore the association between fecal microbiota taxonomic and pathway-related signatures and HOMA-IR index in a Mediterranean population of elderly subjects with overweight/obesity, MetS, and without T2D.

V. METHODOLOGY

1. Study participants: the PREDIMED-Plus trial

The PREDIMED (PREvención con DIeta MEDiterránea)-Plus trial is an ongoing multicenter, parallel-group, randomized, single blind clinical trial counting 6874 participants recruited in 23 Spanish recruiting centers between September 2013 and December 2016.

The trial aims to assess the long-term effects of an intensive weight loss lifestyle intervention based on an energy-restricted MedDiet, physical activity promotion, and behavioral support (IG) on hard CVD events, *versus* a CG following an energy-unrestricted MedDiet without any advice to increase physical activity. The trial was registered at the International Standard Randomized Controlled Trial (http://www.isrctn.com/ISRCTN89898870) and approved by the institutional review board of all participating institutions. A detailed protocol is available at the web page http://predimedplus.com/.

Eligible participants were men (aged 55–75 years) and women (aged 60–75 years), without documented history of CVD at enrollment, with overweight/obesity (BMI ≥27 and <40 kg/m^2) and who met at baseline at least three components of the MetS. More in detail: waist circumference > 102 cm in men and > 88 cm in women; serum triglyceride ≥ 150 mg/dL or drug treatment for elevated triglycerides; HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women, or drug use for low HDL-cholesterol; blood pressure ≥ 130/85 mmHg or antihypertensive drug treatment; and fasting plasma glucose level ≥ 100 mg/dL or hypoglycemic treatment. The MetS criteria were described in 2009 by the harmonized definition of the joint statement from the International Diabetes Federation/National Heart, Lung, and Blood Institute/American Heart Association [115].

Potential eligible candidates entered a 4-week run-in period comprising three screening visits aiming to determine the adherence to study procedures.

Candidates meeting eligibility criteria who had attended all screening visits and correctly filled in the administered questionnaires and records were randomized in a 1:1 ratio to either the IG or the CG, using a centrally controlled, computer-generated random-number internet-based system with stratification by recruiting center, sex, and age (<65, 65–70, and >70 years). The randomization procedure was blinded to all staff members and principal investigators and was audited for all centers. For participant couples sharing the same household, randomization was done by cluster, with the couple as the unit of randomization. In the specific cases of couples in which the first spouse was previously recruited at a different time, the last spouse entering the study was directly assigned (not randomized) to the same study arm as his/her partner.

2. Clinical and dietary assessment

Information about sociodemographic and lifestyle aspects, education level, individual and family medical history, and current medication use was collected.

A 17-item questionnaire [116] was used to assess adherence to the energy-restricted MedDiet at baseline and follow-up visits. This 17-item questionnaire is a modified version of the previously validated 14-item Mediterranean Diet Adherence Screener (MEDAS) questionnaire, which was also used in this trial [117]. In addition, A validated 143- item food frequency questionnaire was also completed to assess food habits [118].

At each visit waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape, body weight was measured twice using high-quality electronic calibrated scales and height was measured twice using a wall-mounted stadiometer. Systolic and diastolic blood pressure was measured 3 times using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan) and the mean of repeated measures was used.

3. Blood sampling and biochemical analysis

Blood samples were collected after an overnight fast. Tubes of serum and plasma were collected, and aliquots were coded and stored at -80°C in a central laboratory until analyses.

Plasma levels of glucose, insulin, total cholesterol, HDL cholesterol and triglycerides were measured using standard enzymatic methods, LDL cholesterol was calculated with the Friedewald formula (whenever triglycerides were less than 300 mg/dL), glycated hemoglobin was measured by a chromatographic method. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) [119] using the following formula:

$$insulin\,(mU/mL) \times FPG(mg/dL)\,/_{405}$$

4. Stool samples collection, microbial DNA extraction and 16S amplicon sequencing

Stool samples were collected at home by participants and kept frozen till the delivery to the laboratory. In case of antibiotic or prebiotic supplements use, samples were collected 15 days after treatment completion. Stool samples were then separated into 250 mg aliquots and stored at −80 °C, until analysis.

Microbial DNA was extracted using the QIAmp PowerFecal DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Previous extraction samples were disrupted in a 5 minutes lysis step using FastPrep-24™ 5G Homogenizer (MP Biomedicals, Santa Ana, CA, USA). After extraction, the DNA was stored at –20°C until further processing. DNA concentration and purity was assessed with the Qubit 2.0 Fluorometer-dsDNA Broad Range Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Bacterial 16S rRNA gene was amplified with the Ion 16S Metagenomics kit (Thermo Fisher Scientific, Waltham, MA, USA), using two sets of primers (V2, V4, V8 and V3, V6-7, V9) covering the multiple hypervariable regions of the gene. Sequencing libraries were created with Ion Plus Fragment Library Kit (Thermo Fisher Scientific, Waltham, MA, USA) and barcoded adapters ligated using the Ion Xpress Barcode Adapters kit (Thermo Fisher Scientific, Waltham, MA, USA). Synthesized libraries were pooled and templated on the automated Ion Chef system (Thermo Fisher Scientific, Waltham, MA, USA) followed by a 400 bp sequencing on the Ion S5 (Thermo Fisher Scientific, Waltham, MA, USA). Sequenced reads were generated in BAM (Binary Alignment Map) format and then converted in FASTQ format using the File Explorer plugin of the Torrent Suite Server software (Thermo Fisher Scientific, Waltham, MA, USA), interfaced with the Ion S5.

5. Statistical analysis

5.1. Fecal microbiota analysis

Sequenced reads in FASTQ format were processed using different bioinformatics pipelines according to the study conducted. These steps are described in the methods session of the manuscripts included in this doctoral thesis.

5.2. Analysis of participants´ clinical characteristics

The clinical characteristics of the study participant were described using the software IBM SPSS Statistics version 23 (SPSS Inc., Chicago, Illinois, USA). Data normality was assessed with Shapiro-Wilk test. Normally distributed variables were described as means and standard deviations, non-normally distributed variables were described as median and interquartile range, whereas categorical variables were described as numbers and percentages. Differences across study groups were evaluated through one-way analysis of variance

(ANOVA) or Kruskal–Wallis non-parametric test for numerical variables, and with Pearson's chi-square test for categorical variables. Student's t-test or Mann–Whitney non-parametric test were used to calculate differences between study groups for numerical variables, Pearson's chi-square test was used for categorical variables. All statistical tests were 2-sided and p-value < 0.05 was deemed statistically significant.

VI. RESULTS

Publication 1

Type: Journal article

Authors: Alessandro Atzeni, Serena Galié, Jananee Muralidharan, Nancy Babio, Francisco José Tinahones, Jesús Vioque, Dolores Corella, Olga Castañer, Josep Vidal, Isabel Moreno-Indias, Laura Torres-Collado, Rebeca Fernández-Carrión, Montserrat Fitó, Romina Olbeyra, Miguel Angel Martínez-González, Monica Bulló, Jordi Salas-Salvadó

Title: Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome

Journal: Microorganisms

Publisher: MDPI

Status: Published on February 10, 2021

microorganisms

MDPI

Article

Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome

Alessandro Atzeni [1,2,3], Serena Galié [1,2,3], Jananee Muralidharan [1,2,3], Nancy Babio [1,2,3], Francisco José Tinahones [3,4], Jesús Vioque [5,6], Dolores Corella [3,7], Olga Castañer [3,8], Josep Vidal [9,10], Isabel Moreno-Indias [3,4], Laura Torres-Collado [5,6], Rebeca Fernández-Carrión [3,7], Montserrat Fitó [3,8], Romina Olbeyra [9], Miguel Angel Martínez-González [3,11,12], Monica Bulló [1,2,3,*,†] and Jordi Salas-Salvadó [1,2,3,*,†]

1 Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, 43201 Reus, Spain; alessandro.atzeni@urv.cat (A.A.); serena.galie@iispv.cat (S.G.); jananee.muralidharan@urv.cat (J.M.); nancy.babio@urv.cat (N.B.)
2 Institut D'Investigació Sanitària Pere Virgili (IISPV), Hospital Universitari de Sant Joan de Reus, 43204 Reus, Spain
3 CIBER de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain; fjtinahones@uma.es (F.J.T.); dolores.corella@uv.es (D.C.); ocastaner@imim.es (O.C.); isabel.moreno@ibima.eu (I.M.-I.); rebeca.fernandez@uv.es (R.F.-C.); mfito@imim.es (M.F.); mamartinez@unav.es (M.A.M.-G.)
4 Unidad de Gestión Clínica de Endocrinología y Nutrición, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, 29010 Málaga, Spain
5 Instituto de Investigación Sanitaria y Biomédica de Alicante, ISABIAL-UMH, 03010 Alicante, Spain; vioque@umh.es (J.V.); l.torres@umh.es (L.T.-C.)
6 CIBER Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain
7 Department of Preventive Medicine, University of Valencia, 46100 Valencia, Spain
8 Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Research Institute (IMIM), 08003 Barcelona, Spain
9 Endocrinology and Nutrition Department, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic Universitari, 08036 Barcelona, Spain; jovidal@clinic.cat (J.V.); rominaolbeyra@gmail.com (R.O.)
10 CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain
11 Department of Preventive Medicine and Public Health, University of Navarra, IdiSNA, 31008 Pamplona, Spain
12 Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA 02115, USA
* Correspondence: monica.bullo@urv.cat (M.B.); jordi.salas@urv.cat (J.S.-S.)
† Senior authors.

check for updates

Citation: Atzeni, A.; Galié, S.; Muralidharan, J.; Babio, N.; Tinahones, F.J.; Vioque, J.; Corella, D.; Castañer, O.; Vidal, J.; Moreno-Indias, I.; et al. Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome. *Microorganisms* **2021**, *9*, 346. https://doi.org/10.3390/microorganisms9020346

Academic Editor: Mar Larrosa
Received: 12 January 2021
Accepted: 6 February 2021
Published: 10 February 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Gut microbiota is essential for the development of obesity and related comorbidities. However, studies describing the association between specific bacteria and obesity or weight loss reported discordant results. The present observational study, conducted within the frame of the PREDIMED-Plus clinical trial, aims to assess the association between fecal microbiota, body composition and weight loss, in response to a 12-month lifestyle intervention in a subsample of 372 individuals (age 55–75) with overweight/obesity and metabolic syndrome. Participants were stratified by tertiles of baseline body mass index (BMI) and changes in body weight after 12-month intervention. General assessments, anthropometry and biochemical measurements, and stool samples were collected. 16S amplicon sequencing was performed on bacterial DNA extracted from stool samples and microbiota analyzed. Differential abundance analysis showed an enrichment of *Prevotella 9*, *Lachnospiraceae* UCG-001 and *Bacteroides*, associated with a higher weight loss after 12-month of follow-up, whereas in the cross-sectional analysis, *Prevotella 2* and *Bacteroides* were enriched in the lowest tertile of baseline BMI. Our findings suggest that fecal microbiota plays an important role in the control of body weight, supporting specific genera as potential target in personalized nutrition for obesity management. A more in-depth taxonomic identification method and the need of metabolic information encourages to further investigation.

Keywords: obesity; gut microbiota; BMI; weight loss; 16S sequencing; clinical trial

1. Introduction

Overweight and obesity are considered a worldwide public health problem which has rapidly increased up to reach global epidemic proportions [1]. Obesity is a complex multifactorial disease, characterized by an anomalous or disproportionate adipose tissue accumulation associated with several metabolic complications [2,3].

In the last few years, gut microbiota has been highlighted as an important factor related to obesity and its associated comorbidities [4]. Causal evidence linking gut microbiota to obesity mostly originates from fecal transplant studies conducted in germ free mice that gained weight when colonized with gut microbes from obese donors [5]. Moreover, the gut microbiota is able to predict post-dieting weight regain in obese mice [6]. A recent systematic review of observational studies has reported differences between the gut microbiota profiles of individuals with obesity and lean individuals, identifying some bacteria potentially involved in the development of obesity [4]. *Bacteroidetes* are commonly less abundant in people with obesity, with this abundance increasing along with weight-loss [7], whereas *Firmicutes* phylum as some of their genera as *Lactobacillus* and *Clostridium* have been associated to metabolic dysregulations related to obesity [8], suggesting that specific bacteria could be beneficial or detrimental to obesity. Whether the gut microbes are related to weight dynamics in humans has been sparsely studied [9]. In a weight-loss study conducted over 49 participants from the DIETFITS randomized either to a low-carbohydrates or low-fat diets, microbiota composition did not predict participants' weight loss at 1 year [10]. In contrast, other trials of shorter duration shown that different relative abundance of specific genera (i.e., *Phascolarctobacterium*, *Dialister*, *Prevotella*-to-*Bacteroidetes* ratio) were associated with a higher or lower weight loss [11,12].

Accordingly, the aim of the present study is to identify, in a large sample size, specific genera associated with baseline body mass index (BMI) and changes in body weight in response to a lifestyle intervention, in an elderly population with overweight/obesity and metabolic syndrome.

2. Materials and Methods

2.1. Participants and Study Design

This study was conducted within the frame of the PREDIMED-Plus clinical trial, that aims to evaluate the long-term effect of an intensive weight-loss lifestyle intervention on cardiovascular disease and mortality in a population with overweight and obesity (BMI 27–40 kg/m^2), aged between 55 and 75 years old and who at least met 3 criteria for metabolic syndrome [13]. Participants were randomized in a 1:1 ratio to an intervention group that encouraged an energy-reduced Mediterranean diet, promoted physical activity, and provided behavioral support, or to a control group that encouraged an energy-unrestricted Mediterranean diet without any other specific advice for losing weight. The PREDIMED-Plus study protocol is available at http://www.predimedplus.com, accessed 18 November 2020, and was registered at the International Standard Randomized Controlled Trial (http://www.isrctn.com/ISRCTN89898870, accessed 18 November 2020). This trial was approved by the institutional review board of all participating institutions, and participants provided written informed consent.

The present observational study included 400 participants (200 participants for each intervention group) recruited in the PREDIMED-Plus centers of Reus and Málaga in Spain, randomly selected, matched by sex, age, and BMI, and with stool samples available at baseline and after 12-month of intervention.

A cross-sectional analysis was conducted stratifying the sample by tertiles of baseline BMI. In addition, a longitudinal analysis was conducted stratifying the sample by tertiles of changes in body weight after 12-month intervention.

2.2. General Assessments, Anthropometric and Biochemical Measurements, Samples Collection

Information about disease prevalence, lifestyle and medication use was collected. At baseline and 12-month timepoint, waist circumference was measured midway between the the lowest rib and the iliac crest using an anthropometric tape, body weight was measured using high-quality electronic calibrated scales, height was measured using a wall-mounted stadiometer. Systolic and diastolic blood pressure was measured 3 times using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan) and the mean value recorded.

Blood samples were collected at both timepoints after an overnight fast. Plasma fasting glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides concentrations were measured using standard enzymatic methods, low-density lipoprotein (LDL) cholesterol concentrations were calculated with the Friedewald formula whenever triglycerides were less than 300 mg/dL, and glycated hemoglobin was measured by a chromatographic method.

Baseline and 12-month timepoint stool samples were collected and kept frozen till the delivery to the laboratory. In case of antibiotic treatment or fiber supplements, samples were collected 15 days after treatment completion. Stool samples were then separated into 250 mg aliquots stored at −80 °C, until analysis.

2.3. Microbial DNA Extraction, 16S Amplicon Sequencing and Data Processing

Microbial DNA was extracted using the QIAmp PowerFecal DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. In the first step of the extraction, an additional lysing of 5 min using FastPrep-24™ 5G Homogenizer (MP Biomedicals, Santa Ana, CA, USA) was conducted. DNA concentration and purity were assessed with the Qubit 2.0 Fluorometer-dsDNA Broad Range Assay Kit (Invitrogen, Carlsbad, CA, USA).

Targeted sequencing libraries were created with the 16S Metagenomics kit (Life Technologies, Carlsbad, CA, USA), using a pool of primers to amplify multiple hypervariable regions (V2, V3, V4, V6-7, V8, V9) of the 16S rRNA gene, in combination with Ion Plus Fragment Library Kit (Life Technologies, Carlsbad, CA, USA), to ligate barcoded adapters. Synthesized libraries were pooled and templated on the automated Ion Chef system (Life Technologies, Carlsbad, CA, USA) followed by a 400 bp sequencing on the Ion S5 (Life Technologies, Carlsbad, CA, USA). Sequenced reads were generated in BAM (Binary Alignment Map) format and then converted in FASTQ format using the File Explorer plugin of the Torrent Suite Server software (Life Technologies, Carlsbad, CA, USA), interfaced with the Ion S5.

A customized Python script [14] was used to separate the reads according to the different hypervariable regions of the 16S rRNA gene, and the V4 data selected and individually processed with the software QIIME (Quantitative Insight into Microbial Ecology) 2, version 2020.2 [15]. Sequenced reads were demultiplexed, trimmed to 265 bp, and denoised into ASVs (amplicon sequence variants) using the denoise-pyro method of the DADA2 plugin [16]. Taxonomic assignment was performed using the consensus-vsearch method of the vsearch plugin [17], against the 16S rRNA gene reference database SILVA 132 [18].

2.4. Statistical Analysis

Baseline characteristics of participant were described as means and standard deviations or median and interquartile range (as appropriate) for quantitative variables, and numbers and percentages for categorical variables. Population was stratified by tertiles of baseline BMI and by tertiles of changes in body weight after 12-month intervention irrespective of the intervention group of the trial. Differences across tertiles were evaluated through one-way analysis of variance (ANOVA) or Kruskal–Wallis test for numerical variables, as appropriate, and with Pearson's chi-square test for categorical variables. Student's t-test or Mann–Whitney U test were used to calculate differences between tertiles for numerical variables, Pearson's chi-square test was used for categorical variables. Statistical analysis

was carried out using IBM SPSS Statistics version 23 (SPSS Inc., Chicago, IL, USA). All statistical tests were 2-sided and *P* value < 0.05 was deemed statistically significant.

ASV counts and taxonomic information generated with QIIME 2, were imported into R (version 3.6.2) and processed with the package Phyloseq, version 1.30.0 [19]. ASVs counts table was filtered at 10% prevalence cut off at genus level for both samples and overall ASVs.

Chao1, Shannon and Simpson indexes were calculated and pairwise comparison using Wilcoxon rank sum test performed to evaluate differences in microbial diversity among tertiles of baseline BMI. Bray-Curtis, Jaccard, Weighted and Unweighted Unifrac distance matrices were calculated and permutational multivariate analysis of variance (PERMANOVA) performed using the adonis function ("vegan" package, version 2.5-6), to test differences in groups compositions, whereas permutation test for homogeneity of multivariate dispersions was performed to test variability among groups.

The log-normalized *Firmicutes*-to-*Bacteroidetes* (F/B) ratio was computed based on the relative abundance between the phylum *Firmicutes* and *Bacteroidetes*, the log-normalized *Prevotella*-to-*Bacteroides* ratio (P/B) was computed based on the relative abundance between the genus *Prevotella* and *Bacteroides*. One-way ANOVA was used to test if F/B and P/B ratios were statistically significant different between tertiles of baseline BMI and tertiles of changes in body weight after 12-month intervention.

Differential abundant significant ASVs (Benjamini–Hochberg adjusted *P* value < 0.05) were identified between tertiles of baseline BMI and tertiles of changes in body weight after 12-month intervention, using Wald's test in the DESeq2 package, version 1.26.0 [20], adjusting for type 2 diabetes prevalence and intervention group as covariates.

3. Results

3.1. Association between Fecal Microbiota and Tertiles of Baseline Body Mass Index

A total of 400 participants, in the framework of the PREDIMED-Plus clinical trial, were randomly selected and matched by age, sex and BMI. From these 400, stool samples at baseline and at 12-month timepoint were available for 372, from which bacterial DNA was extracted and sequenced. Sequence data generated was separated according to the different hypervariable regions of the 16S rRNA gene, and V4 data selected and processed with QIIME 2. Few samples were excluded from the analysis because no information was generated after the denoise step, or because missed or repeated, reducing the number of participants included in the cross-sectional study to 368. Finally, counts table was filtered at 10% prevalence cut off at genus level for both samples and overall ASVs, further reducing the number of participants to 364.

The baseline characteristics of the study population categorized by tertiles of baseline BMI are shown in Table 1. Body weight, BMI, waist circumference, fasting glucose and glycated hemoglobin levels, the prevalence of type 2 diabetes, and the prevalence of metformin or other antidiabetic drugs use, were higher in the tertiles 2 and 3 compared to tertile 1.

Differences in alpha and beta diversity, as well as differences in F/B ratio and P/B ratio between tertiles were not statistically significant (Supplementary Materials, Tables S1–S5).

A total of 5453 ASVs were detected in 364 samples. Statistically significant differential abundant ASVs between tertiles of baseline BMI are summarized in Figure 1, whereas detailed information, including *P* values are listed in Supplementary Materials, Table S7. The analysis revealed one ASV representing the genus *Prevotella* 2, more abundant in tertile 1 versus to tertile 2, one ASV representing the genus *Bacteroides* in tertile 1 versus tertile 3, one ASV representing *Bacteroides* in tertile 2 versus tertile 3 and one ASV representing the genus *Prevotella* 2 in tertile 3 versus tertile 2.

Table 1. Baseline characteristics of the study population according to tertiles of baseline body mass index.

Tertile Min–Max	T1 (n = 121) 25.9–31.5	T2 (n = 122) 31.5–35.0	T3 (n = 121) 35.0–40.3	P Trend [&]
Sex, female	58 (47.9)	57 (46.7)	73 (60.3)	0.064
Age, years	64.9 ± 5.2	64.3 ± 4.8	65.0 ± 5.1	0.591
Intervention group	55 (45.5)	63 (51.6)	66 (54.5)	0.352
Body weight, kg	79.4 ± 9.1	88.4 ± 10.4 **	96.9 ± 12.0 **†	<0.001
BMI, kg/m^2	29.4 ± 1.4	33.1 ± 1.0 **	37.3 ± 1.5 **†	<0.001
Waist circumference, cm	102.2 ± 7.1	109.7 ± 7.4 **	117.5 ± 8.1 **†	<0.001
Smoking				
Current smoker	20 (16.5)	21 (17.2)	15 (12.4)	
Former smoker	48 (39.7)	47 (38.5)	39 (32.2)	0.369
Never smoked	52 (43.0)	54 (44.3)	67 (55.4)	
Education				
Primary school	64 (52.9)	68 (55.7)	64 (52.9)	
Secondary school	37 (30.6)	39 (32.0)	41 (33.9)	0.880
Academic or graduate	20 (16.5)	15 (12.3)	16 (13.2)	
Recruiting center				0.093
Reus	45 (37.2)	39 (32.0)	55 (45.5)	
Malaga	76 (62.8)	83 (68.0)	66 (54.5)	
Hypercholesterolemia	77 (63.6)	82 (67.2)	75 (62.0)	0.685
Hypertension	110 (90.9)	117 (95.9)	116 (95.9)	0.159
T2DM prevalence	17 (14.0)	33 (27.0) *	35 (28.9) *	0.012
Insulin treatment	2 (1.7)	9 (7.4)	10 (8.3)	0.057
Metformin treatment	10 (8.3)	29 (23.8) *	26 (21.5) *	0.003
Other anti diabetic drugs use	12 (9.9)	27 (22.1) *	28 (23.1) *	0.013
Glucose, mg/dL	103.9 ± 19.8	112.4 ± 28.7 *	112.9 ± 25.8 *	0.007
HbA1c, %	5.7 [0.6]	5.9 [0.6] **	5.9 [0.8] *	0.001
Triglycerides, mg/dL	152 [100]	147 [90]	155.5 [78]	0.291
Total cholesterol, mg/dL	204.8 ± 38.6	197.0 ± 37.2	203.0 ± 37.1	0.241
HDL-cholesterol, mg/dL	50.3 ± 12.9	48.0 ± 12.5	48.6 ± 11.9	0.316
LDL-cholesterol, mg/dL	122.6 ± 34.2	114.8 ± 33.1	118.8 ± 33.0	0.193
SBP, mm Hg	139.0 ± 18.2	140.2 ± 14.8	141.3 ± 17.6	0.589
DBP, mm Hg	78.8 ± 9.6	80.6 ± 9.6	77.9 ± 10.5	0.099

Data shown as mean ± SD, median [IQR] or n (%); SD; standard deviation; IQR; interquartile range; BMI, body mass index; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein SBP, systolic blood pressure; DBP, diastolic blood pressure. [&] One-way ANOVA, Pearson's chi-square test or Kruskal–Wallis test used to calculate differences across tertiles; Pearson's chi-square test, Student's t-test or Mann–Whitney test used to calculate differences between tertiles; ** $P < 0.001$ vs. T1; * $P < 0.05$ vs. T1; † $P < 0.001$ vs. T2.

3.2. Association between Fecal Microbiota and Tertiles of Changes in Body Weight after 12-Month Intervention

From 372 participants with available stool samples, 357 were those with available baseline and correspondent 12-month timepoint sample included in the following steps of the analysis. Following the counts table filtering step, 12 samples were excluded from the analysis, further reducing the number of samples to 345.

Baseline characteristics and changes at 12-month timepoint in anthropometric and biochemical parameters, and blood pressure are shown in Table 2. In average, participants in tertile 1 and 2 lose weight (−7.2 ± 3.4 kg and −2.3 ± 1.0 kg, respectively), whereas participants in tertile 3 increased weight during the intervention. A total 82.6%, 54.8% and 13.0% of subjects allocated in tertiles 1, 2 and 3, respectively, belonged to the intensive lifestyle intervention group. There were significant differences at baseline in BMI, waist circumference and glucose levels across tertiles. Glucose levels were higher in those participants in tertile 2 compared to those in the other tertiles. Total body weight, BMI, waist circumference, glucose levels, glycated hemoglobin, and diastolic blood pressure decreased in tertile 1 and increased in tertile 3, with differences in changes significant between both extreme tertiles of body weight changes.

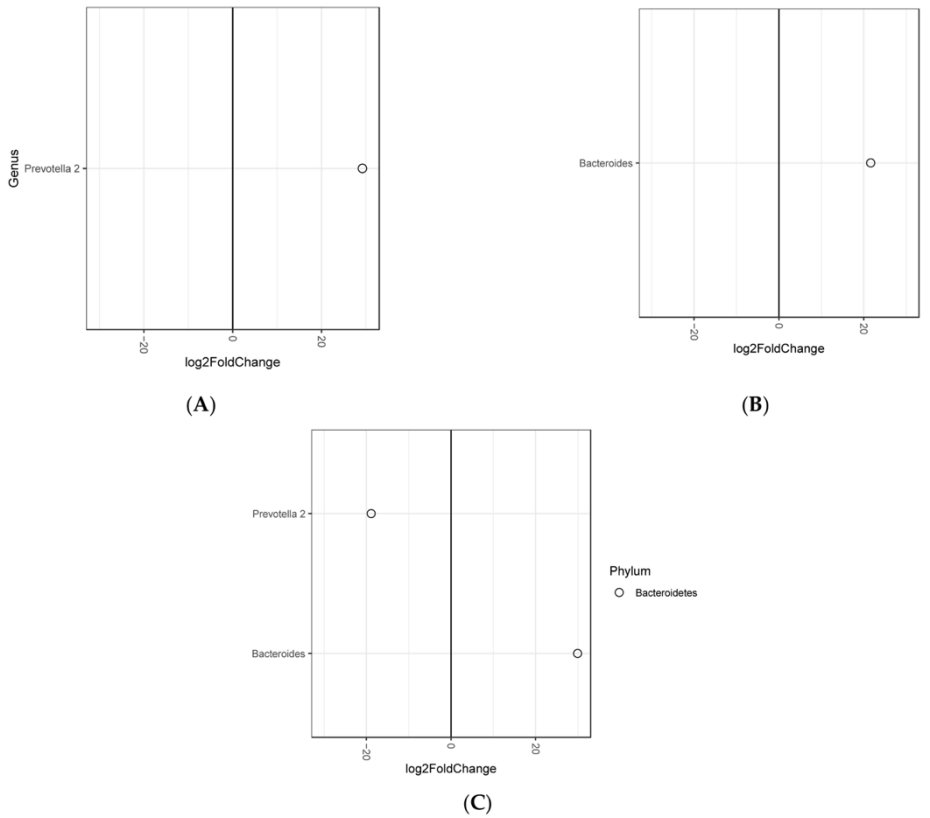


Figure 1. Differential abundant ASVs between tertiles of baseline body mass index. (**A**) tertile 1 versus tertile 2, (**B**) tertile 1 versus tertile 3, (**C**) tertile 2 versus tertile 3. Only ASVs with adjusted *P*-values < 0.05 are depicted.

Differences in F/B ratio and P/B ratio were not statistically significant across tertiles of changes in body weight (Supplementary Materials, Table S6).

A total of 8060 ASVs were detected in 690 samples. Statistically significant differential abundant ASVs determined between tertiles of changes in body weight after 12-month intervention are summarized in Figure 2, whereas detailed information, including *P* values are listed in Supplementary Materials, Table S8. A total of six ASVs were differentially abundant between tertile 1 and tertile 2, of which five (mostly represented by genera Prevotella 9, Bacteroides, and Lachnospiraceae UCG-001) were more abundant in tertile 1, whereas one ASV (represented by Prevotella 2 genus) more abundant in tertile 2. A total of six ASVs were differentially abundant between tertile 1 and tertile 3, all of which (mostly represented by genera Prevotella 9, Lachnospiraceae UCG-001, Bacteroides and uncultured bacteria) were more abundant in tertile 1. A total of 18 ASVs were differentially abundant between tertile 2 and tertile 3, of which two (represented by Bacteroides and Prevotella 2 genus) were more abundant in tertile 2, and 16 (mostly represented by genera Sutterella, Bacteroides, Prevotella 2, Dialister, Prevotella 9) were more abundant in tertile 2.

Table 2. Baseline characteristics and changes of the study population according to tertiles of changes in body weight after 12-month intervention.

Tertile **Min—Max**	**T1 (*n* = 115)** **−24.2–−4.5**	**T2 (*n* = 115)** **−4.5–−0.7**	**T3 (*n* = 115)** **−0.72–11.6**	***P* Trend** [&]
Sex, female	54 (47.0)	62 (53.9)	57 (49.6)	0.567
Age, years	64.4 ± 5.1	64.8 ± 4.8	64.8 ± 5.3	0.788
Recruiting center				0.178
Reus	45 (39.1)	48 (41.7)	35 (30.4)	
Malaga	70 (60.9)	67 (58.3)	80 (69.6)	
Intervention group	95 (82.6)	63 (54.8) **	15 (13.0) **	<0.001
Hypercholesterolemia	69 (60.0)	72 (62.6)	78 (68.7)	0.455
Hypertension	105 (91.3)	110 (95.7)	109 (94.8)	0.345
Type 2 diabetes prevalence	25 (21.7)	35 (30.4)	21 (18.3)	0.081
Insulin treatment	4 (3.5)	10 (8.7)	6 (5.2)	0.226
Metformin treatment	19 (16.5)	26 (22.6)	17 (18.3)	0.268
Other anti diabetic drugs use	19 (16.5)	28 (24.3)	17 (14.8)	0.139
Body weight, kg	89.2 ± 13.0	89.6 ± 14.1	86.1 ± 10.9	0.066
Change in body weight, kg	−7.2 ± 3.4	−2.3 ± 1.0 **	1.5 ± 1.7 **††	<0.001
BMI, kg/m^2	33.3 ± 3.6	33.8 ± 3.6	32.5 (3.1) †	0.018
Change in BMI, kg/m^2	−2.6 ± 1.3	−0.8 ± 0.5 **	0.6 ± 0.7 **††	<0.001
Waist circumference, cm	110.4 ± 10.1	111.6 ± 10.2	107.6 ± 9.8 *†	0.007
Change in waist circumference, cm	−7.4 ± 4.7	−2.4 ± 3.8 **	1.2 ± 3.8 **††	<0.001
Glucose, mg/dL	107.9 ± 22.7	114.9 ± 30.5 *	106.3 ± 21.3 †	0.023
Change in glucose, mg/dL	−7.8 ± 15.8	−1.9 ± 21.1 *	3.6 ± 21.4 **†	<0.001
HbA1c, %	5.9 [0.6]	5.9 [0.9]	5.7 [0.6]	0.086
Changes in HbA1c, %	−0.2 [0.4]	0.0 [0.3] *	0.1 [0.3] **†	<0.001
Triglycerides, mg/dL	137.0 [78.0]	153.0 [98.0]	162.0 [92.0]	0.571
Change in triglycerides, mg/dL	−19.0 [60.0]	−8.5 [60.2]	−4.5 [76.2]	0.595
Total cholesterol, mg/dL	201.5 ± 31.1	195.5 ± 40.2	205.1 ± 40.9	0.169
Change in total cholesterol, mg/dL	−1.6 ± 27.3	−0.8 ± 31.7	−4.9 ± 39.2	0.614
HDL-cholesterol, mg/dL	48.2 ± 13.3	48.0 ± 12.0	50.1 ± 12.2	0.387
Change in HDL-cholesterol, mg/dL	3.0 ± 6.7	3.0 ± 7.2	1.0 ± 8.2	0.065
LDL-cholesterol, mg/dL	120.4 ± 28.7	113.9 ± 34.5	120.7 ± 36.9	0.232
Change in LDL-cholesterol, mg/dL	−1.2 ± 24.2	−0.8 ± 27.1	−5.2 ± 35.3	0.462
SBP, mm Hg	140.1 ± 15.6	141.7 ± 17.3	139.2 ± 17.3	0.531
Change in SBP, mm Hg	−6.8 ± 13.1	−4.1 ± 16.0	−2.0 ± 16.6	0.058
DBP, mm Hg	79.9 ± 9.6	79.0 ± 10.2	79.0 ± 10.1	0.713
Change in DBP, mm Hg	−3.6 ± 8.2	−1.0 ± 8.3 *	−1.1 ± 8.3 *	0.027

Data shown as mean ± SD, median [IQR] or *n* (%); SD; standard deviation; IQR; interquartile range; BMI, body mass index; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein SBP, systolic blood pressure; DBP, diastolic blood pressure. [&] One-way ANOVA, Pearson's chi-square test or Kruskal–Wallis test used to calculate differences across tertiles; Pearson's chi-square test, Student's t-test or Man-Whitney test used to calculate differences between tertiles; ** $P < 0.001$ vs. T1; * $P < 0.05$ vs. T1; †† $P < 0.001$ vs. T2; † $P < 0.05$ vs. T2.

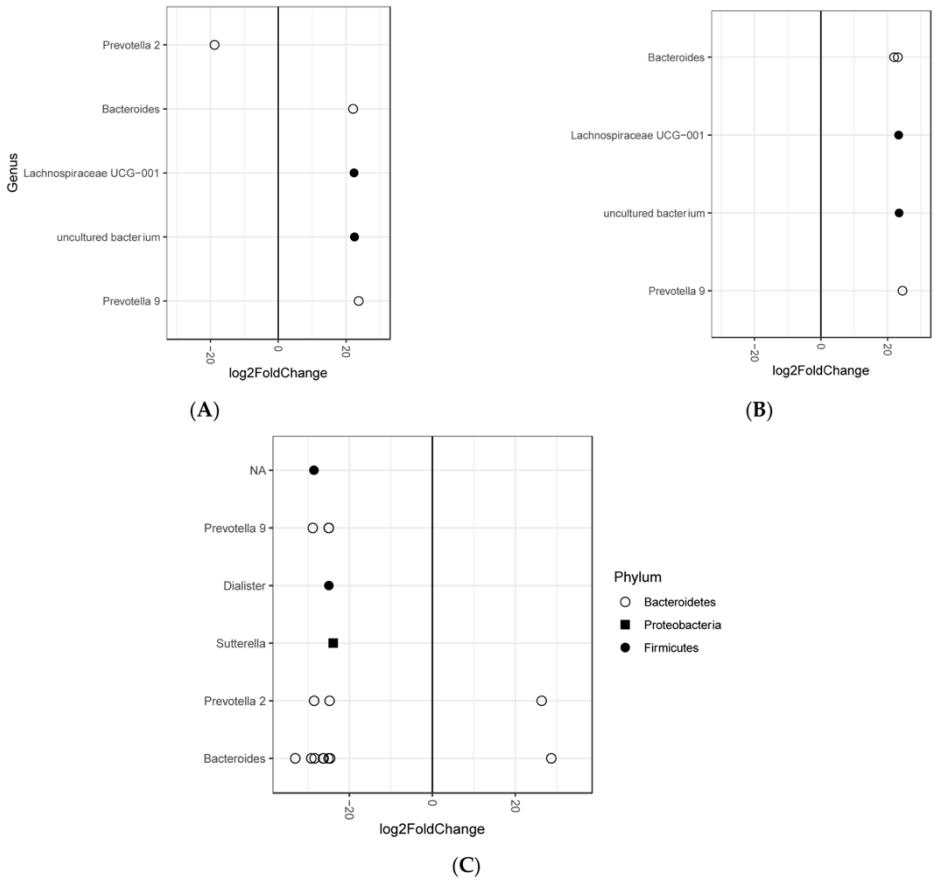


Figure 2. Differential abundant ASVs between tertiles of changes in body weight after 12-month intervention. (**A**) tertile 1 versus tertile 2, (**B**) tertile 1 versus tertile 3, (**C**) tertile 2 versus tertile 3. Only ASVs with adjusted *P*-values < 0.05 are depicted.

4. Discussion

In our study, conducted on subjects with obesity/overweight and metabolic syndrome, we found that a significant differential abundance of ASVs representing *Prevotella* 9, *Lachnospiraceae* UCG-001 and *Bacteroides* genus, was associated with a higher weight loss after 12-month of follow-up. Our findings support the hypothesis that specific components of fecal microbiota may be involved in the control of body weight. Consistently, in the cross-sectional analysis, ASVs representing *Prevotella* 2 and *Bacteroides* genus were significantly differentially abundant in the lowest tertile of baseline BMI.

The role of gut microbiota in the control of body weight was first described by Bäckhed et al. which observed an increase of body fat content and insulin resistance in GF mice colonized with gut microbiota of conventionally raised mice [7]. A drastic reduction in

Bacteroidetes and a proportional increase in *Firmicutes* was described in genetically obese mice compared to lean and wild type animals fed with the same diet, highlighting the gut microbiota's contribution to obesity [21]. Further animal [22] and human studies [7] confirmed these results; however, these findings are not consistent across different studies. A study conducted by Duncan et al., with the objective to examine the associations betweenBMI, weight loss and fecal microbiota, showed no significant differences in the proportion of *Bacteroidetes* between individuals with obesity and healthy individuals [23], whereas other studies, described a higher relative abundance of *Bacteroidetes* in subjects with obesity compared with lean subjects [24,25]. Accordingly, we did not find any association between F/B ratio neither with baseline BMI nor with weight changes, highlighting the need for focusing on a deeper taxonomic level rather than just consider the imbalance in the proportion of *Bacteroidetes* and *Firmicutes* phylum [26].

Studies at genus level showed that *Bacteroides* were lower in individuals with obesity compared to healthy individuals [27]. In a study conducted by Liu et al. *Bacteroides* spp. was found markedly reduced in Chinese individuals with obesity [28]. On the other hand, a comparative analysis of the gut microbiota of lean, normal, individuals with obesity and surgically treated Indian individuals with obesity showed higher levels of *Bacteroides* among subjects with obesity and its abundance positively correlated with BMI [25]. On the contrary, in our study, the *Bacteroides* abundance was significantly enriched in those patients with lower baseline BMI, and those who lost weight after 12-month of lifestyle intervention. *Bacteroides* is known as a mutualist bacterium that could drive the functionality of others [29]. Moreover, *Bacteroides* is able to adapt its metabolic machinery to the food source [30], becoming a key bacterium for dietary and/or weight loss interventions.

The relative abundance of *Prevotella* was found increased in individuals with severe obesity [31], contrarily in our results *Prevotella* genus was found increased in the lowest tertile of baseline BMI and in highest tertile of weight loss. Even though, other studies did not show any correlation between increased abundance of *Prevotella* and BMI [32].

The P/B ratio was demonstrated to be a useful tool to evaluate weight loss success in individuals with obesity exposed to ad libitum high fiber diets [12]. Results showed that individuals with high P/B ratio were more susceptible to lose weight on a diet rich in fiber and whole grains. A more recent study aimed to investigate the differences in weight loss maintenance between subjects with low and high P/B ratio and the potential interactions with markers of glucose metabolism and dietary fiber intake. Results showed that subjects with high P/B ratio were more susceptible to regain body weight than subjects with low P/B ratio, especially when dietary fiber intake was low and glucose metabolism was impaired [33]. Considering these findings, matching diet to gut microbiota profile may be crucial to increasing the effectiveness of weight loss programs. In a recent study conducted by Christiansen et al., healthy overweight subjects exposed to different fiber-rich diet were stratified according to baseline P/B ratios and *Prevotella* abundance. The *Prevotella* abundances correlated inversely with weight changes, whereas P/B ratios did not show any correlation. Subjects with high *Prevotella* abundance lost more weight than subjects with low *Prevotella* abundance when consuming a fiber-rich diet [34]. These outcomes are only partly supported by our results, in which no significant differences were observed in P/B ratio, but *Prevotella* 9 genus was found to be increased after weight loss.

Changes in the gut microbiota of patients with obesity after weight-loss interventions, have been described with divergent results between studies in terms of the bacterial profile involved [7,35]. A study conducted by Korpela et al. presented evidence about the validity of the baseline microbiota information in predicting the host's response to a dietary intervention [36]. Specifically, they identified *Clostridium* clusters and *Bacilli* indicative of the amenability of the gut microbiota to dietary modification, which in turn was associated with the host's lipid metabolism. According to these findings, we also detected an enrichment of uncultured genera belonging to the *Clostridiales* order in those patients with more tendency to lose weight after lifestyle intervention.

Contrary to our expectations in our study we observed that *Lachnospiraceae* UCG-001 genus was more abundant at baseline, in those subjects who lose weight after 12-month intervention, as these genera are producers of short chain fatty acids involved in an improvement in energy efficiency [37]. However, in a recent review, inconsistencies across different studies, about the impact of *Lachnospiaraceae* on the energy efficiency were reported [37], probably because an adequate amount of short chain fatty acids is necessary to control energy intake and expenditure.

A recent review showed inconsistent evidence to support baseline gut microbiota as an accurate predictor of weight loss in obesity, suggesting the need of further investigation with larger scale [38]. A recent study by Fragiadakis et al., aimed to determine if baseline gut microbiota was associated with long-term (12-month) diet weight loss success [39]. After 3 months of weight loss, they show differences in gut microbiota profile, however gut bacteria returned to the original composition at 12 months. Baseline gut microbiota profile was not associated to long-term changes in total body weight, suggesting a resilience to perturbation of the microbiota starting profile. Contrary to the aforementioned study, we have been able to detect differences at genus level after 12-month intervention, supporting long-term effects on weight loss.

In addition to the large sample size and the homogeneity of our study population (all with overweight/obesity and metabolic syndrome), this study has some limitations that deserve comments. First, in our study we did not evaluate short-term changes in body weight and therefore, we cannot determine resilience of the gut microbiota; second, the design of our study did not allow it to establish causality; and finally, as this study was conducted in elderly Spanish individuals with obesity and metabolic syndrome, it cannot be extrapolated to other populations.

5. Conclusions

We identified specific fecal microbiota signatures at genus level potentially related to changes in body weight in response to lifestyle intervention in an elderly population with overweight and obesity. These finding offer a promising novel perspective to support clinicians to tailor personalized interventions for obesity treatment, in which successful strategies can be predicted according to the microbiota composition. In any case, the validity of these microbial signatures has to be reproduced in other populations, taking into account the gut microbiota at species level. Furthermore, metabolic data are necessary to integrate these results and identify potential pathways involved, encouraging the need for further investigation in this field.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-2607/9/2/346/s1, Table S1: Differences in richness metrics, Chao1, Shannon and Simpson, between tertiles of baseline body mass index. Table S2: Differences in beta diversity distances, Bray-Curtis, Jaccard, Weighted Unifrac, Unweighted Unifrac, between tertiles of baseline body mass index. Table S3: Variability in beta diversity distances among tertiles of baseline body mass index. Table S4: Pairwise comparisons of baseline body mass index tertiles mean dispersion. Table S5: Results of log normalized Firmicutes-to-Bacteroidetes ratio and Prevotella-to-Bacteroides ratio between tertiles of baseline body mass index. Table S6: Results of log normalized Firmicutes-to-Bacteroidetes ratio and Prevotella-to-Bacteroides ratio between tertiles of changes in body weight after 12-month intervention. Table S7: Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of baseline body mass index. Table S8: Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of changes in body weight after 12-month intervention. Figure S1: Flowchart of study participants.

Author Contributions: Conceptualization, A.A., F.J.T., J.V. (Jesús Vioque), D.C., J.V. (Josep Vidal)., M.F., M.A.M.-G., M.B., and J.S.-S.; formal analysis, A.A. and S.G.; funding acquisition, F.J.T., J.V. (Jesús Vioque), D.C., J.V. (Josep Vidal), M.F., M.A.M.-G., and J.S.-S.; data curation, A.A., I.M.-I., and M.F.; writing—original draft preparation, A.A.; writing—review and editing, M.B., and J.S.-S.; supervision, M.B., and J.S.-S.; Visualization, S.G., J.M., N.B., F.J.T., J.V. (Jesús Vioque), D.C., O.C., J.V. (Josep Vidal),

I.M.-I., L.T.-C., R.F.-C., M.F., R.O., M.A.M.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713679 and from the Universitat Rovira i Virgili (URV). This work was supported by the official Spanish Institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund (three coordinated FIS projects leaded by J.S.-S., including the following projects: PI13/00462, PI16/00501 and PI19/00576); the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to J.S.-S.; the Recercaixa (number 2013ACUP00194) grant to J.S.-S.; The Fondo de Investigaciones Sanitarias of the Instituto de Salut Carlos III PI17/00215; The Generalitat Valenciana PROMETEO 17/2017. None of the funding sources took part in the design, collection, analysis, interpretation of the data, or writing the report, or in the decision to submit the manuscript for publication. J.S.-S., senior author of this study gratefully acknowledges the financial support by ICREA under the ICREA Academia programme. Food companies Hojiblanca (Lucena, Spain) and Patrimonio Comunal Olivarero (Madrid, Spain) donated extra virgin olive oil; and the Almond Board of California (Modesto, CA), American Pistachio Growers (Fresno, CA), and Paramount Farms (Wonderful Company, LLC, Los Angeles, CA) donated nuts for the PREDIMED-Pilot study.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Hospital Universitari Sant Joan de Reus Ethics Committee (protocol code 13-07-25/7proj2, date of approval: 25/07/2013), and the Comité de Ética de la Investigación Provincial de Málaga (protocol code Predimed+DM/01, date of approval: 27/11/2014).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and analysed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scommittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

Acknowledgments: We thank all the volunteers for the participation and personnel for the contribution in the PREDIMED-Plus trial. We also thank all the investigators of the PREDIMED-Plus study (supplemental Material). CIBEROBN (Centros de Investigación Biomédica en Red: Obesidad y Nutrición) is an initiative of ISCIII, Madrid, Spain. The authors also thank the PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples. Jordi Salas-Salvadó, senior author of this study gratefully acknowledges the financial support by ICREA under the ICREA Academia programme. The research groups thanks for its support to the CIBER-IBIMA-Metagenomics platform, especially to Pablo Rodríguez.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wang, Y.; Beydoun, M.A. The obesity epidemic in the United States—Gender, age, socioeconomic, racial/ethnic, and geographic characteristics: A systematic review and meta-regression analysis. *Epidemiol. Rev.* **2007**, *29*, 6–28. [CrossRef]
2. Ghosh, S.; Bouchard, C. Convergence between biological, behavioural and genetic determinants of obesity. *Nat. Rev. Genet.* **2017**, *18*, 731. [CrossRef] [PubMed]
3. Martin-Rodriguez, E.; Guillen-Grima, F.; Martí, A.; Brugos-Larumbe, A. Comorbidity associated with obesity in a large population: The APNA study. *Obes. Res. Clin. Pract.* **2015**, *9*, 435–447. [CrossRef] [PubMed]
4. Crovesy, L.; Masterson, D.; Rosado, E.L. Profile of the gut microbiota of adults with obesity: A systematic review. *Eur. J. Clin. Nutr.* **2020**. [CrossRef] [PubMed]

5. Bäckhed, F.; Ding, H.; Wang, T.; Hooper, L.V.; Koh, G.Y.; Nagy, A.; Semenkovich, C.F.; Gordon, J.I. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15718–15723. [CrossRef]
6. Thaiss, C.A.; Itav, S.; Rothschild, D.; Meijer, M.T.; Levy, M.; Moresi, C.; Dohnalová, L.; Braverman, S.; Rozin, S.; Malitsky, S.; et al. Persistent microbiome alterations modulate the rate of post-dieting weight regain. *Nature* **2016**, *540*, 544–551. [CrossRef]
7. Ley, R.E.; Turnbaugh, P.J.; Klein, S.; Gordon, J.I. Microbial ecology: Human gut microbes associated with obesity. *Nature* **2006**, *444*, 1022–1023. [CrossRef]
8. Karlsson, F.H.; Tremaroli, V.; Nookaew, I.; Bergström, G.; Behre, C.J.; Fagerberg, B.; Nielsen, J.; Bäckhed, F. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **2013**, *498*, 99–103. [CrossRef] [PubMed]
9. Christensen, L.; Roager, H.M.; Astrup, A.; Hjorth, M.F. Microbial enterotypes in personalized nutrition and obesity management. *Am. J. Clin. Nutr.* **2018**, *108*, 645–651. [CrossRef]
10. Gardner, C.D.; Trepanowski, J.F.; Del Gobbo, L.C.; Hauser, M.E.; Rigdon, J.; Ioannidis, J.P.; Desai, M.; King, A.C. Effect of low-fat VS low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion the DIETFITS randomized clinical trial. *JAMA* **2018**, *319*, 667–679. [CrossRef]
11. Pedrogo, D.A.M.; Jensen, M.D.; Van Dyke, C.T.; Murray, J.A.; Woods, J.A.; Chen, J.; Kashyap, P.C.; Nehra, V. Gut Microbial Carbohydrate Metabolism Hinders Weight Loss in Overweight Adults Undergoing Lifestyle Intervention with a Volumetric Diet. *Mayo Clin. Proc.* **2018**, *93*, 1101–1110.
12. Hjorth, M.F.; Roager, H.M.; Larsen, T.M.; Poulsen, S.K.; Licht, T.R.; Bahl, M.I.; Zohar, Y.; Astrup, A. Pre-treatment microbial Prevotella-to-Bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int. J. Obes.* **2018**, *42*, 580–583. [CrossRef] [PubMed]
13. Alberti, K.G.M.M.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.C.; James, W.P.T.; Loria, C.M.; Smith, S.C., Jr. Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention for the Study of Obesity. *Circulation* **2009**, *120*, 1640–1645. [CrossRef] [PubMed]
14. Mas-Lloret, J.; Obón-Santacana, M.; Ibáñez-Sanz, G.; Guinó, E.; Pato, M.L.; Rodriguez-Moranta, F.; Mata, A.; García-Rodríguez, A.; Moreno, V.; Pimenoff, V.N. Gut microbiome diversity detected by high-coverage 16S and shotgun sequencing of matched stool and colon biopsy samples. *bioRxiv* **2019**, 742635. [CrossRef]
15. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
16. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods.* **2016**, *13*, 581–583. [CrossRef]
17. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **2016**, *4*, e2584. [CrossRef]
18. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2012**, *41*, 590–596. [CrossRef]
19. McMurdie, P.J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **2013**, *8*, e61217. [CrossRef]
20. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 1–21. [CrossRef]
21. Ley, R.E.; Bäckhed, F.; Turnbaugh, P.; Lozupone, C.A.; Knight, R.D.; Gordon, J.I. Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11070–11075. [CrossRef]
22. Murphy, E.F.; Cotter, P.D.; Healy, S.; Marques, T.M.; O'sullivan, O.; Fouhy, F.; Clarke, S.F.; O'toole, P.W.; Quigley, E.M.; Stanton, C.; et al. Composition and energy harvesting capacity of the gut microbiota: Relationship to diet, obesity and time in mouse models. *Gut* **2010**, *59*, 1635–1642. [CrossRef]
23. Duncan, S.H.; Lobley, G.E.; Holtrop, G.; Ince, J.; Johnstone, A.M.; Louis, P.; Flint, H.J. Human colonic microbiota associated with diet, obesity and weight loss. *Int. J. Obes.* **2008**, *32*, 1720–1724. [CrossRef] [PubMed]
24. Schwiertz, A.; Taras, D.; Schäfer, K.; Beijer, S.; Bos, N.A.; Donus, C.; Hardt, P.D. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* **2010**, *18*, 190–195. [CrossRef] [PubMed]
25. Patil, D.P.; Dhotre, D.P.; Chavan, S.G.; Sultan, A.; Jain, D.S.; Lanjekar, V.B.; Gangawani, J.; Shah, P.S.; Todkar, J.S.; Shah, S.; et al. Molecular analysis of gut microbiota in obesity among Indian individuals. *J. Biosci.* **2012**, *37*, 647–657. [CrossRef]
26. John, G.K.; Mullin, G.E. The Gut Microbiome and Obesity. *Curr. Oncol. Rep.* **2016**, *18*, 1–7. [CrossRef]
27. Kasai, C.; Sugimoto, K.; Moritani, I.; Tanaka, J.; Oya, Y.; Inoue, H.; Tameda, M.; Shiraki, K.; Ito, M.; Takei, Y.; et al. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol.* **2015**, *15*, 100. [CrossRef]
28. Liu, R.; Hong, J.; Xu, X.; Feng, Q.; Zhang, D.; Gu, Y.; Shi, J.; Zhao, S.; Liu, W.; Wang, X.; et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med.* **2017**, *23*, 859–868. [CrossRef]
29. Wexler, A.G.; Goodman, A.L. An insider's perspective: Bacteroides as a window into the microbiome. *Nat. Microbiol.* **2017**, *2*, 1–11. [CrossRef]
30. Wexler, H.M. Bacteroides: The good, the bad, and the nitty-gritty. *Clin. Microbiol. Rev.* **2007**, *20*, 593–621. [CrossRef] [PubMed]

31. Zhang, H.; DiBaise, J.K.; Zuccolo, A.; Kudrna, D.; Braidotti, M.; Yu, Y.; Parameswaran, P.; Crowell, M.D.; Wing, R.; Rittmann, B.E.; et al. Human gut microbiota in obesity and after gastric bypass. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2365–2370. [CrossRef] [PubMed]
32. Zupancic, M.L.; Cantarel, B.L.; Liu, Z.; Drabek, E.F.; Ryan, K.A.; Cirimotich, S.; Jones, C.; Knight, R.; Walters, W.A.; Knights, D.; et al. Analysis of the gut microbiota in the old order amish and its relation to the metabolic syndrome. *PLoS ONE* **2012**, *7*, e43052. [CrossRef]
33. Hjorth, M.F.; Christensen, L.; Kjølbæk, L.; Larsen, L.H.; Roager, H.M.; Kiilerich, P.; Kristiansen, K.; Astrup, A. Pretreatment Prevotella-to-Bacteroides ratio and markers of glucose metabolism as prognostic markers for dietary weight loss maintenance. *Eur. J. Clin. Nutr.* **2020**, *74*, 338–347. [CrossRef]
34. Christensen, L.; Vuholm, S.; Roager, H.M.; Nielsen, D.S.; Krych, L.; Kristensen, M.; Astrup, A.; Hjorth, M.F. Prevotella Abundance Predicts Weight Loss Success in Healthy, Overweight Adults Consuming a Whole-Grain Diet Ad Libitum: A Post Hoc Analysis of a 6-Wk Randomized Controlled. *Trial. J. Nutr.* **2019**, *149*, 2174–2181. [CrossRef] [PubMed]
35. Damms-Machado, A.; Mitra, S.; Schollenberger, A.E.; Kramer, K.M.; Meile, T.; Königsrainer, A.; Huson, D.H.; Bischoff, S.C. Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption. *BioMed Res Int.* **2015**, *2015*, 806248. [CrossRef]
36. Korpela, K.; Flint, H.J.; Johnstone, A.M.; Lappi, J.; Poutanen, K.; Dewulf, E.; Delzenne, N.; De Vos, W.M.; Salonen, A. Gut Microbiota Signatures Predict Host and Microbiota Responses to Dietary Interventions in Obese Individuals. *PLoS ONE* **2014**, *9*, e90702. [CrossRef]
37. Vacca, M.; Celano, G.; Calabrese, F.M.; Portincasa, P.; Gobbetti, M.; De Angelis, M. The controversial role of human gut lachnospiraceae. *Microorganisms* **2020**, *8*, 573. [CrossRef]
38. Biesiekierski, J.R.; Jalanka, J.; Staudacher, H.M. Can Gut Microbiota Composition Predict Response to Dietary Treatments? *Nutrients* **2019**, *11*, 1134. [CrossRef] [PubMed]
39. Fragiadakis, G.K.; Wastyk, H.C.; Robinson, J.L.; Sonnenburg, E.D.; Sonnenburg, J.L.; Gardner, C.D. Long-term dietary intervention reveals resilience of the gut microbiota despite changes in diet and weight. *Am. J. Clin. Nutr.* **2020**, *111*, 1127–1136. [CrossRef] [PubMed]

microorganisms

MDPI

Supplementary Materials: Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome

Alessandro Atzeni, Serena Galié, Jananee Muralidharan, Nancy Babio, Francisco José Tinahones, Jesús Vioque, Dolores Corella, Olga Castañer, Josep Vidal, Isabel Moreno-Indias, Laura Torres-Collado, Rebeca Fernández-Carrión, Montserrat Fitó, Romina Olbeyra, Miguel Angel Martínez-González, Monica Bulló* and Jordi Salas-Salvadó *

Table S1. Differences in richness metrics, Chao1, Shannon and Simpson, between tertiles of baseline body mass index.

Differences in Chao1		
	Tertile 1	Tertile 2
Tertile 2	0.95 #	-
Tertile 3	0.95	0.95
Differences in Shannon		
	Tertile 1	Tertile 2
Tertile 2	0.82	-
Tertile 3	0.82	0.82
Differences in Simpson		
	Tertile 1	Tertile 2
Tertile 2	0.92	-
Tertile 3	0.92	0.92

False discovery rate *P*-value tested by Wilcoxon rank sum test.

Table S2. Differences in beta diversity distances, Bray-Curtis, Jaccard, Weighted Unifrac, Unweighted Unifrac, between tertiles of baseline body mass index.

Differences in Bray-Curtis distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.762	0.381	1.062	0.006	0.318
Residuals	361	129.461	0.359		0.994	
Total	363	130.223			1.000	
Differences in Jaccard distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.863	0.431	1.033	0.006	0.323
Residuals	361	150.728	0.417		0.994	
Total	363	152.591			1.000	
Differences in Weighted Unifrac distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.004	0.002	0.633	0.003	0.535
Residuals	361	1.267	0.003		0.996	
Total	363	1.271			1.000	
Differences in Unweighted Unifrac distance						
	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
Tertile	2	0.218	0.109	0.904	0.005	0.716
Residuals	361	43.552	0.121		0.995	
Total	363	43.770			1.000	

Df, degrees of freedom; Sqs, squares; Pr(>F), significance on F-test. Differences in beta diversity distances were tested using PERMANOVA test. Permutations: free; number of permutations 999; terms added sequentially (first to last).

Table S3. Variability in beta diversity distances among tertiles of baseline body mass index.

Bray-Curtis distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0002	0.0001	0.050	999	0.955
Residuals	361	0.911	0.002			
Jaccard distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.00008	0.00004	0.044	999	0.953
Residuals	361	0.316	0.0009			
Weighted Unifrac distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0009	0.0004	0.439	999	0.644
Residuals	361	0.357	0.001			
Unweighted Unifrac distance						
	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.0005	0.0002	0.066	999	0.939
Residuals	361	1.323	0.004			

Df, degrees of freedom; Sq, square; N.Perm, number of permutations; Pr(>F), significance on F-test. Permutation test for homogeneity of multivariate dispersions performed to statistically test variability among groups. Permutation: free; number of permutations: 999; response: distances.

Table S4. Pairwise comparisons of baseline body mass index tertiles mean dispersion.

Bray-Curtis distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.885 [#]	0.871
Tertile 2	0.879	-	0.743
Tertile 3	0.871	0.747	-
Jaccard distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.823	0.929
Tertile 2	0.838	-	0.769
Tertile 3	0.931	0.768	-
Weighted Unifrac distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.374	0.910
Tertile 2	0.392	-	0.435
Tertile 3	0.927	0.438	-
Unweighted Unifrac distance			
	Tertile 1	Tertile 2	Tertile 3
Tertile 1	-	0.762	0.738
Tertile 2	0.779	-	0.977
Tertile 3	0.735	0.972	-

[#] Observed P-value below diagonal. permuted *P*-value above diagonal, calculated using t-test performed on the pairwise group dispersions.

Table S5. Results of log normalized *Firmicutes*-to-*Bacteroidetes* ratio and *Prevotella*-to-*Bacteroides* ratio between tertiles of baseline body mass index.

	N	Mean	Std dev	Min	Max
Log2_FB_ratio					
Tertile 1	121	0.861	1.388	−5.500	3.536
Tertile 2	122	0.864	1.298	−4.917	2.964
Tertile 3	121	0.915	1.310	−3.917	2.839
Log2_PB_ratio					
Tertile 1	121	−0.224	2.786	−11.459	10.071
Tertile 2	122	−0.146	2.543	−9.515	6.104
Tertile 3	121	−0.758	3.154	−12.788	5.687

Log2_FB_ratio one-way ANOVA (F (2, 361) = 0.064, $P = 0.938$).
Log2_PB_ratio one-way ANOVA (F (2, 361) = 1.388, $P = 0.332$).

Table S6. Results of log normalized *Firmicutes*-to-*Bacteroidetes* ratio and *Prevotella*-to-*Bacteroides* ratio between tertiles of changes in body weight after 12-month intervention.

	N	Mean	Std dev	Min	Max
Log2_FB_ratio					
Tertile 1	115	0.783	1.367	−2.96	5.22
Tertile 2	115	1.046	1.223	−2.74	4.21
Tertile 3	115	0.759	1.358	−3.54	4.11
Log2_PB_ratio					
Tertile 1	115	−0.289	2.756	−12.47	6.00
Tertile 2	115	−0.270	2.576	−12.70	6.06
Tertile 3	115	−0.295	2.778	−11.46	5.69
Delta_FB_ratio					
Tertile 1	115	0.041	1.404	−4.05	4.59
Tertile 2	115	−0.229	1.325	−7.16	2.44
Tertile 3	115	0.116	1.478	−6.86	3.83
Delta_PB_ratio					
Tertile 1	115	−0.164	3.297	−12.62	12.47
Tertile 2	115	0.034	2.838	−13.26	9.72
Tertile 3	115	0.293	3.399	−11.79	11.46

Log2_FB_ratio one-way ANOVA (F (2, 342) = 1.680, $P = 0.188$)
Log2_PB_ratio one-way ANOVA (F (2, 342) = 0.003, $P = 0.997$).
Delta_FB_ratio one-way ANOVA (F (2, 342) = 1.931, $P = 0.147$).
Delta_PB_ratio one-way ANOVA (F (2, 342) = 0.598, $P = 0.550$).

Table S7. Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of baseline body mass index.

ASV ID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Genus
T1 vs T2							
5b1b613f2b8a8224ffb8f4df91585f76	10.099	29.200	3.568	8.185	2.7×10^{-16}	1.2×10^{-12}	Prevotella 2
T1 vs T3							
6c03f9ce7c4e3251103817d98a644563	0.442	21.623	3.585	6.032	1.6×10^{-9}	6.9×10^{-6}	Bacteroides
T2 vs T3							
6c03f9ce7c4e3251103817d98a644563	0.442	29.927	3.540	8.453	2.8×10^{-17}	1.2×10^{-13}	Bacteroides
5b1b613f2b8a8224ffb8f4df91585f76	10.099	−18.876	3.541	−5.33	9.8×10^{-18}	2.1×10^{-14}	Prevotella 2

lfcSE, log fold change Standard Error; stat, Wald test statistic; padj, Benjamini-Hochberg adjusted *P*-value.

Table 8. Differential abundant ASVs, and correspondent assigned genera, identified by DESeq2 in gut microbiota, between tertiles of changes in body weight after 12-month intervention.

ASV ID	baseMean	log2FoldChange	lfcSE	stat	*p*-value	padj	Genus
T1 vs T2							
de204b44fc231cd402652f4d87380471	0.234	23.705	3.671	6.457	1.1×10^{-10}	7.3×10^{-7}	Prevotella 9
d7cc2fea278ab0156b4e6e72dcdbe327	0.103	22.482	3.671	6.123	9.2×10^{-10}	2.0×10^{-6}	uncultured
c2a9c6e2349c0166d4d1f321354baae3	0.095	22.355	3.671	6.088	1.1×10^{-9}	2.0×10^{-6}	Lachnospiraceae UCG-001
733ac0a6c00a457793ea9c9d1a428aa9	0.102	22.323	3.671	6.080	1.2×10^{-9}	2.0×10^{-6}	NA
cc5d6923fc7f11241605 33a11bcfbb76	0.078	22.072	3.671	6.011	1.8×10^{-9}	2.5×10^{-6}	Bacteroides
5b1b613f2b8a8224ffb8f4df91585f76	8.046	−18.829	3.670	−5.13	2.9×10^{-7}	3.3×10^{-4}	Prevotella 2
T1 vs T3							
de204b44fc231cd402652f4d87380471	0.234	24.512	4.303	5.697	1.2×10^{-8}	8.3×10^{-5}	Prevotella 9
d7cc2fea278ab0156b4e6e72dcdbe327	0.103	23.473	4.303	5.454	4.9×10^{-8}	1.2×10^{-4}	uncultured
c2a9c6e2349c0166d4d1f321354baae3	0.095	23.330	4.303	5.421	5.9×10^{-8}	1.2×10^{-4}	Lachnospiraceae UCG-001
cc5d6923fc7f11241605 33a11bcfbb76	0.078	23.103	4.303	5.368	7.9×10^{-8}	1.2×10^{-4}	Bacteroides
733ac0a6c00a457793ea9c9d1a428aa9	0.102	23.018	4.303	5.349	8.8×10^{-8}	1.2×10^{-4}	NA
d00c1161004d40e7b94bbbda74a867cf	0.355	22.966	4.303	5.105	3.3×10^{-7}	3.7×10^{-4}	Bacteroides
T2 vs T3							

d00c1161004d40e7b94b bbda74a867cf	0.356	28.676	3.830	7.488	7.0×10^{-14}	1.2×10^{-10}	Bacteroides
5b1b613f2b8a8224ffb8f 4df91585f76	8.046	26.343	3.831	6.876	6.1×10^{-12}	4.7×10^{-9}	Prevotella 2
c24bf620a7e511901684 4066114196fc	0.070	−23.898	3.832	−6.237	4.5×10^{-10}	1.7×10^{-7}	Sutterella
68ec1aefa0103ae766daf c814b38894b	0.142	−24.646	3.832	−6.432	1.3×10^{-10}	5.0×10^{-8}	Bacteroides
26cb357d46b7cb7c7fc6 0dec29530cba	0.077	−24.767	3.832	−6.464	1.0×10^{-10}	4.4×10^{-8}	Prevotella 2
89595c082e6cf4a47edb b41a7d13bd3a	0.095	−24.929	3.832	−6.506	7.7×10^{-11}	3.5×10^{-8}	Dialister
a10c31f88f53ba0b7319 de39d21e0ee6	0.099	−24.931	3.832	−6.506	7.7×10^{-11}	3.5×10^{-8}	Prevotella 9
21ab743a71ced7896203 e4bfeca1289f	0.113	−24.988	3.832	−6.521	6.7×10^{-11}	3.5×10^{-8}	Prevotella 9
579a75cbf324508b5a74 d6533dd9169c	0.181	−25.015	3.832	−6.528	6.6×10^{-11}	3.5×10^{-8}	Bacteroides
fb5d414fd6bd7d6dc753 f0b889a3c473	0.167	−26.198	3.832	−6.837	8.1×10^{-12}	5.0×10^{-9}	Bacteroides
88bff18bedc7d38cd6ff3 b46201c90d0	0.167	−26.198	3.832	−6.837	8.1×10^{-12}	5.0×10^{-9}	Bacteroides
a188c60c7eab567a12ae 4318c1a54694	0.107	−26.339	3.832	−6.874	6.2×10^{-12}	4.7×10^{-9}	Bacteroides
9e7912a029eca5c8e694 a06acaa15ef3	0.111	−28.364	3.832	−7.4	1.3×10^{-13}	1.3×10^{-10}	Bacteroides
a58697475d24d4d265ea a922b346d6f5	0.088	−28.490	3.832	−7.435	1.0×10^{-13}	1.2×10^{-10}	Prevotella 2
4c6f7e347c7157738bafc b4ac98c385f	0.103	−28.547	3.832	−7.450	9.3×10^{-14}	1.2×10^{-10}	NA
eb1e06232c80ba2efd64 dfa172203d5c	0.171	−28.813	3.832	−7.520	5.5×10^{-14}	1.2×10^{-10}	Prevotella 9
d49ea76b83aacea8576b fc30b28f0caa	0.165	−29.180	3.832	−7.615	2.6×10^{-14}	9.0×10^{-11}	Bacteroides
02500c8e7dff746267b9 b6542b9f5140	0.225	−33.036	3.832	−8.622	6.6×10^{-18}	4.5×10^{-14}	Bacteroides

lfcSE, log fold change Standard Error; stat, Wald test statistic; padj, Benjamini-Hochberg adjusted *P*-value.

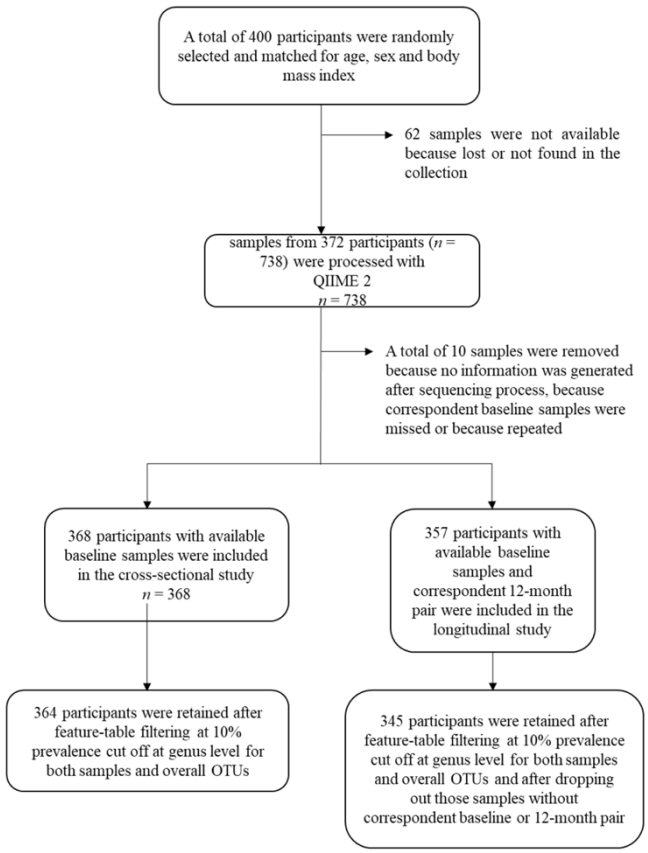


Figure S1. Flowchart of study participants.

Publication 2

Type: Journal article

Authors: Alessandro Atzeni, Thomaz F. S. Bastiaanssen, John F. Cryan, Francisco José Tinahones, Jesús Vioque, Dolores Corella, Montserrat Fitó, Josep Vidal, Isabel Moreno-Indias, Ana M. Gómez Pérez, Laura Torres-Collado, Oscar Coltell, Olga Castañer, Monica Bulló, Jordi Salas-Salvadó

Title: Taxonomic and Functional Fecal Microbiota Signatures Associated with Insulin Resistance in Non-diabetic Subjects with Overweight/Obesity Within the Frame of the PREDIMED-Plus Study

Journal: Frontiers in Endocrinology

Publisher: Frontiers

Status: Accepted on March 21, 2022

Taxonomic and functional fecal microbiota signatures associated with insulin resistance in non-diabetic subjects with overweight/obesity within the frame of the PREDIMED-Plus study

Alessandro Atzeni[1,2,3], Thomaz F. S. Bastiaanssen[4], John F. Cryan[4], Francisco José Tinahones[3,5], Jesús Vioque[6,7], Dolores Corella[3,8], Montserrat Fitó[3,9], Josep Vidal[10,11], Isabel Moreno Indias[3,5], Ana M. Gómez Pérez[3,5], Laura Torres Collado[6,7], Oscar Coltell[3,12], Olga Castañer[3,9], Monica Bulló[1,2,3*], Jordi Salas Salvadó[1,2,3*]

[1]Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Reus, Spain

[2]Institut D'Investigació Sanitària Pere Virgili (IISPV), Hospital Universitari de Sant Joan de Reus, Reus, Spain

[3]CIBER de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain

[4]APC Microbiome Ireland, Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

[5]Unidad de Gestión Clínica de Endocrinología y Nutrición, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Málaga, Spain

[6]Instituto de Investigación Sanitaria y Biomédica de Alicante, ISABIAL-UMH, Alicante, Spain

[7]CIBER Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[8]Department of Preventive Medicine, University of Valencia, Valencia, Spain

[9]Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Research Institute (IMIM), Barcelona, Spain

[10]Endocrinology and Nutrition Department, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic Universitari, Barcelona, Spain

[11]CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[12]Department of Computer Languages and Systems, University Jaume I, Castelló de la Plana, Spain

*** Correspondence:**
Monica Bulló
monica.bullo@urv.cat

Jordi Salas Salvadó
jordi.salas@urv.cat

Keywords: fecal microbiota, insulin resistance, HOMA-IR, 16S sequencing, gut metabolic modules

Abstract

Objective: An altered gut microbiota has been associated with insulin resistance, a metabolic dysfunction consisting of cellular insulin signaling impairment. The aim of the present study is to determine the taxonomic and functional fecal microbiota signatures associated with HOMA-IR index in a population with high cardiovascular risk.

Methods: A total of 279 non-diabetic individuals (55-75 years aged) with overweight/obesity and metabolic syndrome were stratified according to tertiles of HOMA-IR index. Blood biochemical parameters, anthropometric measurements and fecal samples were collected at baseline. Fecal microbial DNA extraction, 16S amplicon sequencing and bioinformatics analysis were performed.

Results: *Desulfovibrio*, *Odoribacter* and *Oscillospiraceae* UCG-002 were negatively associated with HOMA-IR, whereas predicted total functional abundances revealed gut metabolic modules mainly linked to amino acid degradation. *Butyricicoccus*, *Erysipelotrichaceae* UCG-003, *Faecalibacterium* were positively associated with HOMA-IR index, whereas predicted total functional abundances revealed gut metabolic modules mainly linked to saccharide degradation. These bacteria contribute differentially to the gut metabolic modules, being the degree of contribution dependent on insulin resistance. Both taxa and gut metabolic modules negatively associated to HOMA-IR index were linked to mechanisms involving sulfate reducing bacteria, improvement of intestinal gluconeogenesis and production of acetate. Furthermore, both taxa and gut metabolic modules positively associated to HOMA-IR index were linked to production and mechanisms of action of butyrate.

Conclusions: Specific taxonomic and functional fecal microbiota signatures associated with insulin resistance were identified in a non-diabetic population with overweight/obesity at high cardiovascular risk. These findings suggest that tailoring therapies based on specific fecal microbiota profiles could be a potential strategy to improve insulin sensitivity.

INTRODUCTION

Insulin is a peptide hormone that plays an essential role in the process of glucose metabolism regulation. Insulin resistance (IR) can be defined as a pathological condition of cellular insulin signaling impairment with consequent disturbance of intracellular signaling transduction, which affects several organs and tissues (1,2). Visceral adiposity and increased body fat induce the release of relatively high levels of free fatty acids and pro-inflammatory cytokines into circulation, causing the development of hepatic and muscle IR and type 2 diabetes (T2D) (3).

An altered gut microbiota composition has been associated with the pathophysiology of obesity and IR (4). First evidence was reported in 2004 by Bäckhed *et al*., who has observed an increase in body fat and IR in germ-free mice transplanted with gut microbiota from conventionally raised mice donors (5). A few years later, Vrieze *et al*., shown that insulin sensitivity improved in a cohort of human participants with metabolic syndrome (MetS) six weeks after receiving microbiota from lean healthy human donors (6).

The gut microbiome can induce IR through different mechanisms. Cani *et al*., were the first to identify bacterial lipopolysaccharide (LPS) as a causative inflammatory factor of IR (7). LPS increases intestinal permeability with its consequent translocation from the intestinal lumen to

circulation (8,9). At the cell membrane level, LPS binds and activates the toll-like receptor 4 (TLR4), with consequent downstream cell signaling pathways activation which lead to inflammatory response and cytokine expression and secretion (10). A reduction of bacterial species able to produce short chain fatty acids (SCFAs), with well-established anti-inflammatory effects (11–13), also induces IR (4). However, these findings are still not totally clear due to some controversial results, as those described by Perry and colleagues, who observed increased production of acetate associated with IR and obesity in rodents exposed to high caloric diet (14). In addition, the bioconversion of bile acids carried out by bacteria from human gut, including members of *Lactobacilli*, *Bifidobacteria*, *Clostridium* and *Bacteroides*, has been shown contributing to glucose homeostasis (15,16). A study conducted by Pedersen *et al.*, aimed to identify the gut microbial profile of non-diabetic individuals with IR, has demonstrated a positive association between IR and branched-chain amino acids (BCAA) levels (15). Taken together, these studies suggest that gut microbiome may play an important role in modulating IR, though it remains unclear which aspects of the microbiome contribute to this. Therefore, identify potential fecal microbiota profiles and related metabolic pathways linked with the development of IR is crucial, as characterize the intestinal bacterial community related to glucose homeostasis would be a useful strategy to ameliorate IR and related disorders (17). Accordingly, the aim of the present study was to determine the taxonomic and functional fecal microbiota signatures associated with IR in a population of 279 non-diabetic elderly individuals with overweight/obesity and MetS.

PATIENTS AND METHODS

Participants and study design

This cross-sectional study was conducted with baseline data of participants recruited for the PREDIMED-Plus study (18,19), an ongoing parallel-group, randomized and controlled clinical trial conducted in 23 Spanish centers, which aims to evaluate the effect of an intensive weight loss intervention (based on an hypocaloric Mediterranean diet (MedDiet), physical activity promotion and behavioral support) on cardiovascular disease (CVD) events, compared to a control group receiving usual care advice. The trial was registered at the International Standard Randomized Controlled Trial (http://www.isrctn.com/ISRCTN89898870) and approved by the institutional review board of all participating institutions. The full detailed PREDIMED-Plus protocol is available at http://www.predimedplus.com.

PREDIMED-Plus eligible participants were men and women aged 55–75 years, with overweight or obesity (body mass index (BMI) 27–40 kg/m2), who satisfied at least three criteria for the MetS (waist circumference > 102 cm in men and > 88 cm in women; serum triglyceride ≥ 150 mg/dL or drug treatment for elevated triglycerides; high-density lipoprotein (HDL) cholesterol < 40 mg/dL in men and < 50 mg/dL in women or drug use for low HDL cholesterol; blood pressure ≥ 130/85 mmHg or antihypertensive drug treatment; and fasting plasma glucose level ≥ 100 mg/dL or hypoglycemic treatment) and were free of CVD.

A subsample of 400 participants from the PREDIMED-Plus recruiting centers of Reus and Malaga (Spain) were randomized and matched for age, sex and BMI. For the present cross-sectional study, we select 279 individuals from this cohort with available stool samples and without T2D.

Clinical assessments and blood biochemical analysis

Body weight was measured using high-quality electronic calibrated scales, height was measured using a wall-mounted stadiometer, waist circumference was measured midway between the lowest

rib and the iliac crest using an anthropometric tape, blood pressure was measured using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan).

Blood samples were collected after overnight fasting. Plasma levels of glucose, insulin, total cholesterol, HDL cholesterol and triglycerides were measured using standard enzymatic methods, low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula (whenever triglycerides were less than 300 mg/dL), glycated hemoglobin was measured by a chromatographic method. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) (20), using the following formula:

$$insulin\ (mU/mL) \times glucose\ (mg/dL)\Big/_{405}$$

In addition, information regarding lifestyle, education level, disease prevalence and current medication use was collected, whereas a validated 17-item questionnaire (21) was used to assess the adherence to the MedDiet.

Fecal DNA extraction and 16S rRNA gene sequencing

A complete workflow including the analytic steps is shown in Figure 1.

Stool samples were collected at home by participants and kept frozen. In case of antibiotic use or prebiotics supplementation, samples were collected 15 days after completion of treatment. A time range of 15 days was considered wide enough to gut microbiota recovery after therapeutic antibiotic usage, taking into account the exposure to short-term treatment with amoxicillin (22). Once delivered to the laboratory, stool samples were separated into 250 mg aliquots and stored at −80°C until analysis.

Microbial DNA was extracted with the QIAmp PowerFecal DNA kit (Qiagen, Hilden, Germany), following the manufacturer's instructions, and DNA concentration and purity assessed with the Qubit 2.0 Fluorometer-dsDNA Broad Range Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Targeted 16S sequencing libraries were created with the 16S Metagenomics kit (Thermo Fisher Scientific, Waltham, MA, USA), using a pool of primers to amplify the regions V2–V9 of the 16S rRNA gene, and barcoded adapters ligated using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific, Waltham, MA, USA). Synthesized libraries were pooled and templated on the automated Ion Chef system (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced on the Ion S5(Thermo Fisher Scientific, Waltham, MA, USA). Reads generated were subsequently converted in fastq format using the File Explorer plugin of the Torrent Suite Server software (Thermo Fisher Scientific, Waltham, MA, USA), interfaced with the Ion S5. The quality control of fastq files was assessed with FastQC using a quality score of 20 as a threshold (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Statistical analysis

Demultiplexed sequence reads, were imported into R (version 4.0.5), quality filtered, chimera-checked and clustered in amplicon sequence variants (ASVs) using the R package DADA2 (v 1.16.0) (23) whereas taxonomy was assigned using the SILVA reference database release 138 (24). Obtained counts were filtered and transformed using a centered log-ratio (CLR) transformation (25). Principal component analysis (PCA) was performed to evaluate the distribution of the study population. Alpha diversity indices, Chao1 (26), Shannon (27) and Simpson (28) were calculated and differences

between tertiles of HOMA-IR index tested. Beta diversity was computed in terms of Aitchison distance, or Euclidean distance over CLR-transformed counts(25). Permutational multivariate analysis of variance (PERMANOVA) was performed using the adonis function ("vegan" package, version 2.5-6) to test differences between groups composition. The association between taxa and tertiles of HOMA-IR index was tested using generalized linear modelling (GLM), adjusted for 17-item MedDiet adherence score and recruiting center as covariates. Multiple testing correction was performed using Storey's q-value procedure, and $q < 0.1$ deemed as significant (29).

In order to perform an inferential analysis of the functional potential of the microbiome, DADA2 output files were parsed and used as input for PICRUSt2 in order to generate a table of inferred per-sample abundances of KEGG genome orthologs (KO) (30). To assess the diversity in metabolic potential and anaerobic fermentation capacity encoded in (meta-) genomic sequences, we used a manually curated database of human gut metabolic modules (GMM), that describe enzymatic processes annotated using exclusively prokaryotic and archaeal KO (31). GMM counts were filtered, converted to CLR and the association with HOMA-IR index tested with the same GLM setup used for the analysis of taxa.

In addition to the predicted total functional abundances, PICRUSt2 also includes the predicted functional contribution per taxa (using the --stratified flag). This information was used to study the contribution of each taxon of interest to the GMM of interest.

The clinical characteristics of the study population were described using IBM SPSS Statistics version 23 (SPSS Inc., Chicago, Illinois, USA). Data normality was assessed with Shapiro-Wilk test. Normally distributed variables were described by means and standard deviations, non-normally distribute variables by median and interquartile range, whereas categorical variables were described as number and percentages. Differences across tertiles were evaluated through one-way analysis of variance (ANOVA) parametric test or Kruskal-Wallis non-parametric test for numerical variables, and with Pearson's chi-square test for categorical variables. Student's t parametric test or Mann-Whitney non-parametric test was used to calculate differences between tertiles for numerical variables. All statistical tests were 2-sided and $p < 0.05$ was deemed statistically significant.

RESULTS

Clinical characteristics of the study population

Clinical characteristics of the study population categorized by tertiles of HOMA-IR index are shown in Table 1. Body weight and waist circumference were significantly lower in those participants in tertile 1 (T1) of HOMA-IR index than those in tertile 2 (T2) or tertile 3 (T3); BMI, triglycerides, glucose, insulin and glycated hemoglobin were significantly lower in those participants in T1 compared to T2 and T3 and participants in T2 *versus* those in T3; HDL-cholesterol was significantly higher in T1 *versus* T2 and T3, and in T2 *versus* T3; MedDiet adherence score was significantly higher in T2 vs T3.

Microbiome diversity is not associated with HOMA-IR index

PCA analysis over the CLR-transformed microbiome count data revealed that the first two components account approximately 12.22% and 8.46% respectively. These figures are approximately in line with literature (32). PCA plots shown that the microbiome sample distribution did not cluster based on tertiles of HOMA-IR index (Supplementary figure 1). There were no statistically significant differences across tertiles of HOMA-IR index in the alpha diversity indices Chao1, Shannon and

Simpson (Supplementary figure 2, Supplementary Table 1). The results of the PERMANOVA analysis and the permutation test for homogeneity of multivariate dispersions using Aitchison distance were also not statistically significant across tertiles of HOMA-IR index (Supplementary table 2).

Several microbial genera and GMM are associated with HOMA-IR index

Taxa and GMM significantly associated with HOMA-IR index are summarized in Table 2. Genera *Desulfovibrio*, *Odoribacter* and *Oscillospiraceae* UCG-002 were negatively associated with the highest tertile of HOMA-IR index, whereas genera *Butyricicoccus*, *Erysipelotrichaceae* UCG-003 and *Faecalibacterium* were positively associated with the highest tertile of HOMA-IR index. In addition, 21 GMM generated from the predicted total functional abundances were associated with the highest tertile of HOMA-IR index, 8 of these GMM were negatively associated with the highest tertile of HOMA-IR, whereas 14 GMM were positively associated.

Altered functions are regulated by different microbes based on HOMA-IR status

The contribution of each taxon to the GMM of interest has been displayed at family level (at genus level in case of taxa of interest) per sample (Supplementary figure 3, Supplementary figure 4) and per tertile of HOMA-IR index (Supplementary figure 5, Supplementary figure 6). For the generation of stacked barplots, just the main contributors are represented with a cutoff of 0.3, it means that if a bacterium never contributes more than 30% to any function is not considered for the plot and is categorized as “other taxa”. However, the “other taxa” were not excluded from the statistical analysis.

Just those taxa significantly contributing ($q < 0.1$) to those GMM associated with the highest tertile of HOMA-IR index in from the predicted total functional abundances were depicted.

Taxa contribute differentially to the GMM of interest depending on IR: in case of negative association with HOMA-IR index, *Desulfovibrio*, *Odoribacter*, *Oscillospiraceae* UCG-002, significantly contributes to aspartate degradation and glycine degradation (Table 3); in case of positive association with HOMA-IR, *Butyricicoccus*, *Erysipelotrichaceae* UCG-003, *Faecalibacterium*, significantly contribute to glutamine degradation II, lactaldehyde degradation, lactate production and fructan degradation (Table 4).

DISCUSSION

In the current study, we identified 6 different taxa consistently associated with IR. In addition, exploring the total predicted metagenome we found 21 GMM associated with IR. Finally, we explored the predicted taxa contribution to the GMM of interest, showing that bacteria contribute differentially to the function and that the degree of contribution is also dependent on IR. Therefore, we identified taxonomic signatures and potential metabolic pathways related to IR in a non-diabetic population at high cardiovascular risk. The relatively large number of altered GMM found may be understood in light of the altered gut metabolic environment one would expect to typically accompany a higher IR, thus drastically altering the potential substrates for the microbiome. Microbial genera *Desulfovibrio*, *Odoribacter* and *Oscillospiraceae* UCG-002, were found negatively associated with IR. Notably, *Desulfovibrio*, a genus that is typically associated with worsened host health (33), was negatively associated with IR. Bacteria of the genus *Desulfovibrio* are the most represented sulfate-reducing bacteria (SRB) residing in the human gut. The excessive presence of SRB in the gut, has been associated with the development of disorders, such as inflammatory bowel

disease (34) and ulcerative colitis (33). Also, 16S pyrosequencing results shown that members of *Desulfovibrionaceae* family were found significantly abundant in murine models of MetS and in animals with diet-induced obesity (35). In contrast, a significant increase of *Desulfovibrio* was described in the fecal microbiota of healthy subjects compared to individuals with obesity analysed with 16S rRNA sequencing (36), and reduced *Desulfovibrio* was also found in the gut microbiota assessed with quantitative PCR of preschool children with obesity compared to healthy controls, echoing our findings (37). In a study conducted to assess the nutritional effects as well as the adherence to the MedDiet on the gut microbiota of healthy adults analyzed with 16S rRNA gene sequencing, *Desulfovibrio* was found more abundant in lean individuals (38). Accordingly, we found *Desulfovibrio* negatively associated with the highest tertile of HOMA-IR index, who includes those subjects with a significantly higher BMI. Hydrogen sulfide (H_2S) is a gas metabolite produced by SRB in the gut that directly activates the secretion of glucagon-like peptide-1 (GLP-1), a peptide hormone involved in glucose homeostasis and appetite regulation. Mice treated with a specific SRB supplemented prebiotic diet has elevated levels of *Desulfovibrio piger*, detected with targeted real-time PCR, and increased concentrations of H_2S in feces and colon, with consequent stimulation of GLP-1 and enhancement of insulin secretion, improved oral glucose tolerance, and reduced food intake (39). Mice treated with liraglutide, an injectable GLP-1 receptor agonist (GLP-1 RA) used in the treatment of T2D, with demonstrated efficacy in improving glycemic control, has been shown to produce substantial changes in the gut microbiota assessed with pyrosequencing of 16S rRNA with enrichment of several genera, of whom *Desulfovibrio* (40). These findings support the potential involvement of *Desulfovibrio* in glucose homeostasis through the production of H_2S and activation of GLP-1 and can partially explain the negative association between this genus and IR observed in our study.

In the present study HOMA-IR index was negatively associated with GMM linked to amino acid breakdown. The fraction of amino acids that are not absorbed by the colonic mucosa are metabolized by the large intestinal microbiota, resulting in the generation of metabolites and end-products such as SCFA, and hydrogen sulfate ion (HO_4S^-) (41). Accordingly, we observed that HOMA-IR index was negatively associated to functions linked to cysteine degradation, resulting in the production of H_2S . Glycine and glutamine are intestinal gluconeogenesis substrates, and it has been observed that intestinal gluconeogenesis protects against diabetes and obesity by suppressing hepatic gluconeogenesis and positively regulating glucose homeostasis (42). Furthermore, glutamine immunomodulatory effects have been described (43). In addition, we observed that in case of functions linked to aspartate degradation I and glycine degradation II, taxa of interest contribute significantly to the negative association with HOMA-IR index. Aspartate and glycine are precursors for the synthesis of acetate (44), who has been demonstrated to play a regulatory role in body weight control, and insulin sensitivity through effects on lipid metabolism and glucose homeostasis (45).

Odoribacter is an anaerobic nonmotile Gram-negative, succinate-consuming and SCFA-producing rod-shaped bacteria. This genus has been linked to a decrease in clinical parameters associated with CVD risk (46). The results of a cross-sectional study conducted in a cohort of Spanish subjects shown that obesity was associated with elevated levels of circulating succinate concomitant with impaired glucose metabolism. This increase was associated with specific changes in gut microbiota, assessed with 16S sequencing, including lower relative abundance of succinate-consuming *Odoribacteraceae* family in obese individuals (47). Odoribacter spp. were found to be associated with a healthy fasting serum lipid profile in women with obesity (48). Wang *et al.* shown that a high-fat diet induces reduction in the relative abundance of *Odoribacter* in mice fecal microbiota analysed with 16SrRNA gene sequencing, then suppressed by the use of capsaicin, suggesting that changes in the abundance of *Odoribacter* may play an important role in the effects of treatments for glucose

intolerance and obesity (49). Our findings are in agreement with the beneficial characteristics described for this genus in relation to IR, as we observed a negative association with high HOMA-IR index in our study population.

Oscillospiraceae family negatively correlates with BMI and anti-insulin autoantibodies in type 1 diabetes in children (50) . In addition, members of the family *Oscillospiraceae* were found depleted in obese children (51). Accordingly, we find UCG-002 from *Oscillospiaraceae* family negatively associated with the highest tertile of HOMA-IR index, that includes those subjects with significantly higher BMI, and other variables related to obesity and IR.

Therefore, our results suggest that genera negatively associated with IR, may be involved in mechanisms improving insulin sensitivity in our study population.

On the other hand, in our study we also find some genera positively associated with IR: *Faecalibacterium*, *Butyricicoccus* and *Erysipelotricaceae* UCG-003. Some *Faecalibacterium* strains, like *F. prausnitzii*, are butyrate producers used as health biomarkers in humans for its immunomodulatory properties and more specifically for its anti-inflammatory effects (52). Hippe *et al*., analyzed through molecular experiments based on qPCR, the different *F. prausnitzii* phylotypes and their butyrate producing capacity in non-diabetic individuals with obesity, to determine whether an excess of butyrate production can be a risk factor for the development T2D. Results shown that the overproduction of butyrate induced by different *F. prausnitzii* phylotypes discriminates between obese developing T2D or not, suggesting possible different inflammatory genesis patterns in the host (53). Other studies have suggested that the disruption in the relative proportions of butyrate-producing bacteria, such as *F. prausnitzi*, in gut microbial populations may contribute to weight gain and IR (54).

A similar argument can be extended to another genus found positively associated with HOMA-IR index, *Butyricicoccus*, which includes butyrate-producing strains considered beneficial for gut health (55). Despite the large evidence supporting the beneficial effects of butyrate on gut health, its effect on obesity is still a debated topic. Some studies shown that butyrate induces obesity providing the substrate for energy expenditure but also by engaging in signaling pathways involved in glycolipid metabolism (56). This last observation supports our findings showing butyrate-producing taxa such as *Faecalibacterium* and *Butyricicoccus* positively associated with impaired metabolism and IR.

In accordance, our findings also reveal that HOMA-IR index was positively associated with functions linked to mono-, di-, and polysaccharides degradation. Non-digestible carbohydrates are fermented by colonic microbes, leading to the production SCFAs such as butyrate (57).

We observed that taxa of interest contribute significantly to the GMM positively associated with HOMA-IR index linked to fructan degradation, glutamine degradation II, lactaldehyde degradation and lactate production. Indeed, dietary fibers are the main source of oligosaccharides such as fructan, which can promote the growth of butyrate-producing bacteria (58). In addition, lactate and lactaldehyde are fermentation products intermediary in the production of butyrate from carbohydrates (59,60). Accordingly, it seems that the action of some taxa of interest is mediated by functions mainly linked to butyrate production, and that this metabolite is playing a role in the association observed with IR.

Erysipelotrichaceae have been found enriched in case of inflammatory diseases and obesity, probably because of its involvement in host lipid metabolism (61). In addition, it was found associated with impaired glucose metabolism (62). Accordingly, we observed a positive association

between this genus and high levels of IR. Bacteria belonging to *Erysipelotrichaceae* family use proteins and saccharides as main substrates for lactate production, one of the functional metabolic pathways involved in our study (60).

Unsurprisingly, we discovered that the taxa associated with IR were inferred to contribute to several of the GMM also associated with IR. However, we also found that the degree to which these taxa contributed to the respective functions was dependent on IR as well. This may have major implications for our understanding of the role of the microbiome in IR and indeed other conditions altering host gut metabolism. The altered gut metabolic environment promotes the relative abundance of different microbes that perform the same functions, in a manner similar to that described by Zhang and colleagues in their work on ecological guilds (63).

However, both in the taxonomic and functional aspect, previous studies shown discrepancies, perhaps partially explained by the inherent biases in metagenomic sequencing which make it problematic to estimate bacterial abundance from sequencing data (64). It is also important to mention that to overpass such bias we used a compositional approach for the microbiome analysis (65). Furthermore, is also worth to mention that the sequencing approach conducted in this study is also prone to bias, as described by Barb and colleagues (66). More complete and complex studies, including deepen microbial community profiling together with biochemical characterization of the environment, are needed in order to better understand the taxonomic and functional signatures related to IR.

There were several limitations in this study. First, due to the nature of 16S sequencing, we were limited to a genus-level resolution. Similarly, though PICRUSt2 has been shown to be reasonably reliable, especially in humans, we were restricted by the number and quality of the genomes present in the database used to infer the functional potential of the microbiome. For the same reasons, we were unable to assess the non-bacterial fraction of the microbiome. Whole genome shotgun metagenomics addresses both of these limitations, as it enables the detection and relative quantification of non-bacterial microbes as well as the bacteriome, and also enables analysis at a higher resolution and should be considered in the future. Another important point to consider is the large inherent variance in the microbiome, which is often confounded by host variables (67). The PREDIMED-Plus cohort by its nature consists late-middle aged and overweight participants. It is likely that some of the findings cannot be generalized to all ages and weights. Furthermore, this study only had a single microbiome measurement per participant, thus rendering stability and volatility analysis of the microbiome beyond our reach (68,69). We eagerly await future studies and subsequent meta-analyses that will shed further light on this. Furthermore, given the observational nature of this study, it was not possible to conclude on causality or directionality. Future experiments using the fecal microbiota transplantation (FMT) technique could be considered to elucidate this last aspect.

CONCLUSIONS

In the current study we were able to identify taxa and functions associated with IR and to suggest that bacteria contribute differentially to these functions, also depending on IR. We observed genera *Desulfovibrio*, *Odoribacter* and *Oscillospiraceae* UCG-002 negatively associated with IR through mechanism of amino acid degradation that involves H_2S, activation of gluconeogenesis, immunomodulation and beneficial effects of acetate. On the other hand, we observed that the positive association with IR involves genera such as *Feacalibaterium* and *Butyricicoccus* and GMM linked with the production of butyrate. To conclude, these findings are promising especially for the

perspective of tailoring therapeutic strategies based on the identification of specific signatures of the gut bacterial community and using this information to improve the treatments for IR and other metabolic impairments related to obesity. However, some further investigation, especially under the metabolic point of view, are needed in order to strengthen these findings.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, A.A., F.J.T., J.V. (Jesús Vioque), D.C., J.V. (Josep Vidal)., M.F., M.B., and J.S.-S.; formal analysis, A.A. and T.F.B.; funding acquisition, F.J.T., J.V. (Jesús Vioque), D.C., J.V. (Josep Vidal), M.F., and J.S.-S.; writing—original draft preparation, A.A.; writing—review and editing, T.F.B., M.B., and J.S.-S.; supervision, M.B., and J.S.-S.; all authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713679 and from the Universitat Rovira i Virgili (URV). This work was supported by the official Spanish Institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund (three coordinated FIS projects leaded by J.S.-S., including the following projects: PI13/00462, PI16/00501 and PI19/00576); the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to J.S.-S.; the Recercaixa (number 2013ACUP00194) grant to J.S.-S.; The Fondo de Investigaciones Sanitarias of the Instituto de Salut Carlos III PI17/00215; The Generalitat Valenciana PROMETEO 17/2017. None of the funding sources took part in the design, collection, analysis, interpretation of the data, or writing the report, or in the decision to submit the manuscript for publication. J.S.-S., senior author of this study gratefully acknowledges the financial support by ICREA under the ICREA Academia programme. Food companies Hojiblanca (Lucena, Spain) and Patrimonio Comunal Olivarero (Madrid, Spain) donated extra virgin olive oil; and the Almond Board of California (Modesto, CA), American Pistachio Growers (Fresno, CA), and Paramount Farms (Wonderful Company, LLC, Los Angeles, CA) donated nuts for the PREDIMED-Pilot

ACKNOWLEDGMENTS

We thank all the volunteers for the participation and personnel for the contribution in the PREDIMED-Plus trial. We also thank all the investigators of the PREDIMED-Plus study (supplemental Material). CIBEROBN (Centros de Investigación Biomédica en Red: Obesidad y Nutrición) is an initiative of ISCIII, Madrid, Spain. The authors also thank the PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples. Jordi Salas-Salvadó, senior author of this study gratefully acknowledges the financial support by ICREA under the ICREA Academia programme. The research groups thanks for its support to the CIBER-IBIMA-Metagenomics platform, especially to Pablo Rodríguez.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scommittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

REFERENCES

1. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, Shulzhenko N. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* (2020) **51**:102590. doi:10.1016/j.ebiom.2019.11.051

2. Martyn JAJ, Kaneki M, Yasuhara S, Warner DS, Warner MA. Obesity-induced Insulin Resistance and Hyperglycemia. *Anesthesiology* (2008) **109**:137–148. doi:10.1097/ALN.0b013e3181799d45

3. Fahed M, Abou Jaoudeh MG, Merhi S, Mosleh JMB, Ghadieh R, Ghadieh R, Al Hayek S, Al Hayek S, El Hayek Fares JE. Evaluation of risk factors for insulin resistance: A cross sectional study among employees at a private university in Lebanon. *BMC Endocr Disord* (2020) **20**:1–14. doi:10.1186/s12902-020-00558-9

4. Saad MJA, Santos A, Prada PO. Linking gut microbiota and inflammation to obesity and insulin resistance. *Physiology* (2016) **31**:283–293. doi:10.1152/physiol.00041.2015

5. Bäckhed F, Ding H, Wang T, Hooper L V., Gou YK, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* (2004) **101**:15718–15723. doi:10.1073/pnas.0407076101

6. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* (2012) doi:10.1053/j.gastro.2012.06.031

7. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* (2007) doi:10.2337/db06-1491

8. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermúdez-Humarán LG, Smirnova N, Bergé M, Sulpice T, Lahtinen S, et al. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: Molecular mechanisms and probiotic treatment. *EMBO Mol Med* (2011) doi:10.1002/emmm.201100159

9. Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, Hirabara SM, Castoldi Â, Vieira P, Camara NOS, et al. Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. *PLoS Biol* (2011) doi:10.1371/journal.pbio.1001212

10. Medzhitov R, Horng T. Transcriptional control of the inflammatory response. *Nat Rev Immunol* (2009) **9**:692–703. doi:10.1038/nri2634

11. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. "The Role of Short-Chain Fatty Acids in Health and Disease," in, 91–119. doi:10.1016/B978-0-12-800100-4.00003-9

12. Li M, van Esch BCAM, Wagenaar GTM, Garssen J, Folkerts G, Henricks PAJ. Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *Eur J Pharmacol* (2018) **831**:52–59. doi:10.1016/j.ejphar.2018.05.003

13. Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). *Acta Biochim Pol* (2019) doi:10.18388/abp.2018_2648

14. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, Petersen KF, Kibbey RG, Goodman AL, Shulman GI. Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. *Nature* (2016) doi:10.1038/nature18309

15. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab* (2016) **24**:41–50. doi:10.1016/j.cmet.2016.05.005

16. Gérard C, Vidal H. Impact of Gut Microbiota on Host Glycemic Control. *Front Endocrinol (Lausanne)* (2019) **10**:1–13. doi:10.3389/fendo.2019.00029

17. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BAH, Forslund K, Hildebrand F, Prifti E, Falony G, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* (2016) **535**:376–381. doi:10.1038/nature18646

18. Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, Basora J, Fitó M, Corella D, Serra-Majem L, Wärnberg J, Romaguera D, Estruch R, et al. Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular risk factors: One-year results of the PREDIMED-Plus trial. *Diabetes Care* (2019) doi:10.2337/dc18-0836

19. Sayón-Orea C, Razquin C, Bulló M, Corella D, Fitó M, Romaguera D, Vioque J, Alonso-Gómez ÁM, Wärnberg J, Martínez JA, et al. Effect of a Nutritional and Behavioral Intervention on Energy-Reduced Mediterranean Diet Adherence among Patients with Metabolic Syndrome: Interim Analysis of the PREDIMED-Plus Randomized Clinical Trial. *JAMA - J Am Med Assoc* (2019) doi:10.1001/jama.2019.14630

20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) **28**:412–419. doi:10.1007/BF00280883

21. Schröder H, Zomeño MD, Martínez-González MA, Salas-Salvadó J, Corella D, Vioque J, Romaguera D, Martínez JA, Tinahones FJ, Miranda JL, et al. Validity of the energy-restricted Mediterranean Diet Adherence Screener. *Clin Nutr* (2021) **40**:4971–4979. doi:10.1016/j.clnu.2021.06.030

22. MacPherson CW, Mathieu O, Tremblay J, Champagne J, Nantel A, Girard SA, Tompkins TA. Gut Bacterial Microbiota and its Resistome Rapidly Recover to Basal State Levels after Short-term Amoxicillin-Clavulanic Acid Treatment in Healthy Adults. *Sci Rep* (2018) **8**:1–14. doi:10.1038/s41598-018-29229-5

23. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* (2016) doi:10.1038/nmeth.3869

24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* (2013) doi:10.1093/nar/gks1219

25. Aitchison J, Barceló-Vidal C, Martín-Fernández JA, Pawlowsky-Glahn V. Logratio analysis and compositional distance. *Math Geol* (2000) **32**:271–275. doi:10.1023/A:1007529726302

26. Chao A. Estimating the Population Size for Capture-Recapture Data with Unequal Catchability. *Biometrics* (1987) **43**:783. doi:10.2307/2531532

27. Shannon CE. A Mathematical Theory of Communication. *Bell Syst Tech J* (1948) **27**:623–656. doi:10.1002/j.1538-7305.1948.tb00917.x

28. Simpson EH. Measurment of Diversity. *Nature* (1949) **163**:688. Available at: https://doi.org/10.1038/163688a0

29. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci* (2003) **100**:9440–9445. doi:10.1073/pnas.1530509100

30. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* (2020) **38**:685–688. doi:10.1038/s41587-020-0548-6

31. Vieira-Silva S, Falony G, Darzi Y, Lima-Mendez G, Garcia Yunta R, Okuda S, Vandeputte D, Valles-Colomer M, Hildebrand F, Chaffron S, et al. Species-function relationships shape ecological properties of the human gut microbiome. *Nat Microbiol* (2016) **1**:1–8. doi:10.1038/nmicrobiol.2016.88

32. Structure, function and diversity of the healthy human microbiome. *Nature* (2012) **486**:207–214. doi:10.1038/nature11234

33. Kushkevych I, Dordević D, Kollár P. Analysis of physiological parameters of Desulfovibrio strains from individuals with colitis. *Open Life Sci* (2018) **13**:481–488. doi:10.1515/biol-2018-0057

34. Rowan F, Docherty NG, Murphy M, Murphy B, Coffey JC, O'Connell PR. Desulfovibrio Bacterial Species Are Increased in Ulcerative Colitis. *Dis Colon Rectum* (2010) **53**:1530–1536. doi:10.1007/DCR.0b013e3181f1e620

35. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, et al. Interactions between gut microbiota, host genetics and diet relevant to development of

metabolic syndromes in mice. *ISME J* (2010) **4**:232–241. doi:10.1038/ismej.2009.112

36. Andoh A, Nishida A, Takahashi K, Inatomi O, Imaeda H, Bamba S, Kito K, Sugimoto M, Kobayashi T. Comparison of the gut microbial community between obese and lean peoples using 16S gene sequencing in a Japanese population. *J Clin Biochem Nutr* (2016) **59**:65–70. doi:10.3164/jcbn.15-152

37. Karlsson CLJ, Önnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* (2012) **20**:2257–2261. doi:10.1038/oby.2012.110

38. Garcia-Mantrana I, Selma-Royo M, Alcantara C, Collado MC. Shifts on gut microbiota associated to mediterranean diet adherence and specific dietary intakes on general adult population. *Front Microbiol* (2018) **9**:1–11. doi:10.3389/fmicb.2018.00890

39. Pichette J, Fynn-Sackey N, Gagnon J. Hydrogen sulfide and sulfate prebiotic stimulates the secretion of GLP-1 and improves glycemia in male mice. *Endocrinology* (2017) **158**:3416–3425. doi:10.1210/en.2017-00391

40. Wang L, Li P, Tang Z, Yan X, Feng B. Structural modulation of the gut microbiota and the relationship with body weight: compared evaluation of liraglutide and saxagliptin treatment. *Sci Rep* (2016) **6**:33251. doi:10.1038/srep33251

41. Neis EPJG, Dejong CHC, Rensen SS. The role of microbial amino acid metabolism in host metabolism. *Nutrients* (2015) doi:10.3390/nu7042930

42. Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, De Michieli F, Paschetta E, Musso G. Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence? *Curr Diab Rep* (2018) **18**: doi:10.1007/s11892-018-1057-6

43. Orsso CE, Peng Y, Deehan EC, Tan Q, Field CJ, Madsen KL, Walter J, Prado CM, Tun HM, Haqq AM. Composition and functions of the gut microbiome in pediatric obesity: Relationships with markers of insulin resistance. *Microorganisms* (2021) **9**:1–18. doi:10.3390/microorganisms9071490

44. Zhao J, Zhang X, Liu H, Brown MA, Qiao S. Dietary Protein and Gut Microbiota Composition and Function. *Curr Protein Pept Sci* (2018) **20**:145–154. doi:10.2174/1389203719666180514145437

45. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* (2019) **11**: doi:10.3390/nu11081943

46. Gomez-Arango LF, Barrett HL, McIntyre HD, Callaway LK, Morrison M, Dekker Nitert M. Increased Systolic and Diastolic Blood Pressure is Associated with Altered Gut Microbiota Composition and Butyrate Production in Early Pregnancy. *Hypertension* (2016) **68**:974–981. doi:10.1161/HYPERTENSIONAHA.116.07910

47. Serena C, Ceperuelo-Mallafré V, Keiran N, Queipo-Ortuño MI, Bernal R, Gomez-Huelgas R, Urpi-Sarda M, Sabater M, Pérez-Brocal V, Andrés-Lacueva C, et al. Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *ISME J* (2018)

12:1642–1657. doi:10.1038/s41396-018-0068-2

48. Brahe LK, Le Chatelier E, Prifti E, Pons N, Kennedy S, Hansen T, Pedersen O, Astrup A, Ehrlich SD, Larsen LH. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutr Diabetes* (2015) **5**:e159-7. doi:10.1038/nutd.2015.9

49. Wang Y, Tang C, Tang Y, Yin H, Liu X. Capsaicin has an anti-obesity effect through alterations in gut microbiota populations and short-chain fatty acid concentrations. *Food Nutr Res* (2020) **64**:1–14. doi:10.29219/fnr.v64.3525

50. Biassoni R, Di Marco E, Squillario M, Barla A, Piccolo G, Ugolotti E, Gatti C, Minuto N, Patti G, Maghnie M, et al. Gut Microbiota in T1DM-Onset Pediatric Patients: Machine-Learning Algorithms to Classify Microorganisms as Disease Linked. *J Clin Endocrinol Metab* (2020) **105**:e3114–e3126. doi:10.1210/clinem/dgaa407

51. Maya-Lucas O, Murugesan S, Nirmalkar K, Alcaraz LD, Hoyo-Vadillo C, Pizano-Zárate ML, García-Mena J. The gut microbiome of Mexican children affected by obesity. *Anaerobe* (2019) **55**:11–23. doi:10.1016/j.anaerobe.2018.10.009

52. Lenoir M, Martín R, Torres-Maravilla E, Chadi S, González-Dávila P, Sokol H, Langella P, Chain F, Bermúdez-Humarán LG. Butyrate mediates anti-inflammatory effects of Faecalibacterium prausnitzii in intestinal epithelial cells through Dact3. *Gut Microbes* (2020) **12**:1–16. doi:10.1080/19490976.2020.1826748

53. Hippe B, Remely M, Aumueller E, Pointner A, Magnet U, Haslberger AG. Faecalibacterium prausnitzii phylotypes in type two diabetic, obese, and lean control subjects. *Benef Microbes* (2016) **7**:511–517. doi:10.3920/BM2015.0075

54. Barlow GM, Yu A, Mathur R. Role of the gut microbiome in obesity and diabetes mellitus. *Nutr Clin Pract* (2015) **30**:787–797. doi:10.1177/0884533615609896

55. Ma Q, Li Y, Wang J, Li P, Duan Y, Dai H, An Y, Cheng L, Wang T, Wang C, et al. Investigation of gut microbiome changes in type 1 diabetic mellitus rats based on high-throughput sequencing. *Biomed Pharmacother* (2020) **124**:109873. doi:10.1016/j.biopha.2020.109873

56. Liu H, Wang J, He T, Becker S, Zhang G, Li D, Ma X. Butyrate: A double-edged sword for health? *Adv Nutr* (2018) **9**:21–29. doi:10.1093/advances/nmx009

57. Blandino G, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. *Diabetes Metab* (2016) **42**:303–315. doi:10.1016/j.diabet.2016.04.004

58. Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. *Eur Rev Med Pharmacol Sci* (2016) **20**:4742–4749.

59. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* (2017) **19**:29–41. doi:10.1111/1462-2920.13589

60. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. *Microbiome* (2019) **7**:1–15.

doi:10.1186/s40168-019-0704-8

61. Kaakoush NO. Insights into the role of Erysipelotrichaceae in the human host. *Front Cell Infect Microbiol* (2015) **5**:1–4. doi:10.3389/fcimb.2015.00084

62. Lippert K, Kedenko L, Antonielli L, Kedenko I, Gemeier C, Leitner M, Kautzky-Willer A, Paulweber B, Hackl E. Gut microbiota dysbiosis associated with glucose metabolism disorders and the metabolic syndrome in older adults. *Benef Microbes* (2017) **8**:545–556. doi:10.3920/BM2016.0184

63. Zhang C, Yin A, Li H, Wang R, Wu G, Shen J, Zhang M, Wang L, Hou Y, Ouyang H, et al. Dietary Modulation of Gut Microbiota Contributes to Alleviation of Both Genetic and Simple Obesity in Children. *EBioMedicine* (2015) **2**:968–984. doi:10.1016/j.ebiom.2015.07.007

64. McLaren MR, Willis AD, Callahan BJ. Consistent and correctable bias in metagenomic sequencing experiments. *Elife* (2019) **8**:1–31. doi:10.7554/eLife.46923

65. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome datasets are compositional: And this is not optional. *Front Microbiol* (2017) **8**:1–6. doi:10.3389/fmicb.2017.02224

66. Barb JJ, Oler AJ, Kim H-S, Chalmers N, Wallen GR, Cashion A, Munson PJ, Ames NJ. Development of an Analysis Pipeline Characterizing Multiple Hypervariable Regions of 16S rRNA Using Mock Samples. *PLoS One* (2016) **11**:e0148047. doi:10.1371/journal.pone.0148047

67. Vujkovic-Cvijin I, Sklar J, Jiang L, Natarajan L, Knight R, Belkaid Y. Host variables confound gut microbiota studies of human disease. *Nature* (2020) **587**:448–454. doi:10.1038/s41586-020-2881-9

68. Vandeputte D, De Commer L, Tito RY, Kathagen G, Sabino J, Vermeire S, Faust K, Raes J. Temporal variability in quantitative human gut microbiome profiles and implications for clinical research. *Nat Commun* (2021) **12**:6740. doi:10.1038/s41467-021-27098-7

69. Bastiaanssen TF., Gururajan A, van de Wouw M, Moloney GM, Ritz NL, Long-Smith CM, Wiley NC, Murphy AB, Lyte JM, Fouhy F, et al. Volatility as a Concept to Understand the Impact of Stress on the Microbiome. *Psychoneuroendocrinology* (2021) **124**:105047. doi:10.1016/j.psyneuen.2020.105047

LIST OF FIGURES

Figure 1. Workflow showing the fecal samples analytic process and bioinformatics pipeline. Bacterial DNA was extracted from frozen fecal and 16S amplicon sequencing performed. Resulting raw sequences in fastq format were imported into R environment and processed with DADA2 package. Output files followed 3 different pipelines: (1) processed in order to obtain information about alpha and beta diversity and differential abundant taxa; (2) processed with PICRUSt2 in order to obtain the predicted total functional abundances, then gut metabolic modules (GMM) were computed and the association with HOMA-IR determined; (3) processed with PICRUSt2 in order to obtain the predicted contribution per genus of each GMM previously computed.

Figure 2. Potential mechanisms explaining the association between fecal microbiome and IR. The negative association with IR seems to be linked with glucose homeostasis, induced by an increase in amino acids breakdown and by an increase in sulfate-reducing bacteria, with consequent promotion of intestinal gluconeogenesis, acetate synthesis and H_2S production, in addition to an improved succinate metabolism. The positive association with IR seems to be linked to an increase in saccharides degradation that can induce the growth of butyrate-producing bacteria and bring to a disproportion in butyrate synthesis and an impairment in glycolipid metabolism.

TABLES

Table 1. Characteristics of the study population according to tertiles of HOMA-IR index.

	Tertile 1 (n = 93)	Tertile 2 (n = 93)	Tertile 3 (n = 93)	*p* trend[&]
Female sex	51 (54.8)	53 (57.0)	47 (50.5)	0.668
Age (years)	66.0 [7.0]	65.0 [7.0]	65.0 [8]	0.619
Recruiting center				0.817
Málaga	33 (35.5)	36 (38.7)	32 (34.4)	
Reus	60 (64.5)	57 (61.3)	61 (65.6)	
Body weight (kg)	82.9 [12.6]	87.6 [18.3] *	90.0 [16.4] **	< 0.001
BMI (kg/m^2)	30.7 [4.6]	33.3 [4.8] **	34.3 [6.0] ** †	< 0.001
Waist circumference (cm)	105.9 ± 8.2	109.0 ± 10.3 *	111.7 ± 9.8 **	< 0.001
SBP (mmHg)	139.8 [22.1]	138.0 [19.0]	140.7 [31.3]	0.852
DBP (mmHg)	79.8 ± 10.3	79.8 ± 8.3	79.1 ± 11.1	0.868
Total cholesterol (mg/dL)	209.0 [48.0]	204.0 [51.0]	200.0 [42.0]	0.641
HDL-cholesterol (mg/dL)	54.0 [17.0]	49.0 [11.0] *	46.0 [11.0] ** †	< 0.001
LDL-cholesterol (mg/dL)	124.0 [42.0]	117.0 [44.0]	116.0 [38.0]	0.211
Triglycerides (mg/dl)	118.5 [69.0]	153.0 [83.0] **	175.0 [107.0] **	< 0.001
FPG (mg/dL)	93.0 [15.0]	100.0 [17.0] **	108.0 [20.0] ** †	< 0.001
Fasting insulin (mU/mL)	10.8 [3.4]	16.6 [3.0] **	27.6 [10.5] ** ††	< 0.001
Glycated hemoglobin (%)	5.6 [0.4]	5.7 [0.5] *	5.8 [0.6] ** †	< 0.001
HOMA-IR index	2.4 [0.7]	4.1 [1.0] **	7.3 [2.7] ** ††	< 0.001
MedDiet adherence score	8.0 [3.0]	8.0 [2.0]	7.0 [3.0] †	0.009
Hypercholesterolemia	55 (59.1)	63 (67.7)	56 (60.2)	0.419
Hypertension	87 (93.5)	86 (92.5)	88 (94.6)	0.837
Smoking habits				0.282
Current smoker	12 (12.9)	13(14.0)	15 (16.1)	
Former smoker	29 (31.2)	30 (32.3)	40 (43.0)	
Never smoker	52 (55.9)	50 (53.8)	38 (40.9)	
Education				0.641
Primary school	50 (53.8)	56 (60.2)	46 (49.5)	
Secondary school	30 (32.3)	28 (30.1)	34 (36.6)	
University	13 (14.0)	9 (9.7)	13 (14.0)	

HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure, HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; MedDiet, Mediterranean diet. Data presented as mean ± SD, median [IQR] or number (percentage). [&]One-way ANOVA, Pearson's chi-square test or Kruskal-Wallis test was used to calculate differences across tertiles, Student's t-test or Mann-Whitney test was used to calculate differences between tertiles. ** $p < 0.001$ vs tertile 1; * $p < 0.05$ vs tertile 1; †† $p < 0.001$ *versus* tertile 2; † $p < 0.05$ *versus* tertile 2.

Table 2. Taxa and GMM associated with tertile 3 of HOMA-IR index

Taxa	Effect size	*q*-value	Association with T3
Desulfovibrio	–0.724	0.023	Negative
Odoribacter	–0.832	0.043	Negative
Oscillospiraceae UCG-002	–0.806	0.088	Negative
Erysipelotrichaceae UCG-003	0.487	0.088	Positive

Faecalibacterium	0.530	0.088	Positive
Butyricicoccus	0.605	0.088	Positive
GMM			
Alanine degradation I	–0.201	0.053	Negative
Pectine degradation II	–0.101	0.053	Negative
Acetate to acetyl CoA	–0.080	0.053	Negative
Aspartate degradation I	–0.071	0.053	Negative
Glycine degradation	–0.063	0.069	Negative
Cysteine degradation II	–0.061	0.070	Negative
Glutamine degradation I	–0.145	0.072	Negative
Isoleucine degradation	–0.069	0.094	Negative
Fructan degradation	0.132	0.047	Positive
Galactose degradation	0.040	0.053	Positive
Sucrose degradation I	0.049	0.053	Positive
Pyruvate formate lyase	0.049	0.053	Positive
Melibiose degradation	0.054	0.053	Positive
Threonine degradation II	0.056	0.053	Positive
Ethanol production I	0.085	0.053	Positive
Lactate production	0.094	0.053	Positive
Fructose degradation	0.111	0.053	Positive
Threonine degradation I	0.044	0.056	Positive
Lactaldehyde degradation	0.069	0.070	Positive
Bifidobacterium shunt	0.025	0.072	Positive
Glutamine degradation II	0.028	0.096	Positive
Sucrose degradation II	0.038	0.098	Positive

GLM was used to calculate the association between taxa and tertile 3 (T3) of HOMA-IR and between predicted total functional abundances GMM and T3 of HOMA-IR. Adjusted Storey's *q*-values $q < 0.1$ deemed as significant.

Table 3. Taxa contribution per tertile to the GMM negatively associated with HOMA-IR index.

GMM	Taxa	T1	T2	T3	Effect size	*q*-value
Aspartate degradation I	Marinifilaceae_Odoribacter	0.0038	0.0031	0.0023	–0.6466	0.0209
	Erysipelatoclostridiaceae	0.0049	0.0067	0.0099	0.7242	0.0251
	Oscillospirales_Oscillospiraceae UCG-002	0.0535	0.0550	0.0413	–0.7908	0.0251
	Peptostreptococcaceae	0.0021	0.0039	0.0052	0.6368	0.0818
	Butyricicoccaceae	0.0017	0.0021	0.0030	0.4768	0.0872
	Desulfovibrionaceae_Desulfovibrio	0.0068	0.0054	0.0043	–0.6658	0.0872
Glycine degradation	Marinifilaceae_Odoribacter	0.0042	0.0034	0.0023	–0.5255	0.0605

T1, tertile 1 of HOMA-IR; T2, tertile 2 of HOMA-IR; T3, tertile 3 of HOMA-IR. The contribution was described at family level (at genus level in case of taxa of interest) with established cutoffs of 0.3. GLM was used to calculate differences between tertiles of HOMA-IR and adjusted Storey's q-values $q < 0.1$ deemed as significant.

Table 4 | Taxa contribution per tertile to the GMMs positively associated with HOMA-IR index

GMM	Taxa	T1	T2	T3	Effect size	*q*-value

Fructan degradation	Butyricicoccaceae_Butyricicoccus	0.0058	0.0072	0.0067	0.5303	0.0993
	Bacteroidaceae	0.0288	0.0303	0.0438	–0.5268	0.0993
	Erysipelatoclostridiaceae	0.0333	0.0330	0.0290	–0.8646	0.0993
Fructose degradation	Anaerovoracaceae	0.0003	0.0002	0.0005	0.4756	0.0112
	Erysipelatoclostridiaceae	0.0146	0.0131	0.0138	–0.8037	0.0516
	UCG-010	0.0063	0.0047	0.0022	–0.6337	0.0600
Glutamine degradation II	Oscillospirales_Oscillospiraceae UCG-002	0.0518	0.0492	0.0368	–0.4279	0.0205
	Butyricicoccaceae_Butyricicoccus	0.0006	0.0008	0.0009	0.6283	0.0304
	Marinifilaceae	0.0050	0.0040	0.0033	–0.6122	0.0304
	UCG-010	0.0025	0.0017	0.0008	–0.6740	0.0367
	Erysipelatoclostridiaceae_ Erysipelotrichaceae_UCG-003	0.0052	0.0078	0.0093	0.7995	0.0611
	Ruminococcaceae_Faecalibacterium	0.0065	0.0095	0.0095	0.5042	0.0638
	Erysipelatoclostridiaceae	0.0079	0.0066	0.0074	–0.7875	0.0638
	Muribaculaceae	0.0062	0.0034	0.0060	–0.7419	0.0638
	Peptostreptococcaceae	0.0004	0.0007	0.0007	0.4752	0.0638
Lactaldehyde degradation	Tannerellaceae	0.0008	0.0003	0.0002	–0.4656	0.0235
	Erysipelatoclostridiaceae_ Erysipelotrichaceae_UCG-003	0.0073	0.0112	0.0130	0.6334	0.0619
	Erysipelatoclostridiaceae	0.0122	0.0124	0.0104	–0.7217	0.0619
Lactate production	Butyricicoccaceae_Butyricicoccus	0.0027	0.0035	0.0038	0.6145	0.0267
	Oscillospirales_Oscillospiraceae UCG-002	0.1743	0.1601	0.1281	–0.4463	0.0267
	Erysipelatoclostridiaceae	0.0208	0.0192	0.0182	–0.7919	0.0391
	UCG-010	0.0058	0.0048	0.0020	–0.6081	0.0391
Threonine degradation II	Marinifilaceae	0.0177	0.0154	0.0134	–0.6546	0.0166
	Selenomonadaceae	0.0023	0.0082	0.0122	0.6122	0.0678

T1, tertile 1 of HOMA-IR; T2, tertile 2 of HOMA-IR; T3, tertile 3 of HOMA-IR. The contribution was described at family level (at genus level in case of taxa of interest) with established cutoffs of 0.3. GLM was used to calculate differences between tertiles of HOMA-IR and adjusted Storey's *q*-values $q < 0.1$ deemed as significant.

Figure 1. Workflow showing the fecal samples analytic process and bioinformatics pipeline

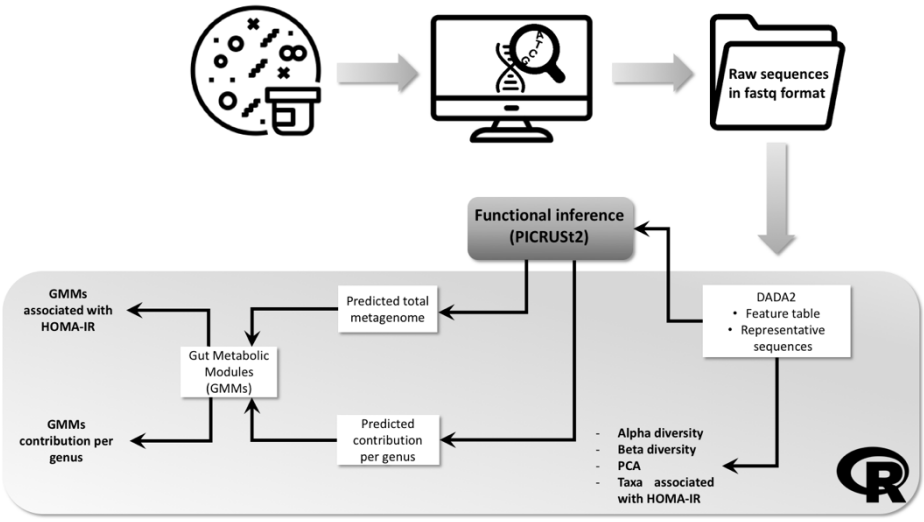
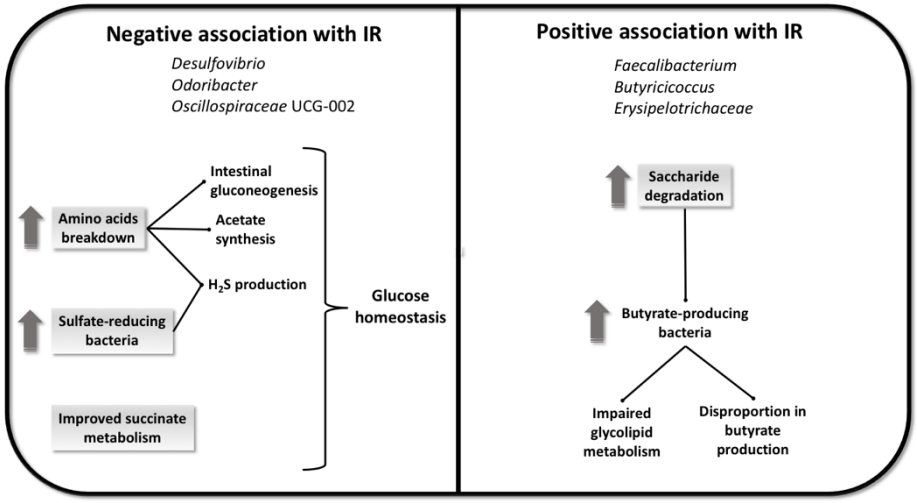


Figure 2. Potential mechanisms explaining the association between fecal microbiome and insulin resistance



SUPPLEMENTAL MATERIAL

Figure S1 | Principal component analysis plot.

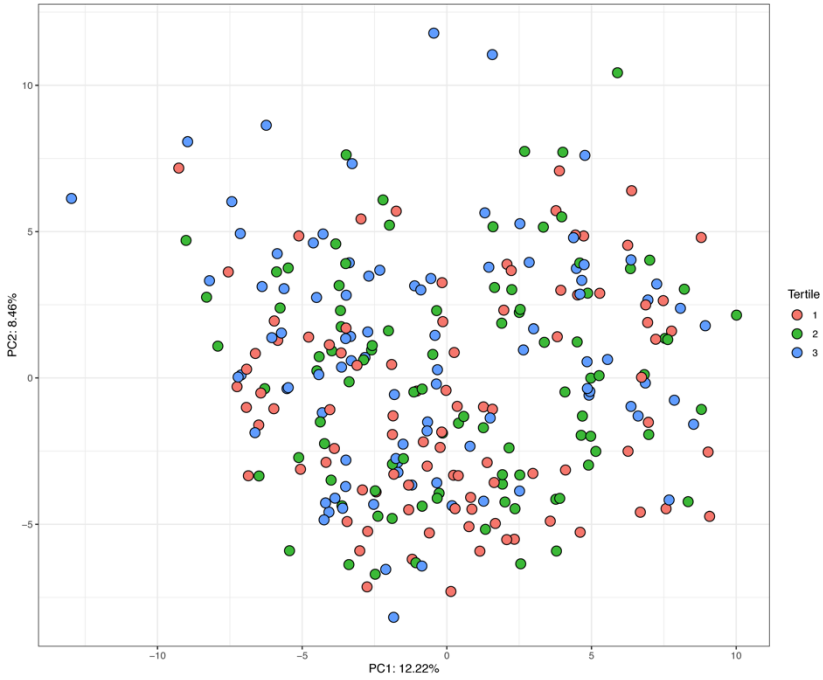


Figure S2 | Box plot showing differences in Chao1, Shannon and Simpson indices between tertiles of HOMA-IR index.

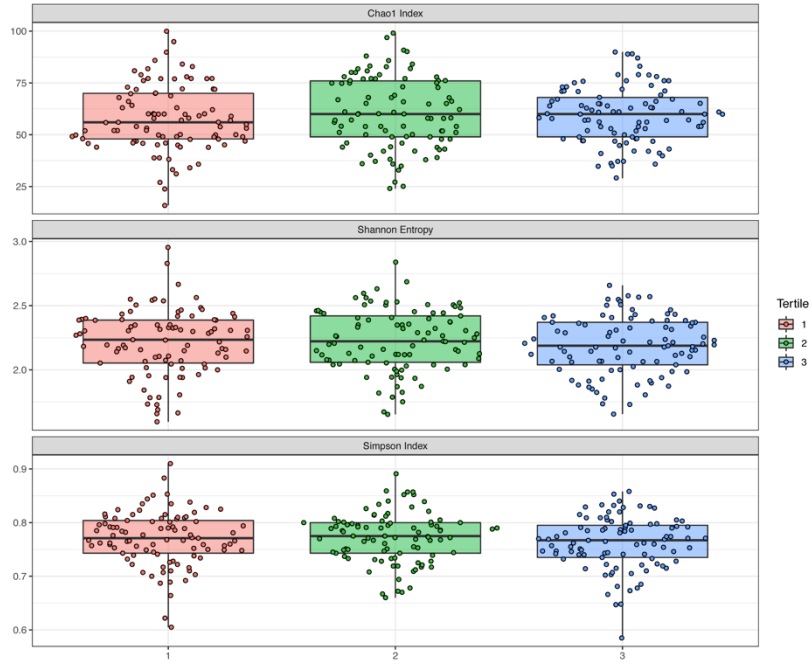


Table S1 | Differences in alpha diversity indices Chao1, Simpson, Shannon, between tertiles of HOMA-IR index.

	Tertile 1	**Tertile 2**	**Tertile 3**	***p*-value**
Chao1	60.0 ± 16.2	57.2 ± 15.1	57.9 ± 15.5	0.446
Simpson	0.8 [0.1]	0.8 [0.1]	0.9 [0.1]	0.720
Shannon	2.6 ± 0.4	2.6 ± 0.4	2.6 ± 0.4	0.855

Data presented as mean ± SD or median [IQR]. One-way ANOVA test and Kruskal-Wallis test used to calculate differences across tertiles of HOMA-IR; $p < 0.05$ deemed as significant.

Table S2 | Results of PERMANOVA analysis and permutation test for homogeneity of multivariate dispersions using Aitchison distance.

	Df	**Sums of squares**	**Mean squares**	**F.Model**	**R2**	**Pr(>F)**
Tertiles	2	281	140.26	0.8247	0.00589	0.802198
Recruiting c.	1	545	544.59	3.2022	0.01144	0.000999 ***
Residuals	274	46769	170.07		0.98266	
Total	278	47594			1.00000	

Figure S3 | Taxa contribution (per sample) to the GMMs negatively associated with HOMA-IR index. Bacteria that did not contribute more than 30% to any function were classified as "other taxa".

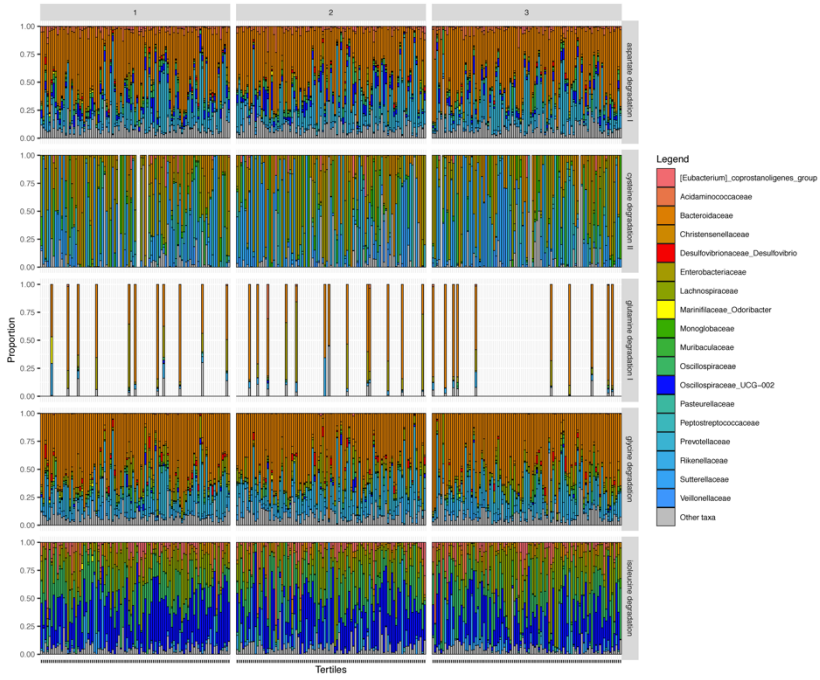


Figure S4 | Taxa contribution (per sample) to the GMMs positively associated with HOMA-IR index. Bacteria that did not contribute more than 30% to any function were classified as "other taxa".

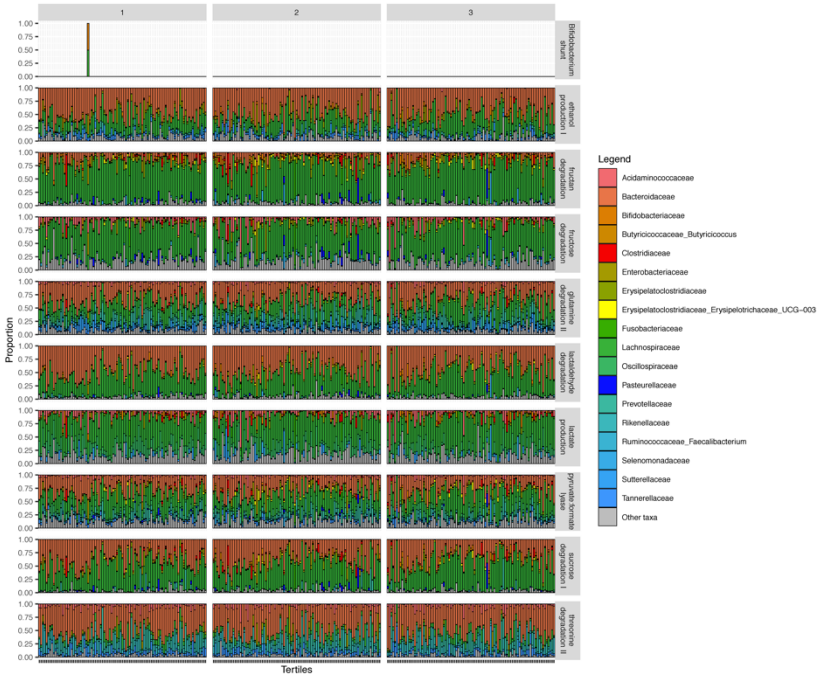


Figure S5 | Taxa contribution (per tertile) to the GMMs negatively associated with HOMA-IR index. Bacteria that did not contribute more than 30% to any function were classified as "other taxa".

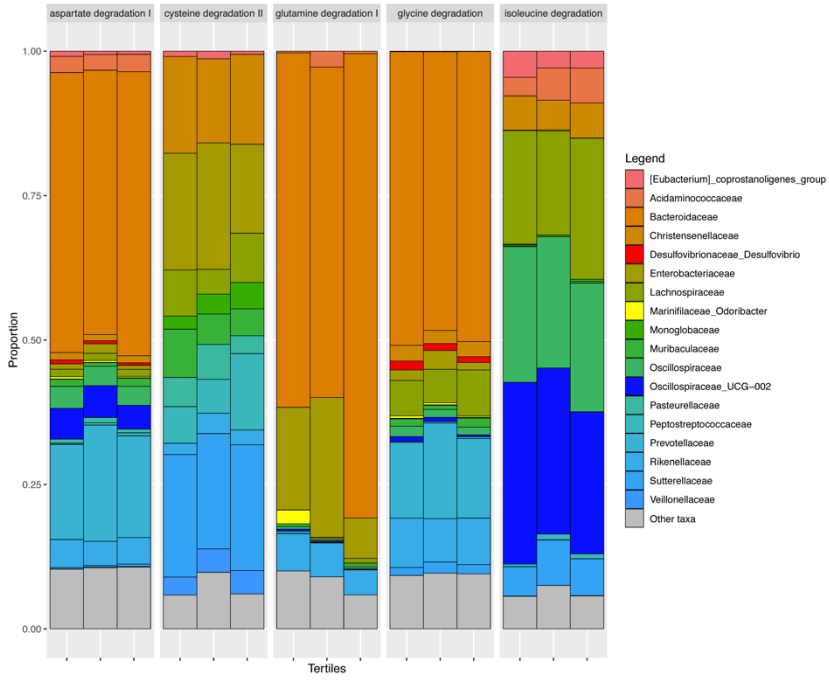
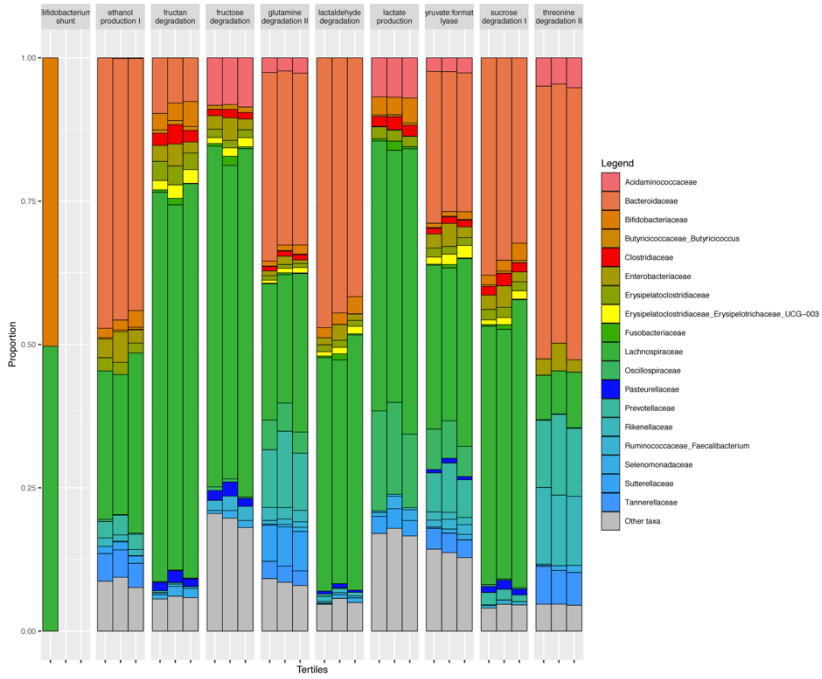


Figure S6 | Taxa contribution (per tertile) to the GMMs positively associated with HOMA-IR index. Bacteria that did not contribute more than 30% to any function were classified as "other taxa".



VII. DISCUSSION

The main purpose of this doctoral thesis is to identify specific fecal microbiota signatures potentially involved in the control of body weight and associated with insulin resistance in a population of elderly subjects (aged 55-75) with overweight/obesity and MetS exposed to a lifestyle intervention based on MedDiet, physical activity and behavioral support. We first explored the association between fecal microbiota, BMI and changes in body weight after 12-month of lifestyle intervention; second, we explored the association between fecal microbiota (taxonomic and pathway-associated) and insulin resistance in subjects without T2D. This study was conducted within the frame of the PREDIMED-Plus clinical trial and includes a subsample of participants with available fecal microbiota information at baseline and after 12-month follow-up.

Fecal microbiota signatures associated with body weight control

In the first study enclosed in this doctoral thesis we observed an association between genera *Prevotella* 2 and *Bacteroides* and lower BMI. Moreover, we observed an association between genera *Prevotella* 9, *Lachnospiraceae* UCG-001, and *Bacteroides*, and higher weight loss after 12-month lifestyle intervention. These findings suggest that specific taxonomic features of the gut bacterial community may be involved in the control of body weight in response to a lifestyle intervention.

Our findings are in line with some evidences observed in previous studies describing reduced abundance of genus *Bacteroides* in individuals with obesity [120,121]. The role of *Bacteroides* in weight loss modulation in response to dietary intervention can be partially explained by the capacity of this genus to adapt its metabolic machinery to the food source [122]. In addition, the mutualistic nature of *Bacteroides* suggest that it may drive the functionality of other bacteria in an ecosystem [123]. Moreover, Christensen and colleagues shown no correlation between *Prevotella*-to-*Bacteroides* ratio and changes in body weight in overweight subjects exposed to different fiber rich diets, but they

observed higher weight loss in those subjects with high *Prevotella* abundance compared to subjects with low *Prevotella* abundance [124]. Accordingly, we observed no association between *Prevotella*-to *Bacteroides* ratio and changes in body weight after intervention, and an enrichment in *Prevotella* in case of weight loss. An enrichment in genus *Lachnospiraceae* UCG-001 was also observed in the highest tertile of weight loss after 12-month follow-up, highlighting the importance of this genus as SCFA producer. However, there are still too many discordant opinions about the involvement of *Lachnospiraceae* in energy efficiency improvement, probably because a proportionate amount of SCFAs is necessary in order to control energy intake and expenditure [125]. In addition, we also observed an enrichment of uncultured genera from *Clostridiales* order associated with weight loss after 12-month follow-up. Interestingly, Korpela and colleagues identified, among different bacteria, *Clostridium* cluster linked to dietary modifications and association with host's lipid metabolism [126]. Taking together these findings, it emerges that matching diet to gut microbiota profiles may be crucial in order to increase the effectiveness of weight loss programs. We also explored the association between Firmicutes-to-Bacteroides ratio, baseline BMI and changes in body weight after 12-month follow-up without observing any significant results. Accordingly, Duncan and colleagues shown no association in the proportion between these two major phyla among fecal bacteria neither with obesity nor after weight loss interventions exposure [82]. However, the lack of consensus regarding the link between Firmicutes-to-Bacterodetes ratio, obesity, and control of body weight, highlights the need of deeper taxonomic exploration of the gut microbiota [127]. Finally our findings suggest differences in the gut microbiota at 12-month follow-up, supporting long-term effects on weight loss, in contrast with other studies in which shifts in the gut microbiota composition in response to weight loss intervention were observed after 3-month follow-up after returning to the original composition at 12 months, suggesting no association to long-term changes in body weight [128].

Fecal microbiota signatures associated with insulin resistance

In the second part of this doctoral thesis, we identified cross-sectionally fecal microbiota taxonomic signatures and related metabolic pathways associated with insulin resistance in participants with overweight/obesity and MetS without T2D. Specifically, we observed genera *Desulfovibrio*, *Odoribacter,* and *Oscillospiraceae* UCG-002, negatively associated with higher HOMA-IR index, whereas genera *Faecalibacterium*, *Butyricicoccus*, and *Erysipelotricaceae* UCG-003, positively associated with higher HOMA-IR index. In addition, exploring the total metagenome in the cohort, we observed a total of 21 gut metabolic modules (GMMs) associated with HOMA-IR index. The relatively large number of altered GMMs found may be understood in light of the altered gut metabolic environment and would expect to typically accompany a higher insulin resistance, thus drastically altering the potential substrates for the microbiome. Furthermore, we observed that bacteria contribute differentially to the GMMs of interest and the degree of contribution is also dependent on HOMA-IR index. This may have major implications for our understanding of the role of the microbiome in insulin resistance and indeed other conditions altering host gut metabolism. Perhaps the altered gut metabolic environment promotes the relative abundance of different microbes that perform the same functions, as described by Zhang and colleagues [129].

We first described fecal microbiota taxonomic and pathway-related signatures negatively associated with HOMA-IR index. Among these results, we observed that genus *Desulfovibrio* was negatively associated with the highest tertile of HOMA-IR index, who includes those subjects with a significantly higher BMI, in accordance with some previous studies where *Desulfovibrio* was found associated with BMI < 25 in healthy adults with greater adherence to the MedDiet [101]. This association may be partially explained by the production of hydrogen sulfide (H_2S), a gas metabolite produced by SRB that can directly stimulate

glucose homeostasis and appetite regulation through GLP-1 activation [18,19]. We also observed a negative association with *Odoribacter* and higher HOMA-IR index in our study population. *Odoribacter* is a succinate-consuming bacterium. Elevated levels of circulating succinate have been associated with obesity and impaired glucose metabolism in a cohort of Spanish subjects. This increase was associated with specific changes in the gut microbiota related to succinate metabolism and a lower relative abundance of *Odoribacteraceae* [131]. Among the genera negatively associated with higher HOMA-IR index and BMI we also observed an enrichment of *Oscillospiraceae* genus, which have been found correlated with BMI and depleted in case of obesity [132,133]. The exploration of the predicted total metagenome revealed that higher HOMA-IR index was negatively associated with GMMs linked to amino acids breakdown. The fraction of amino acids that are not absorbed by the colonic mucosa are metabolized by the bacteria in the large intestine, resulting in the production of different end-products which may play a role in the modulation of obesity and insulin resistance [134]. Specifically, we observed that higher HOMA-IR index was negatively associated with a GMM linked to cysteine degradation, resulting in the production of H_2S, that as we previously mentioned can improve glucose homeostasis. Moreover, we observed negative association with GMMs linked with glycine and glutamine degradation, which are intestinal gluconeogenesis substrates. It has been proposed that intestinal gluconeogenesis protects against diabetes and obesity by suppressing hepatic gluconeogenesis and positively regulating glucose homeostasis [135]. Finally, we observed that for GMMs linked to aspartate degradation and glycine degradation, taxa of interest contribute significantly to the negative association with HOMA-IR index. Aspartate and glycine are precursors for the synthesis of acetate, who has been demonstrated to play a regulatory role in body weight control, and insulin sensitivity through effects on lipid metabolism and glucose homeostasis [136,137].

Following we explored fecal microbiota taxonomic and pathway-related signatures positively associated with HOMA-IR index. Some *Faecalibacterium* strains, such as *F. prausnitzii* are butyrate producers with immunomodulatory properties and anti-inflammatory effects [138]. Surprisingly we found *Feacalibacterium* positively associated with higher HOMA-IR index. In support to our findings, Hippe and colleagues shown that the overproduction of butyrate induced by different *F. prausnitzii* phylotypes, observed in a cohort of non-diabetic individuals with obesity, discriminates between obese developing T2D or not, suggesting possible different inflammatory genetic patterns in the host [139]. In addition, we find positive association between butyrate-producing genus *Butyricicoccus* and higher HOMA-IR index. Accordingly, the analysis of the total predicted metagenome revealed positive association between higher HOMA-IR index and GMMs linked to mono-, di- and polysaccharide degradation which leads to the production of SCFAs such as butyrate. Moreover, taxa of interest significantly contribute to the GMMs positively associated with higher HOMA-IR index linked to fructan degradation, glutamine degradation, lactaldehyde degradation and lactate production. Indeed, dietary fibers are the main source of oligosaccharides such as fructans, which can promote the growth of butyrate-producing bacteria [88]. In addition, lactate and lactaldehyde are fermentation products intermediary in the production of butyrate from carbohydrates [140,141]. Accordingly, it seems that the action of some taxa of interest is mediated by functions mainly linked to butyrate production, and that this metabolite is playing a role in the association observed with insulin resistance. Despite the large evidence supporting the beneficial effects of butyrate on gut health, its effect on obesity remain unclear. In accordance to our findings, it has been shown that butyrate can induce obesity providing the substrate for energy expenditure but also by engaging in signaling pathways involved in glycolipid metabolism [142]. However, due the controversial results especially considering literature mainly supporting the beneficial role of butyrate, we cannot

discard reverse causality. Among taxa positively associated with higher HOMA-IR index we also observed an enrichment in *Erysipelotrichaceae*, which have been found involved in host lipid and glucose metabolism impairment [143,144]. Bacteria belonging to *Erysipelotrichaceae* family use proteins and saccharides as main substrates for lactate production, one of the functional metabolic pathways that we found negatively associated with higher HOMA-IR index.

Strengths and limitations of the study

The studies enclosed in this doctoral thesis present some strengths and limitations that deserve comments. Among its strengths it is important to mention that these studies were conducted within the frame of a large lifestyle intervention aiming to provide a clinical approach for long-term weight loss maintenance and improvement of related metabolic disorders, with high sustainability for the participants included in the study. Under this point of view, it is also important to highlight that the study setup provides an ideal scenario to explore specific gut microbial changes related to pathological conditions such as obesity and insulin resistance. The large sample size, the long-term setup of the intervention and the longitudinal assessment in some analyses are additional strengths of the present studies.

These studies also include some limitations that deserve mention, especially because important for the interpretation of the results but also for the improvement of potential future studies related. The observational design of this study did not make possible to conclude on causality or directionality, as these results indicate just associations. Even if considering potential confounders, we cannot exclude residual confounding in the study of association. In addition, considering the lack of consensus in several aspects related to the association between gut microbiota, obesity and insulin resistance we cannot discard reverse causality. As this study was conducted in elderly Spanish individuals with overweight/obesity and MetS, our results cannot be extrapolated to other

populations. Also, the resilience of the gut microbiota cannot be determined, as these studies did not evaluate short-term changes. Under the methodological point of view, the nature of 16S sequencing limits the taxonomic profiling to genus-level resolution and reduces the quantity and quality of accessible information used to infer the functional potential of the microbiome. Furthermore, we lack metabolomics data, which is crucial in order to make our results more consistent. To conclude, the absence of a standardized computational approach to study the microbiota makes challenging to compare and reproduce the results generated with the other available studies.

Future insights

The above-mentioned limitations should be considered as first encouragement of further future studies. The large number of studies showing discrepancies in the taxonomic and functional aspect can be partially explained by the inherent biases in metagenomic sequencing which make it problematic to estimate bacterial abundance from sequencing data. Regardless, whole genome shotgun metagenomics enables analysis at a higher resolution and should be considered for future improvements. The taxonomic identification of the gut microbiota and possible associations with disease status could be validated with metabolomics techniques, which can provide detailed information on functional and metabolic profiles and offer a valid tool to help tailor personalized interventions in which successful strategies can be predicted according to this set of information. In addition, it has been observed that pathology-related gut microbiota alterations are linked with epigenetic changes, adding further opportunities of potential future investigations.

A large sample size clinical trial such as the PREDIMED-Plus, which also includes a huge collection of clinical and biochemical data provides the ideal platform to study several types of outcomes mediated by the gut microbiota. In addition, the consortium also benefits of a large collection of clinical samples

which makes feasible the possibility to improve these studies with multi-omics approaches. A big variety of opportunities are currently opened also considering the rapid evolution of computational and statistical methods that can be implemented.

VIII. CONCLUSIONS

In the first study enclosed in this doctoral thesis we observed that specific fecal microbiota taxonomic features were associated with lower BMI and higher weight loss after a 12-month lifestyle intervention in a population of elderly individuals with overweight, obesity and MetS, suggesting that specific member of the gut bacterial community may be involved in the control of body weight in response to a lifestyle intervention.

In the second study enclosed in this doctoral thesis we observed that specific fecal microbiota genera and related predicted metabolic pathways were negatively associated with higher HOMA-IR index. This association could be linked to improved glucose homeostasis, induced by an increase in amino acids breakdown that could induce intestinal gluconeogenesis and acetate synthesis; by an increase in SRB and consequent H_2S production; by improved succinate metabolism. In addition, specific fecal microbiota genera and related predicted metabolic pathways, were positively associated with higher HOMA-IR index. This association could be linked to an increased saccharide degradation that may stimulate the growth of butyrate-producing bacteria with consequent abnormal production of butyrate and impaired glycolipid metabolism.

Taking together these findings emerges that the identification of specific gut microbiota taxonomic signatures could be useful in order to adapt the strategies for obesity management by predicting treatments' success according to the microbiota composition, but also to target potential bacterial candidates for the development of therapies for insulin resistance. The effectiveness of these strategies should be validated in the future with appropriate and well-designed animal and human studies.

IX. REFERENCES

1. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med [Internet]. 2016;8(1]:1–12. Available from: http://dx.doi.org/10.1186/s13073-016-0303-2

2. WHO. Obesity and Overweight. 2020 [Internet]. [cited 2021 Nov 8]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight

3. Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Collazo-Clavell ML, Korinek J, et al. Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes [Internet]. 2008 Jun 19;32(6):959–66. Available from: http://www.nature.com/articles/ijo200811

4. Wang Y, Beydoun MA. The obesity epidemic in the United States - Gender, age, socioeconomic, racial/ethnic, and geographic characteristics: A systematic review and meta-regression analysis. Epidemiologic Reviews. 2007.

5. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med [Internet]. 2017 Jul 6;377(1):13–27. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1614362

6. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in Obesity and Severe Obesity Prevalence in US Youth and Adults by Sex and Age, 2007-2008 to 2015-2016. JAMA [Internet]. 2018 Apr 24;319(16):1723. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2018.3060

7. Kelly T, Yang W, Chen C-S, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes [Internet]. 2008 Sep 8;32(9):1431–7. Available from: http://www.nature.com/articles/ijo2008102

8. Seidell JC, Halberstadt J. The global burden of obesity and the challenges of prevention. Ann Nutr Metab. 2015;66(suppl 2):7–12.

9. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2224–60.

10. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne). 2021;12(September):1–9.

11. Obri A, Claret M. The role of epigenetics in hypothalamic energy balance control: implications for obesity. Cell Stress [Internet]. 2019 Jul 8;3(7):208–20. Available from: http://www.cell-stress.com/researcharticles/2019a-obri-cell-stress/

12. Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics [Internet]. 2015 Jul 4;33(7):673–89. Available from: http://link.springer.com/10.1007/s40273-014-0243-x

13. Levine JA. Poverty and Obesity in the U.S. Diabetes [Internet]. 2011 Nov 24;60(11):2667–8. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db11-1118

14. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med. 2017;376(3):254–66.

15. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and Metabolic Implications of Regional Differences among Fat Depots. Cell Metab [Internet]. 2013 May;17(5):644–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413113001113

16. Heymsfield SB, Hu HH, Shen W, Carmichael O. Emerging Technologies

and their Applications in Lipid Compartment Measurement. Trends Endocrinol Metab [Internet]. 2015 Dec;26(12):688–98. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043276015002039

17. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. Clin Liver Dis [Internet]. 2004 Aug;8(3):521–33. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1089326104000340

18. Jiang SZ, Lu W, Zong XF, Ruan HY, Liu Y. Obesity and hypertension. Exp Ther Med. 2016;12(4):2395–9.

19. Piché ME, Tchernof A, Després JP. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. Circ Res. 2020;1477–500.

20. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. Circulation [Internet]. 2014 Jun 24;129(25_suppl_2). Available from: https://www.ahajournals.org/doi/10.1161/01.cir.0000437739.71477.ee

21. Expert panel report: Guidelines (2013) for the management of overweight and obesity in adults. Obesity [Internet]. 2014 Jul;22(S2):S41–410. Available from: https://onlinelibrary.wiley.com/doi/10.1002/oby.20660

22. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, et al. Pharmacological Management of Obesity: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab [Internet]. 2015 Feb;100(2):342–62. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2014-3415

23. Gadde KM, Martin CK, Berthoud H, Heymsfield SB. Obesity: Pathophysiology and Management. J Am Coll Cardiol [Internet]. 2018 Jan;71(1):69–84. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S0735109717415841

24. Gadde KM, Pritham Raj Y. Pharmacotherapy of Obesity: Clinical Trials to Clinical Practice. Curr Diab Rep [Internet]. 2017 May 4;17(5):34. Available from: http://link.springer.com/10.1007/s11892-017-0859-2

25. Piché M-È, Auclair A, Harvey J, Marceau S, Poirier P. How to Choose and Use Bariatric Surgery in 2015. Can J Cardiol [Internet]. 2015 Feb;31(2):153–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0828282X14016626

26. Martyn JAJ, Kaneki M, Yasuhara S, Warner DS, Warner MA. Obesity-induced Insulin Resistance and Hyperglycemia. Anesthesiology [Internet]. 2008 Jul 1;109(1):137–48. Available from: https://pubs.asahq.org/anesthesiology/article/109/1/137/9035/Obesity-induced-Insulin-Resistance-and

27. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature [Internet]. 2001 Dec;414(6865):799–806. Available from: http://www.nature.com/articles/414799a

28. Fahed M, Abou Jaoudeh MG, Merhi S, Mosleh JMB, Ghadieh R, Ghadieh R, et al. Evaluation of risk factors for insulin resistance: A cross sectional study among employees at a private university in Lebanon. BMC Endocr Disord. 2020;20(1):1–14.

29. Draznin B. Molecular Mechanisms of Insulin Resistance. Contemp Endocrinol. 2020;55–66.

30. Lowell BB, Shulman GI. Mitochondrial Dysfunction and Type 2 Diabetes. Science (80-) [Internet]. 2005 Jan 21;307(5708):384–7. Available from: https://www.science.org/doi/10.1126/science.1104343

31. Ueno M, Carvalheira JBC, Tambascia RC, Bezerra RMN, Amaral ME, Carneiro EM, et al. Regulation of insulin signalling by hyperinsulinaemia:

role of IRS-1/2 serine phosphorylation and the mTOR/p70 S6K pathway. Diabetologia [Internet]. 2005 Mar 4;48(3):506–18. Available from: http://link.springer.com/10.1007/s00125-004-1662-6

32. Li Y, Soos TJ, Li X, Wu J, DeGennaro M, Sun X, et al. Protein Kinase C θ Inhibits Insulin Signaling by Phosphorylating IRS1 at Ser1101. J Biol Chem [Internet]. 2004 Oct;279(44):45304–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925820724543

33. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res [Internet]. 2005 Nov;46(11):2347–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022227520328728

34. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, et al. SOCS-3 Inhibits Insulin Signaling and Is Up-regulated in Response to Tumor Necrosis Factor-α in the Adipose Tissue of Obese Mice. J Biol Chem [Internet]. 2001 Dec;276(51):47944–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925819403049

35. Hotamisligil GS. Inflammation and metabolic disorders. Nature [Internet]. 2006 Dec 13;444(7121):860–7. Available from: http://www.nature.com/articles/nature05485

36. Petersen KF, Shulman GI. Etiology of Insulin Resistance. Am J Med [Internet]. 2006 May;119(5):S10–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002934306001008

37. Wondmkun YT. Obesity, insulin resistance, and type 2 diabetes: Associations and therapeutic implications. Diabetes, Metab Syndr Obes Targets Ther. 2020;13:3611–6.

38. Shimoda Y, Okada S, Yamada E, Pessin JE, Yamada M. Tctex1d2 Is a

Negative Regulator of GLUT4 Translocation and Glucose Uptake. Endocrinology [Internet]. 2015 Oct 1;156(10):3548–58. Available from: https://academic.oup.com/endo/article/156/10/3548/2422876

39. Jeschke MG, Boehning D. Endoplasmic reticulum stress and insulin resistance post-trauma: similarities to type 2 diabetes. J Cell Mol Med [Internet]. 2012 Mar;16(3):437–44. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1582-4934.2011.01405.x

40. Ye J. Mechanisms of insulin resistance in obesity. Front Med [Internet]. 2013 Mar 9;7(1):14–24. Available from: http://link.springer.com/10.1007/s11684-013-0262-6

41. Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA, et al. Asian Indians Have Enhanced Skeletal Muscle Mitochondrial Capacity to Produce ATP in Association With Severe Insulin Resistance. Diabetes [Internet]. 2008 May 1;57(5):1166–75. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db07-1556

42. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet [Internet]. 2017 Jun;389(10085):2239–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673617300582

43. WHO. Diabetes.2019 [Internet]. [cited 2021 Nov 16]. Available from: https://www.who.int/health-topics/diabetes#tab=tab_1

44. Huda MN, Kim M, Bennett BJ. Modulating the Microbiota as a Therapeutic Intervention for Type 2 Diabetes. Front Endocrinol (Lausanne). 2021;12(April).

45. Abdul M, Khan B, Hashim MJ, King JK, Govender RD, Mustafa H, et al. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020;10:107–11.

46. Schlienger J-L. Type 2 diabetes complications. Presse Med [Internet]. 2013 May;42(5):839–48. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0755498213004582

47. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care [Internet]. 2021 Jan;44(Supplement 1):S15–33. Available from: http://care.diabetesjournals.org/lookup/doi/10.2337/dc21-S002

48. Barlow GM, Yu A, Mathur R. Role of the gut microbiome in obesity and diabetes mellitus. Nutr Clin Pract. 2015;30(6):787–97.

49. Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, et al. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. Cell Metab. 2020;32(3):379-390.e3.

50. Upadhyaya S, Banerjee G. Type 2 diabetes and gut microbiome: At the intersection of known and unknown. Gut Microbes. 2015;6(2):85–92.

51. Pircalabioru GG, Picu A, Petcu L, Popa M, Chifiriuc MC. The Intricate Relationship between Diabetes, Diet and the Gut Microbiota. In: Pathophysiology - Altered Physiological States [Internet]. InTech; 2018. Available from: http://www.intechopen.com/books/pathophysiology-altered-physiological-states/the-intricate-relationship-between-diabetes-diet-and-the-gut-microbiota

52. Pfeiffer AFH, Klein HH. The Treatment of Type 2 Diabetes. Dtsch Arztebl Int [Internet]. 2014 Jan 31;111(5):69–82. Available from: https://www.aerzteblatt.de/10.3238/arztebl.2014.0069

53. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science (80-). 2005;307(5717):1915–20.

54. van de Wouw M, Schellekens H, Dinan TG, Cryan JF. Microbiota-Gut-

Brain Axis: Modulator of Host Metabolism and Appetite. J Nutr. 2017;147(5):727–45.

55. Natividad JMM, Verdu EF. Modulation of intestinal barrier by intestinal microbiota: Pathological and therapeutic implications. Pharmacological Research. 2013.

56. Blandino G, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. Diabetes Metab [Internet]. 2016;42(5):303–15. Available from: http://dx.doi.org/10.1016/j.diabet.2016.04.004

57. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016.

58. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, et al. The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res [Internet]. 2010 May;51(5):1101–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022227520410661

59. Heiss CN, Olofsson LE. Gut Microbiota-Dependent Modulation of Energy Metabolism. Journal of Innate Immunity. 2018.

60. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano G, Gasbarrini A, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms. 2019 Jan;7(1):14.

61. McBurney MI, Davis C, Fraser CM, Schneeman BO, Huttenhower C, Verbeke K, et al. Establishing What Constitutes a Healthy Human Gut Microbiome: State of the Science, Regulatory Considerations, and Future Directions. J Nutr. 2019;149(11):1882–95.

62. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G,

Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci [Internet]. 2010 Jun 29;107(26):11971–5. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1002601107

63. Fan W, Huo G, Li X, Yang L, Duan C. Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Infants During the Six Months of Life. J Microbiol Biotechnol [Internet]. 2014 Feb 28;24(2):133–43. Available from: http://www.jmb.or.kr/journal/view.html?doi=10.4014/jmb.1309.09029

64. Dogra SK, Doré J, Damak S. Gut Microbiota Resilience: Definition, Link to Health and Strategies for Intervention. Front Microbiol. 2020;11(September):2014–21.

65. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.

66. Bäckhed F, Ding H, Wang T, Hooper L V., Gou YK, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101(44):15718–23.

67. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci [Internet]. 2007 Jan 16;104(3):979–84. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.0605374104

68. Crovesy L, Masterson D, Rosado EL. Profile of the gut microbiota of adults with obesity: a systematic review. Eur J Clin Nutr [Internet]. 2020; Available from: http://dx.doi.org/10.1038/s41430-020-0607-6

69. Lee CJ, Sears CL, Maruthur N. Gut microbiome and its role in obesity and insulin resistance. Ann N Y Acad Sci. 2019;1–16.

70. Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. Curr Opin Pharmacol [Internet]. 2019;49:1–5. Available from: https://doi.org/10.1016/j.coph.2019.03.011

71. Bianchi F, Duque ALRF, Saad SMI, Sivieri K. Gut microbiome approaches to treat obesity in humans. Appl Microbiol Biotechnol [Internet]. 2019 Feb 16;103(3):1081–94. Available from: http://link.springer.com/10.1007/s00253-018-9570-8

72. Castaner O, Goday A, Park YM, Lee SH, Magkos F, Shiow SATE, et al. The gut microbiome profile in obesity: A systematic review. Int J Endocrinol. 2018;2018.

73. Patterson EE, Ryan PM, Cryan JF, Dinan TG, Paul Ross R, Fitzgerald GF, et al. Gut microbiota, obesity and diabetes. Postgrad Med J. 2016;92(1087):286–300.

74. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;

75. Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: Metabolism and perspective in obesity. Gut Microbes. 2018;9(4):308–25.

76. Medzhitov R, Horng T. Transcriptional control of the inflammatory response. Nature Reviews Immunology. 2009.

77. Saad MJA, Santos A, Prada PO. Linking gut microbiota and inflammation to obesity and insulin resistance. Physiology. 2016;31(4):283–93.

78. Sugita H, Kaneki M, Tokunaga E, Sugita M, Koike C, Yasuhara S, et al. Inducible nitric oxide synthase plays a role in LPS-induced hyperglycemia and insulin resistance. Am J Physiol Metab [Internet]. 2002 Feb 1;282(2):E386–94. Available from:

https://www.physiology.org/doi/10.1152/ajpendo.00087.2001

79. Velloso LA, Folli F, Saad MJ. TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. Endocrine Reviews. 2015.

80. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5. Science (80-) [Internet]. 2010 Apr 9;328(5975):228–31. Available from: https://www.science.org/doi/10.1126/science.1179721

81. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature [Internet]. 2012 Jan 18;481(7381):278–86. Available from: http://www.nature.com/articles/nature10759

82. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes. 2008;32(11):1720–4.

83. Chambers ES, Preston T, Frost G, Morrison DJ. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. Curr Nutr Rep. 2018;7(4):198–206.

84. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med [Internet]. 2009 Aug 26;15(8):930–9. Available from: http://www.nature.com/articles/nm.2002

85. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-Mediated Bile Acid Sensing Controls Glucose Homeostasis. Cell Metab. 2009;

86. Rodriguez-Castaño GP, Caro-Quintero A, Reyes A, Lizcano F. Advances in Gut Microbiome Research, Opening New Strategies to Cope with a

Western Lifestyle. Front Genet [Internet]. 2017 Jan 10;7. Available from: http://journal.frontiersin.org/article/10.3389/fgene.2016.00224/full

87. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;

88. Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, et al. The role of diet on gut microbiota composition. Eur Rev Med Pharmacol Sci. 2016;20(22):4742–9.

89. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci [Internet]. 2010 Aug 17;107(33):14691–6. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1005963107

90. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature [Internet]. 2012 Jun 9;486(7402):222–7. Available from: http://www.nature.com/articles/nature11053

91. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science (80-) [Internet]. 2011 Oct 7;334(6052):105–8. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1208344

92. Enck P, Zimmer JM, Lange B, Zimmermann K, Schwiertz A, Rusch K, et al. A Vegan or Vegetarian Diet Substantially Alters the Human Fecal Microbiome Composition. Gastroenterology [Internet]. 2018 May;140(5):S-306. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0016508511612298

93. Gentile CL, Weir TL. The gut microbiota at the intersection of diet and human health. Science (80-) [Internet]. 2018 Nov 16;362(6416):776–80.

Available from: http://www.sciencemag.org/lookup/doi/10.1126/science.aau5812

94. Galilea-Zabalza I, Buil-Cosiales P, Salas-Salvadó J, Toledo E, Ortega-Azorín C, Díez-Espino J, et al. Mediterranean diet and quality of life: Baseline cross-sectional analysis of the PREDIMED-PLUS trial. Rohrmann S, editor. PLoS One [Internet]. 2018 Jun 18;13(6):e0198974. Available from: https://dx.plos.org/10.1371/journal.pone.0198974

95. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr [Internet]. 2010 Nov 1;92(5):1189–96. Available from: https://academic.oup.com/ajcn/article/92/5/1189/4597540

96. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. N Engl J Med [Internet]. 2013 Apr 4;368(14):1279–90. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1200303

97. Merra G, Noce A, Marrone G, Cintoni M, Tarsitano MG, Capacci A, et al. Influence of mediterranean diet on human gut microbiota. Nutrients. 2021;13(1):1–12.

98. Tosti V, Bertozzi B, Fontana L. Health Benefits of the Mediterranean Diet: Metabolic and Molecular Mechanisms. Journals Gerontol - Ser A Biol Sci Med Sci. 2018;73(3):318–26.

99. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut. 2016;65(11):1–10.

100. Gutiérrez-Díaz I, Fernández-Navarro T, Sánchez B, Margolles A, González S, Mitsou EK, et al. Metabolomics and Microbiomes as

Potential Tools to Evaluate the Effects of the Mediterranean Diet. Food Funct. 2017/08/09. 2018;9(5):2347–56.

101. Garcia-Mantrana I, Selma-Royo M, Alcantara C, Collado MC. Shifts on gut microbiota associated to mediterranean diet adherence and specific dietary intakes on general adult population. Front Microbiol. 2018;9(MAY):1–11.

102. Ghosh TS, Rampelli S, Jeffery IB, Santoro A, Neto M, Capri M, et al. Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. Gut. 2020 Feb 17;gutjnl-2019-319654.

103. Galié S, García-Gavilán J, Camacho-Barcía L, Atzeni A, Muralidharan J, Papandreou C, et al. Effects of the Mediterranean Diet or Nut Consumption on Gut Microbiota Composition and Fecal Metabolites and their Relationship with Cardiometabolic Risk Factors. Mol Nutr Food Res. 2021;65(19):1–9.

104. Cho KY. Lifestyle modifications result in alterations in the gut microbiota in obese children. BMC Microbiol [Internet]. 2021 Dec 6;21(1):10. Available from: https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-020-02002-3

105. Stanislawski MA, Frank DN, Borengasser SJ, Ostendorf DM, Ir D, Jambal P, et al. The gut microbiota during a behavioral weight loss intervention. Nutrients. 2021;13(9).

106. Muralidharan J, Moreno-Indias I, Bulló M, Lopez JV, Corella D, Castañer O, et al. Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study. Am J Clin

Nutr. 2021;114(3):1148–58.

107. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. Cell [Internet]. 2014 Jan;156(1–2):84–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S009286741301550X

108. Troy-Fioramonti S, Demizieux L, Gresti J, Muller T, Vergès B, Degrace P. Acute Activation of Cannabinoid Receptors by Anandamide Reduces Gastrointestinal Motility and Improves Postprandial Glycemia in Mice. Diabetes [Internet]. 2015 Mar;64(3):808–18. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db14-0721

109. Carlucci C, Petrof EO, Allen-Vercoe E. Fecal Microbiota-based Therapeutics for Recurrent Clostridium difficile Infection, Ulcerative Colitis and Obesity. EBioMedicine [Internet]. 2016 Nov;13:37–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2352396416304509

110. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science (80-). 2013;

111. Alang N, Kelly CR. Weight Gain After Fecal Microbiota Transplantation. Open Forum Infect Dis [Internet]. 2015 Jan 1;2(1). Available from: https://academic.oup.com/ofid/article/doi/10.1093/ofid/ofv004/1461242

112. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;

113. Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra A V., Udayappan SD,

et al. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. Cell Metab [Internet]. 2017 Oct;26(4):611-619.e6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413117305594

114. Merrick B, Allen L, Masirah M Zain N, Forbes B, Shawcross DL, Goldenberg SD. Regulation, risk and safety of Faecal Microbiota Transplant. Infect Prev Pract [Internet]. 2020;2(3):100069. Available from: https://doi.org/10.1016/j.infpip.2020.100069

115. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome. Circulation [Internet]. 2009 Oct 20;120(16):1640–5. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.109.192644

116. Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort Profile: Design and methods of the PREDIMED-Plus randomized trial. Martínez-González, M A, Buil-Cosiales, P, Corella, D, Bulló, M, Fitó, M, Vioque, J, … Fandos Sánchez, M (2019) Cohort Profile Des methods PREDIMED-Plus randomized trial Int J Epidemiol 48(2), 387-388o https [Internet]. 2019 Apr 1;48(2):387-388o. Available from: https://academic.oup.com/ije/article/48/2/387/5202210

117. Schröder H, Zomeño MD, Martínez-González MA, Salas-Salvadó J, Corella D, Vioque J, et al. Validity of the energy-restricted Mediterranean Diet Adherence Screener. Clin Nutr. 2021;40(8):4971–9.

118. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010;103(12):1808–16.

119. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.

120. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, et al. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. BMC Gastroenterol. 2015;

121. Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. Nat Med. 2017;23(7):859–68.

122. Wexler HM. Bacteroides: The good, the bad, and the nitty-gritty. Clinical Microbiology Reviews. 2007.

123. Wexler AG, Goodman AL. An insider's perspective: Bacteroides as a window into the microbiome. Nature Microbiology. 2017.

124. Christensen L, Vuholm S, Roager HM, Nielsen DS, Krych L, Kristensen M, et al. Prevotella Abundance Predicts Weight Loss Success in Healthy, Overweight Adults Consuming a Whole-Grain Diet Ad Libitum: A Post Hoc Analysis of a 6-Wk Randomized Controlled Trial. J Nutr. 2019;149(12):2174–81.

125. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The controversial role of human gut lachnospiraceae. Microorganisms. 2020;8(4):1–25.

126. Korpela K, Flint HJ, Johnstone AM, Lappi J, Poutanen K, Dewulf E, et al. Gut Microbiota Signatures Predict Host and Microbiota Responses to Dietary Interventions in Obese Individuals. Bereswill S, editor. PLoS One

[Internet]. 2014 Mar 6;9(3):e90702. Available from: https://dx.plos.org/10.1371/journal.pone.0090702

127. John GK, Mullin GE. The Gut Microbiome and Obesity. Curr Oncol Rep [Internet]. 2016;18(7). Available from: http://dx.doi.org/10.1007/s11912-016-0528-7

128. Fragiadakis GK, Wastyk HC, Robinson JL, Sonnenburg ED, Sonnenburg JL, Gardner CD. Long-term dietary intervention reveals resilience of the gut microbiota despite changes in diet and weight. Am J Clin Nutr. 2020;111(6):1127–36.

129. Zhang C, Yin A, Li H, Wang R, Wu G, Shen J, et al. Dietary Modulation of Gut Microbiota Contributes to Alleviation of Both Genetic and Simple Obesity in Children. EBioMedicine [Internet]. 2015 Aug;2(8):968–84. Available from: http://dx.doi.org/10.1016/j.ebiom.2015.07.007

130. Pichette J, Fynn-Sackey N, Gagnon J. Hydrogen sulfide and sulfate prebiotic stimulates the secretion of GLP-1 and improves glycemia in male mice. Endocrinology. 2017;158(10):3416–25.

131. Serena C, Ceperuelo-Mallafré V, Keiran N, Queipo-Ortuño MI, Bernal R, Gomez-Huelgas R, et al. Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. ISME J [Internet]. 2018;12(7):1642–57. Available from: http://dx.doi.org/10.1038/s41396-018-0068-2

132. Biassoni R, Di Marco E, Squillario M, Barla A, Piccolo G, Ugolotti E, et al. Gut Microbiota in T1DM-Onset Pediatric Patients: Machine-Learning Algorithms to Classify Microorganisms as Disease Linked. J Clin Endocrinol Metab [Internet]. 2020 Sep 1;105(9):e3114–26. Available from: https://academic.oup.com/jcem/article/105/9/e3114/5871462

133. Maya-Lucas O, Murugesan S, Nirmalkar K, Alcaraz LD, Hoyo-Vadillo

C, Pizano-Zárate ML, et al. The gut microbiome of Mexican children affected by obesity. Anaerobe [Internet]. 2019;55:11–23. Available from: https://doi.org/10.1016/j.anaerobe.2018.10.009

134. Neis EPJG, Dejong CHC, Rensen SS. The role of microbial amino acid metabolism in host metabolism. Nutrients. 2015.

135. Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, et al. Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence? Curr Diab Rep. 2018;18(10).

136. Zhao J, Zhang X, Liu H, Brown MA, Qiao S. Dietary Protein and Gut Microbiota Composition and Function. Curr Protein Pept Sci. 2018;20(2):145–54.

137. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients. 2019;11(8).

138. Lenoir M, Martín R, Torres-Maravilla E, Chadi S, González-Dávila P, Sokol H, et al. Butyrate mediates anti-inflammatory effects of Faecalibacterium prausnitzii in intestinal epithelial cells through Dact3. Gut Microbes. 2020;12(1):1–16.

139. Hippe B, Remely M, Aumueller E, Pointner A, Magnet U, Haslberger AG. Faecalibacterium prausnitzii phylotypes in type two diabetic, obese, and lean control subjects. Benef Microbes. 2016;7(4):511–7.

140. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol. 2017;19(1):29–41.

141. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. Microbiome. 2019;7(1):1–15.

142. Liu H, Wang J, He T, Becker S, Zhang G, Li D, et al. Butyrate: A double-

edged sword for health? Adv Nutr. 2018;9(1):21–9.

143. Kaakoush NO. Insights into the role of Erysipelotrichaceae in the human host. Front Cell Infect Microbiol. 2015;5(NOV):1–4.

144. Lippert K, Kedenko L, Antonielli L, Kedenko I, Gemeier C, Leitner M, et al. Gut microbiota dysbiosis associated with glucose metabolism disorders and the metabolic syndrome in older adults. Benef Microbes [Internet]. 2017 Aug 24;8(4):545–56. Available from: https://www.wageningenacademic.com/doi/10.3920/BM2016.0184

X. APPENDICES

Scientific contributions belonging to this doctoral thesis

Atzeni, Alessandro, Serena Galié, Jananee Muralidharan, Nancy Babio, Francisco José Tinahones, Jesús Vioque, Dolores Corella, *et al*. 2021. "Gut Microbiota Profile and Changes in Body Weight in Elderly Subjects with Overweight/Obesity and Metabolic Syndrome." Microorganisms 9 (2): 346.

Atzeni, Alessandro, Thomaz F. S. Bastiaanssen, John F. Cryan, Francisco José Tinahones, Jesús Vioque, Dolores Corella, Montserrat Fitó, Josep Vidal, Isabel Moreno Indias, Ana M. Gómez Pérez, Laura Torres Collado, Oscar Coltell, Olga Castañer, Monica Bulló, Jordi Salas Salvadó. "Taxonomic and functional fecal microbiota signatures associated with insulin resistance in non-diabetic subjects with overweight/obesity within the frame of the PREDIMED-Plus study." Frontiers in Endocrinology. Accepted on March 21, 2022.

Other scientific contributions

Muralidharan, Jananee, Isabel Moreno-Indias, Mónica Bulló, Jesús Vioque Lopez, Dolores Corella, Olga Castañer, Josep Vidal, **Alessandro Atzeni**, *et al*. 2021. "Effect on Gut Microbiota of a 1-y Lifestyle Intervention with Mediterranean Diet Compared with Energy-Reduced Mediterranean Diet and Physical Activity Promotion: PREDIMED-Plus Study." The American Journal of Clinical Nutrition 114 (3): 1148–58.

Galié, Serena, Jesús García-Gavilán, Lucía Camacho-Barcía, **Alessandro Atzeni**, Jananee Muralidharan, Christopher Papandreou, Pierre Arcelin, *et al*. 2021. "Effects of the Mediterranean Diet or Nut Consumption on Gut Microbiota Composition and Fecal Metabolites and Their Relationship with Cardiometabolic Risk Factors." Molecular Nutrition and Food Research 65 (19): 1–9.

Kaźmierczak-Siedlecka, Karolina, Karolina Skonieczna-Żydecka, Jarosław

Biliński, Giandomenico Roviello, Luigi F. Iannone, **Alessandro Atzeni**, Bartosz K. Sobocki, and Karol Połom. 2021. "Gut Microbiome Modulation and Faecal Microbiota Transplantation Following Allogenic Hematopoietic Stem Cell Transplantation" Cancers 13, no. 18: 4665.

Book chapter: Alasalvar, Cesarettin, Jordi Salas-Salvadó, Emilio Ros, and Joan Sabate. 2020. Health benefits of nuts and dried fruits. CRC Press. Chapter 13, Nuts: Gut Health and Microbiota. Serena Galié, Monica Bullo, **Alessandro Atzeni**, Pablo Hernandez-Alonso, Jananee Muralidharan, and Jordi Salas-Salvadó

Others: Alessandro Atzeni, Jordi Salas-Salvadó (2019) Multi-metabolite biomarker panels to study nuts intake. Nutfruit Magazine. International Nut Council.

Participation in national and international conferences

Nutrition Winter School 2020, Jan 27 – Jan 31, 2020, Levi (Finland).

Poster communication: **Alessandro Atzeni**, "Gut microbiota as predictive biomarker of changes in body weight".

28th European Congress on Obesity (ECO ONLINE 2021), May 10 – May 13, 2021.

Poster communication: **Alessandro Atzeni**, "Fecal microbiota signatures associated with body composition and weight loss in elderly subjects with overweight/obesity and metabolic syndrome".

International mobility

APC Microbiome Ireland, Apr 19 – July 31, 2021, Cork (Ireland)

Bioinformatics analysis of 16S dataset for the identification of fecal microbiota

taxonomic and functional signatures associated with insulin resistance in non-diabetic subjects within the frame of the PREDIMED-Plus trial.

National mobility

9th FISABIO Summer School, July 1 – July 5, 2019, Valencia

Biomedical research and public health (basic computational skills for genomic analysis)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No. 713679.

This PhD dissertation has been possible with the support of the Universitat Rovira i Virgili and the Fundació Catalunya la Pedrera.

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni

UNIVERSITAT ROVIRA I VIRGILI
GUT MICROBIOTA SIGNATURES ASSOCIATED WITH WEIGHT LOSS AND INSULIN RESISTANCE IN AN ELDERLY MEDITERRANEAN POPULATION WITH OVERWEIGHT/OBESITY AND METABOLIC SYNDROME
Alessandro Atzeni