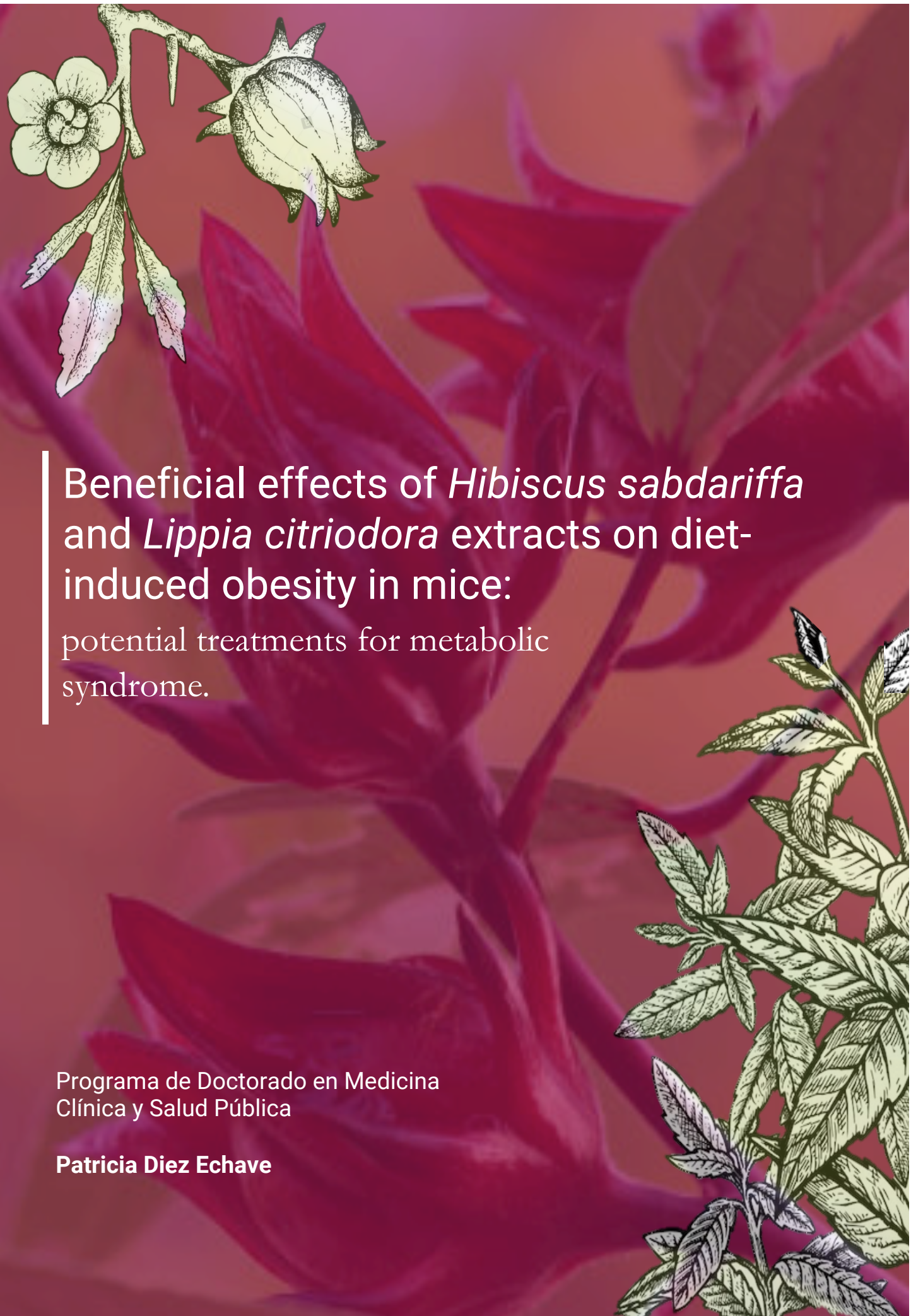

Beneficial effects of *Hibiscus sabdariffa* and *Lippia citriodora* extracts on diet-induced obesity in mice:

potential treatments for metabolic syndrome.

Programa de Doctorado en Medicina Clínica y Salud Pública

Patricia Diez Echave

UNIVERSIDAD DE GRANADA

FACULTAD DE FARMACIA

Departamento de Farmacología

Beneficial effects of *Hibiscus sabdariffa* and *Lippia citriodora* extracts on diet-induced obesity in mice: potential treatments for metabolic syndrome.

Patricia Diez Echave

Bajo la dirección de los Doctores

Julio Juan Gálvez Peralta

María Elena Rodríguez Cabezas

Granada, 2020

Editor: Universidad de Granada. Tesis Doctorales
Autor: Patricia Díez Echave
ISBN: 978-84-1306-580-9
URI: http://hdl.handle.net/10481/63450

Toda historia
es una historia interminable

INDEX

RESUMEN

INTRODUCCIÓN

El síndrome metabólico, enfermedad que afecta a un 25% de la población adulta mundial (1), es un conjunto de alteraciones metabólicas como son la hiperglucemia asociada a la resistencia a la insulina, obesidad (adiposidad visceral), dislipidemia con niveles reducidos de colesterol unido a lipoproteínas de alta densidad (colesterol HDL) y niveles elevados de triglicéridos en plasma, e hipertensión (2). Entre todos, la obesidad es considerada el eje central, y se trata de una enfermedad compleja y multifactorial, que se desarrolla como consecuencia de un desequilibro en el balance energético. Como consecuencia, hay un exceso de acumulación de energía en forma de grasa, sobre todo en el tejido adiposo, lo cual hace que los adipocitos aumenten en tamaño y/o número. En los últimos años se ha observado la presencia de un estado inflamatorio subclínico en los pacientes obesos, que se produce como consecuencia de este incremento en la masa del tejido adiposo, junto a un aumento de la producción de mediadores pro-inflamatorios que son estimulados por señales de origen exógeno y/o endógeno (3). El tejido adiposo está constituido por fibroblastos, preadipocitos, adipocitos y macrófagos, siendo estos últimos los que contribuyen significativamente al proceso inflamatorio sistémico con la producción de mediadores pro-inflamatorios (4). De esta manera, existe una asociación íntima y altamente coordinada entre las vías inflamatorias y metabólicas (3). Por otro lado, se ha demostrado que la obesidad está asociada a un desequilibro en la composición y función de la microbiota intestinal, proceso denominado como disbiosis, y que se relaciona con un aumento de la permeabilidad intestinal y por lo tanto el paso de componentes bacterianos, como es el LPS, al torrente sanguíneo, provocando una endotoxemia metabólica que a su vez contribuye al desarrollo del estado inflamatorio crónico (5).

Actualmente, y desgraciadamente, los fármacos disponibles con una actuación global frente al síndrome metabólico son de eficacia limitada y presentan reacciones adversas (6), por lo que es necesaria la investigación de nuevas estrategias terapéuticas que aúnen eficacia y seguridad. Diferentes estudios han puesto de manifiesto la tendencia actual por parte de los pacientes con alteraciones inflamatorias y/o metabólicas de emplear medicinas alternativas y/o complementarias en el tratamiento de sus enfermedades. Este puede ser el caso del uso de extractos vegetales procedentes de plantas medicinales. Estos tratamientos son generalmente seguros al mostrar escasas reacciones adversas, mientras que su uso en la medicina tradicional avalaría su eficacia en estas situaciones,

probablemente debido a su composición, consistente en una mezcla de principios bioactivos que pueden actuar simultáneamente sobre distintas dianas terapéuticas (7).

Entre ellas, es destacable el potencial papel que pueden tener los extractos polifenólicos obtenidos de *Hibiscus sabdariffa* o de *Lippia citriodora*. Los usos tradicionales de *H. sabdariffa* se derivan de su actividad antioxidante, antihipertensiva y antidiabética (8), mientras que en el caso de *L. citriodora*, se describen propiedades antioxidantes (9) y mejora del metabolismo lipídico (10), justificando la inclusión de los extractos de estas plantas medicinales en estos estudios.

OBJETIVOS

El presente trabajo de tesis doctoral pretende evaluar el efecto de un extracto del cáliz de *H. sabdariffa*, y otro extracto de las hojas de *L. citriodora*, bien caracterizados desde un punto de vista químico, en un modelo experimental de síndrome metabólico en ratones, y evaluar los posibles mecanismos responsables de los efectos beneficiosos.

De esta manera, se propusieron los siguientes objetivos:

1. Evaluar el efecto de los extractos sobre el peso corporal, el perfil bioquímico plasmático y la tolerancia a la glucosa.
2. Determinar los efectos antiinflamatorios de los extractos en los tejidos metabólicos (hígado y grasa) y en la función de barrera epitelial intestinal.
3. Valorar los efectos de los extractos en la composición de la microbiota intestinal.
4. Estudiar el impacto sobre la disfunción endotelial vascular asociada a la obesidad.

METODOLOGÍA

Los extractos objeto de nuestros estudios fueron proporcionados por el Centro Tecnológico de Investigación y Desarrollo del Alimento Funcional (CIDAF), donde se realizó su caracterización química. Todos los protocolos que impliquen experimentación animal fueron aprobados por el Comité de Ética de la Universidad de Granada (Ref. No. 28/03/2016/030).

El modelo experimental de síndrome metabólico utilizado consistió en la inducción de obesidad en ratones mediante la ingesta de una dieta enriquecida en grasa. Para ello, se

usaron ratones C57BL/6J de 5 semanas de edad que consumieron una dieta estándar o una dieta rica en grasa en la que el 60% del aporte calórico provenía de grasa de origen animal. Los ratones se dividieron aleatoriamente en 6 grupos experimentales: grupo control sano (dieta estándar), grupo control sano (dieta estándar) al que se administró la dosis mayor del extracto, grupo obeso (dieta rica en grasa) y 3 grupos obesos (dieta rica en grasa) que fueron tratados con distintas dosis de cada uno de los extractos (*H. Sabdariffa* o *L. citriodora*): 1, 10 y 25 mg/kg. El tratamiento duró 6 semanas. Durante el periodo experimental se midió el peso corporal, así como el consumo de comida y bebida semanalmente. Una semana antes de la finalización del ensayo, se realizó un test de tolerancia a la glucosa. Al final del tratamiento se tomaron muestras plasmáticas y tisulares (grasa epididimal, hígado, intestino y aorta), así como muestras del contenido intestinal. Las muestras plasmáticas se usaron para determinaciones bioquímicas que incluyeron los niveles de glucosa, colesterol (cLDL y cHDL) e insulina. El grado de resistencia a insulina se evaluó con el cálculo de HOMA-IR. Las muestras de tejido adiposo e hígado se utilizaron, tras la extracción de ARN, para evaluar la expresión de diferentes biomarcadores que se ven modificados en un estado de obesidad: marcadores inflamatorios (*Tnf-α*, *Il-1β*, *Il-6*, *Mcp-1*), proteínas implicadas en el metabolismo energético, como la leptina, la proteína transportadora de membrana de glucosa (*Glut-4*), y la proteína quinasa dependiente de AMP (*Ampk*), involucrada en procesos metabólicos. También se extrajo ARN de las muestras intestinales con el objetivo de evaluar la expresión de diferentes marcadores relacionados con la función de la barrera intestinal, como las mucinas *Muc-1*, *Muc-2*, *Muc-3*, o las proteínas *Zo-1*, *Occludin*, y *Tff-3*. La funcionalidad endotelial se evaluó usando segmentos de las aortas de los ratones. Por último, a partir de las muestras fecales se extrajo el ADN bacteriano genómico en su totalidad, y se llevó a cabo la secuenciación de este material genético por pirosecuenciación de amplicones obtenidos a partir de la amplificación del gen 16S del ARNr.

RESULTADOS Y DISCUSIÓN

Los efectos beneficiosos de los extractos se evidenciaron en el modelo de obesidad inducida por dieta rica en grasa (*high-fat diet*-HFD) en ratones.

Extracto de *Hibiscus sabdariffa* (HSE)

Los resultados revelaron que los ratones del grupo control alimentados con una dieta rica en grasa ganaron mayor peso corporal en comparación con los ratones alimentados

con una dieta estándar. Los ratones obesos tratados con HSE redujeron significativamente el aumento de peso, aun siendo la ingesta de energía similar a los ratones obesos no tratados, disminuyendo por tanto su eficiencia energética, lo que excluiría que el extracto ejerciera su efecto por una acción anorexigénica. En lo que se refiere al perfil bioquímico, el tratamiento con el extracto redujo la glucemia basal y la resistencia a la insulina, y mostró una mejoría en el perfil lipídico comparado con los ratones obesos.

Como se comentó en la introducción, la obesidad está asociada con un estado de inflamación sistémica, que afecta tanto al hígado como al tejido adiposo (11, 12). En ambos tejidos, la expresión de las citoquinas pro-inflamatorias *Tnf-α*, *Il-1β*, *Il-6* y *Mcp-1* en hígado, y de *Tnf-α* e *Il-6* en grasa aumentaron en los ratones obesos no tratados, lo que se ha relacionado a una alteración en la vía de señalización de la insulina (13). En lo que se refiere a hígado, la expresión de todos los marcadores mejoró significativamente con las distintas dosis de HSE, mientras que en grasa solo lo hizo la dosis más alta ensayada (25 mg/kg). Además, distintas investigaciones han puesto de manifiesto el importante papel de la ruta de la c-Jun N-terminal kinase (JNK) en la producción de la inflamación en tejidos metabólicos (14). En este sentido, la expresión de *Jnk-1* fue significativamente más alta en ratones obesos del grupo control en comparación con los ratones no obesos; el tratamiento con HSE disminuyó la expresión de *Jnk-1*de forma significativa. Por otro lado, la leptina es una adipoquina secretada por el tejido adiposo, teniendo un papel fundamental en la integración del metabolismo sistémico (15). La expresión de esta adipoquina, así como la de su receptor, se ven alteradas tanto en hígado como en grasa de ratones obesos cuando se compara con los ratones no obesos, mostrando un estado de intolerancia a la leptina, manifestada por hiperleptinemia y reducción de la expresión de sus receptores. La administración del extracto mejora la expresión de estos receptores en hígado, pero no en grasa, aunque la expresión de la adipoquina se redujo significativamente a las dosis más altas ensayadas. Además, se considera que la leptina juega un papel pro-inflamatorio, por lo que la mejora en su expresión podría también explicar la mejora en la disminución de mediadores pro-inflamatorios y resistencia a la insulina (16).

Asimismo, el transportador de glucosa GLUT-4 y la proteína AMPK juegan un papel importante en el metabolismo glucídico, y por lo tanto en la resistencia a la insulina observada en ratones obesos. Además, se ha descrito que AMPK también reprime la

activación de la vía NF-κB, reprimiendo así la expresión de citoquinas pro-inflamatorias (17). Las expresiones de ambos marcadores están disminuidas en los ratones obesos no tratados. En el caso de la primera, la administración de las dosis más altas de HSE incrementaron la expresión de *Glut-4* en grasa hasta los niveles basales obtenidos en el grupo control no obeso. En el caso de *Ampk*, su expresión sólo mejoró en hígado, mientras que en grasa no obtuvo ningún efecto significativo.

De igual forma, la mejora en la expresión de marcadores de función de barrera epitelial del intestino, como *Muc-1*, *Muc-3*, *Zo-1* o *Tff-3* demostraron que HSE es capaz de mejorar la función de barrera intestinal, ya que contrarrestó la menor expresión obtenida en los ratones obesos. En estrecha relación con esto último, la expresión hepática de *Tlr-4*se encontró incrementada en los ratones obesos del grupo control, lo que es indicativo de la existencia de endotoxemia metabólica que se podría desarrollar como consecuencia de la translocación de componentes bacterianos debido una permeabilidad intestinal aumentada en situaciones de obesidad (18). El tratamiento de los ratones obesos se asoció con una disminución significativa de la expresión de *Tlr-4* en hígado.

Por último, la obesidad se ha asociado con una alteración en la composición de la microbiota intestinal, lo que puede considerarse una diana para el tratamiento de la obesidad. De hecho, y en comparación con animales delgados, la obesidad se asocia con un incremento significativo en la relación existente entre los dos principales grupos de bacterias dominantes en el intestino, *Firmicutes* y *Bacteroidetes*, que se representa como F/B y es considerada como un potencial marcador dela situación de disbiosis intestinal (19). Las dosis más altas de HSE fueron capaces de revertir la situación de disbiosis que caracteriza a los ratones obesos.

Extracto de *Lippia citriodora* (LCE)

De forma similar a lo indicado en el estudio anterior con HSE, los ratones no tratados y alimentados con la dieta rica en grasa ganaron mayor peso que los que recibieron una dieta estándar. La administración de LCE redujo significativamente este aumento de peso, aunque no se observaron diferencias en el consumo de alimento, por lo que el efecto estaría relacionado con la disminución de eficiencia energética. Además, la administración de este extracto también mejoró las alteraciones en el metabolismo glucídico y lipídico observadas en los ratones obesos no tratados.

En este segundo estudio también se observa la presencia de un estado inflamatorio subclínico, con un aumento en la expresión de *Tnf-α* and *Il-6* tanto en hígado como en grasa. La administración del extracto mejoró la expresión de los dos marcadores en ambos tejidos, aunque solo la dosis más baja fue capaz de disminuir la expresión de *Tnf-α*. Además, la expresión de *Jnk-1*, relacionada con la estimulación de producción de citoquinas pro-inflamatorias, está también disminuida en ratones obesos tratados con LCE, en comparación con los ratones obesos no tratados. La disminución de estos marcadores inflamatorios también podría estar relacionada con la mejora en la situación de intolerancia a la glucosa asociada a la obesidad.

El estado de resistencia a la leptina también se pudo observar en este estudio, ya que los ratones obesos no tratados presentaron un estado de hiperleptinemia y niveles de expresión de su receptor reducidas, tanto en hígado como en grasa. Esta resistencia a la leptina mejoró con el tratamiento con LCE, pudiendo también contribuir a la mejora del estado de resistencia a la insulina.

La alteración en el metabolismo glucídico asociado a la reducción de la expresión de *Glut-4* también se observa en este segundo experimento, donde los ratones obesos no tratados presentan una expresión reducida, expresión que aumenta con el tratamiento con LCE. En el mismo sentido, la expresión de *Ampk*, relacionada con la alteración en el metabolismo glucídico celular, también está reducida en los ratones obesos no tratados, mientras que aumenta a niveles similares a los ratones sanos en el hígado, y parcialmente en grasa, mejorando la tolerancia a la glucosa.

Al evaluar la expresión de los marcadores asociados con la permeabilidad intestinal, se pudo observar una alteración en la función barrera en los ratones obesos no tratados, con una disminución en la expresión de los marcadores *Muc-2* and *Muc-3, Occludin* y *Zo-1*. La administración de LCE mejoró esta función de barrera intestinal, al incrementar la expresión colónica de estas proteínas, limitando así el acceso de componentes bacterianos al torrente sanguíneo. Esto se pudo corroborar al evaluar la expresión de *Tlr-4*, de forma que el tratamiento a ratones obesos con LCE resultó en la disminución de su expresión en comparación con los obesos no tratados, donde su expresión está aumentada al compararla con la de los ratones no obesos.

En referencia a la alteración de la composición de la microbiota intestinal asociada a obesidad, los ratones obesos tratados con LCE tienen una composición más parecida a aquellos grupos control alimentados con dieta estándar, que al grupo obeso no tratado,

mostrando una restauración en la relación *Firmicutes*/*Bacteroidetes*. Especial atención se ha prestado al papel de *Akkermansia muciniphila* en la obesidad, una bacteria degradadora de mucina cuya abundancia está inversamente relacionada con el peso corporal (20). En este segundo estudio, se observó una menor abundancia del género *Akkermansia* en los ratones obesos no tratados, cosa que se revirtió con las dosis mayores del extracto (10 y 25 mg/kg).

Por último, se sabe que la obesidad está estrechamente relacionada con el desarrollo de alteraciones cardiovasculares, entre ellas la disfunción endotelial (21). De hecho, los anillos aórticos procedentes de los ratones obesos del grupo control mostraron respuestas vasodilatadoras a acetilcolina dependientes del endotelio significativamente menores en comparación con los ratones no obesos, lo que se considera un índice de la disfunción endotelial asociada a obesidad. En este caso, solo la dosis más alta del extracto ensayado fue capaz de aumentar esta respuesta vasodilatadora a acetilcolina en ratones obesos.

CONCLUSIONES

La administración de ambos extractos mostró una mejora en la disminución de obesidad inducida por una dieta rica en grasa, mejorando la resistencia a la insulina, y por lo tanto una mejora en el metabolismo glucídico y lipídico. Estos efectos beneficiosos están relacionados con una reducción en la respuesta inflamatoria sistémica y la restauración de la función de la permeabilidad intestinal alterada que se asocian a la obesidad. La capacidad de modular la microbiota intestinal por parte de los extractos ensayados puede tener un papel determinante en las acciones descritas. Por lo tanto, ambos extractos pueden ser candidatos para su uso futuro como complementos nutricionales en el manejo del síndrome metabólico.

PREFACE

Human feeding and lifestyle habits have undergone several changes during its life on Earth, living the 99% of its existence as a hunter-gatherer. In the prehistoric time, hominids were omnivores, although they were considered to eat more vegetables than meat, so their diet mainly consisted in wild-fruits, stems, and roots. 2 million years ago, the *Australopithecus*, the first hominid, started to walk upright, which enabled them to peak fruits and seeds from trees, and hunt bigger animals, thus expanding their diet. Then, our ancestors started to regularly scavenge and have access to animal fat and protein, including that inside the bone marrow of the long bones of dead animals, which resulted in further development of the brain and in the shortening of the digestive tract. A million years ago, *Homo erectus* "discovered" the fire and with it, the cooking of the food, that supposed a change in their lives and drove evolution. It improved the nutritional value of their diet and had an important hygienic impact, because the bacteria and toxins present in food could be eliminated. The progressive acquisition of technology allowed the human being to expand the hunt, to fish and shells, as well as to develop tools to process vegetables, such as grinding stones and mortars. At that time, the human diet had 37% of the energy derived from protein, 41% carbohydrates, and 22% fat, but, importantly, it had a relationship of favourable polyunsaturated/saturated fats ratio (1.4) and very low cholesterol content. This is called the Palaeolithic diet, which contributed to stabilise the genomic structure of the modern man; in other words, this is the one that best responds to the genetic structure that we inherited from our ancestors. Then, two critical events happened. The first took place 11,000 years ago, with the arrival of the agriculture and livestock; which promoted the dependence on cereals that contributed approximately to 90% of the diet, and hence an impoverishing of the diet, which contained very small amounts of animal protein. The second, in the mid-18th century, when industrial revolution made available the consumption of new types of foods, including refined sugars and vegetable oils. This was linked to an increase in energy consumption and a decrease in energy expenditure, as well as a reduction in the intake of fibre and complex carbohydrates. Of note, these last changes in diet have happened in a very short period of time compared with all the feeding history of the human being, without parallel changes in the genetic structure. This phenomenon is known as "evolutionary discordance". In terms of genetics, the modern man lives in a nutritional environment that differs from that for which our genetic constitution was selected. On the last decades, the wide availability of cheap sources of energy and sedentary lifestyle have revealed a susceptibility to current epidemics of

chronic diseases, such as obesity, diabetes, hypertension, dyslipidemia or different types of cancer, set of diseases encompassed in the term of **Metabolic Syndrome**.

INTRODUCTION

THE METABOLIC SYNDROME

Metabolic syndrome (MetS) is one of the main public health problems of our time, and it is defined by its related metabolic dysfunctions, such as obesity (specially visceral adiposity), hypertension, insulin resistance, glucose intolerance, dyslipidemia (high levels of triglycerides (TG) and low levels of high-density lipoprotein (HDL)-cholesterol) and atherosclerosis, which contribute to increase the incidence of cardiovascular diseases (CVD), Type 2 Diabetes Mellitus (T2DM) and cancer (2).

1. Concept

MetS was first described 80 years ago, when the physician Eskil Kylin observed an usual co-occurrence of hypertension and diabetes mellitus in adults, suggesting a shared mechanism for the appearance of both diseases (22). Years after, in 1947, Jean Vague described an association of visceral obesity with metabolic abnormalities related to CVD and T2DM (23). It was in 1988 when Gerald M. Reaven first introduced the concept of insulin resistance as a base for CVD and T2DM, naming it as Syndrome X (24). Reaven did not include visceral obesity in his definition, but in 1989, Norman Kaplan defined "the deadly quartet", which resulted from the combination of visceral adiposity, glucose intolerance, hypertriglyceridemia and hypertension (25). It was soon after reappointed as insulin resistance syndrome, since many authors considered that insulin resistance was the basic pathophysiological mechanism (26). Thereafter, although the term MetS is widely used to refer to this syndrome, many organisms have defined it in a different way, which are described and summarized in Table 1. The World Health Organization (WHO) was the first to describe the MetS (27). They considered insulin resistance as the central metabolic dysfunction to develop MetS, evidenced by Impaired Fasting Glucose (IFG) levels above 100 mg/dl and/or Impaired Glucose Tolerance (IGT) (glucose levels above 140 mg/dl for 120 minutes after ingestion of 75 grams of glucose load during an oral glucose tolerance test), and/or high HOMA-IR value. Apart from insulin resistance, two of these criteria had to be present: central obesity, dyslipidaemia, hypertension or microalbuminuria. One year after, The European Group of Insulin Resistance (EGIR) redefined MetS (28), by considering insulin resistance as a central metabolic dysfunction, and in which fasting plasma insulin levels should be higher than the 75th percentile, plus two additional criteria among obesity, dyslipidemia and hypertension. Later on, in 2001, the National

Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) did not consider insulin resistance as a central dysfunction (29); instead, three of the next five criteria should coincide: waist circumference over 100 cm (men) or 90 cm (women), blood pressure over 130/85 mmHg, fasting TG levels above 150 mg/dl, fasting HDL-cholesterol levels under 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar above 100 mg/dl. This is the definition most commonly used for MetS. In 2005, the International Diabetes Foundation (IDF) published new criteria for MetS. In general, it includes the same criteria as others, but obesity is required to be present, instead of insulin resistance (30).

Table 1: Criteria to define the MetS depending on different organisms

	NCEP ATP III	**WHO**	**EGIR**	**IDF**
Absolutely required	None	Insulin resistance* (IGR, IFG, T2D, or other evidence of IR)	Hyperinsulinemia♯(plasma insulin >75th percentile)	Central obesity (waist circumference§): ≥ 94 cm (M), ≥80 cm (F)
Criteria	Any three of the five criteria below	Insulin resistance or diabetes, plus two of the five criteria below	Hyperinsulinemia, plus two of the four criteria below	Obesity, plus two of the four criteria below
Obesity	Waist circumference: >40 inches (M), > 35 inches (F)	Waist/hip ratio: >0,90 (M), >0,85 (F); or BMI >30 kg/m^2	Waist circumference: ≥94 cm (M), ≥80 cm (F)	Central obesity already required
Hyperglycemia	Fasting glucose ≥100 mg/dl or Rx	Insulin resistance already required	Insulin resistance already required	Fasting glucose ≥ 100 mg/dl
Dyslipidemia	TG ≥ 150 mg/dl or Rx	TG ≥ 150 mg/dl or HDL-C: >35 mg/dl (M), > 39 mg/dl (F)	TG ≥ 177 mg/dl or HDL-c > 39 mg/dl	TG ≥ 150 mg/dl or Rx
Dyslipidemia (second separate criteria)	TG ≥ 150 mg/dl or Rx	TG ≥ 150 mg/dl or HDL-C: <35 mg/dl (M), < 39 mg/dl (F)	TG ≥ 177 mg/dl or HDL-C <39 mg/dl	HDL cholesterol: < 40 mg/dl (M), <50 mg/dl (F); or Rx
Hypertension	>130 mmHg systolic or > 85 mmHg diastolic or Rx	≥ 140/90 mmHg	≥ 140/90 mmHg or Rx	>130 mmHg systolic or >85 mmHg diastolic or Rx
Other criteria	-	Microalbuminuria$^+$	-	-

+ Urinary albumin excretion of ≥ 20 ug/min or albumin-to-creatinine ratio of ≥ 30mg/g.

♯ Reliable only in patients without T2D.

§ Criteria for central obesity (waist circumference) are specific for each population; values given are for European men and women.

Rx pharmacologic treatment.

2. Epidemiology

During the past few decades, the number of people with MetS has augmented worldwide, associated with the global epidemic of obesity and diabetes. It is estimated that over a 1,4 billion people in the world, a quarter of the population, have MetS (1); although the prevalence estimates vary since it depends on the inclusion criteria, as well as on other factors like the characteristics of the population (sex, age, race or ethnicity). Furthermore, lifestyle and socioeconomic factors may influence the prevalence across sex, age, and race/ethnicity cohorts.

While in many countries the prevalence rates of MetS are very similar in women and men, there are some where it is more frequent in women (31-33), and others where the men prevalence is higher (34, 35). But even in studies with participants in the same age-groups, there is great variation between the two genders: for example, in studies that comprise people over 20 years old, the prevalence goes from 8% in India to 24% in USA in men, and from 7% in France to 43% in Iran in women (36). Different factors can explain these discrepancies, including dissimilar socioeconomic status, work-related activities, and cultural views on body fat. Regarding age, and as expected, MetS prevalence grows with it, as there is an increment in obesity, and, specially, in central obesity. For instance, in Iran, the prevalence in the 20-29 year age group is less than 10% for both genders, but it increases to 38% and 67% in men and women, respectively, in the 60-69 year age group (37). Of note, it has been reported more and more higher rates of obesity in youngers, which is associated with earlier onset of the disease; in fact, T2DM and the MetS can be evident even in childhood (38-40). Currently, in USA, 1 in 10 children under the age of 5 is obese (41). Moreover, obesity-associated morbidity is occurring at earlier ages, and this could be an important predictor of future risk of adult diseases including atherosclerosis, CVD, cancer and T2DM (42). This situation is very alarming so appropriate preventative measures should be undertaken.

Sex and age associated differences in MetS also depend on race and ethnicity-related variances. For example, the effect of ethnic origin on the MetS can be observed in the Chinese population, which has a multi-ethnic population. Korean and Hui ethnicities have higher prevalence of MetS, while the Tibetan ethnicity shows a lower one (43). Beside this, very little studies have assessed the impact of socioeconomic status, tobacco, alcohol, and level of education in MetS prevalence. However, it is evident that

it is higher in developed countries, sedentary people, smokers, low-socioeconomic status groups, and people with unhealthy dietary lifestyles (44).

3. Etiopathogenesis

The etiopathogenesis of the MetS is complex and many factors may be involved. It is evident that genetics and environmental factors contribute to its aetiology (45, 46). In this sense, two factors are decisive for its development: weight increase, as a consequence of unhealthy lifestyle, and predisposition to accumulate intra-abdominal fat, including in liver, pancreas, heart and other organs (30, 47). On the other hand, MetS is considered as a state of sub-clinic inflammation derived from obesity or overweight, which is a key factor for the development of insulin resistance (48, 49). Finally, insulin resistance would be the trigger for comorbidities associated with MetS, such as hypertension, glucose intolerance, dyslipidemia and atherosclerosis (50). Anyway, the specific causes conditioning the grouping of metabolic dysfunctions are still unknown, being all dysfunctions related and contributing independently to the development of MetS.

4. Central features of the MetS

The current definitions of MetS consider five main characteristics: insulin resistance, glucose intolerance, visceral obesity, dyslipidemia and endothelial dysfunction. Nevertheless, low-grade systemic inflammation appears to also play an important role in the pathophysiology of this condition.

4.1. Insulin resistance

Insulin resistance is the most recognized and consolidated hypothesis to explain the pathophysiology of MetS. Insulin exerts different biological effects: boost muscle and fat glucose uptake, muscle and liver synthesis of protein and glycogen, and lipid synthesis and storage in liver and fat, restrains fatty acid oxidation, glycogenolysis and gluconeogenesis (51). Thus, insulin increases glucose uptake reducing circulating glucose and lipid levels and increasing its conversion into glycogen or fat (52). When insulin resistance occurs, adipose, muscle and liver cells response to insulin is defective, so the plasma levels of glucose and lipids remain elevated. The mechanisms behind insulin resistance can be found at different cellular levels, from the membrane to the nucleus, and can include insulin receptor (IR) desensitization, inhibition of functionality

or expression of Insulin Recepor Substrate (IRS)-1, suppression of Phosphoinositide 3-Kinase (PI3K) cascades, and deregulation of Foxo1 transcriptional activity, which may inhibit IR (53). Although, traditionally, insulin resistance has been explained with a glucocentric view, one of the key factors for the development of insulin resistance is an excess of circulating fatty acids. One of the principal actions of insulin is lipolysis inhibition, but once insulin resistance is developed, lipolysis is not inhibited and the levels of circulating lipids increases, thus generating a vicious circle with the consequent worsening of the metabolic function (54).

Physiological insulin signalling is mediated by insulin binding to the IR, which phosphorylates and displays binding sites for numerous signalling patterns. Then, there is a parallel and balanced activation of the PI3K-Akt pathway (metabolic arm) and the Ras-MAPK pathway (mitogenic arm), which stimulates endothelial cell growth and metabolism and improves the vascular function. The metabolic effects consist on glucose transport, glycogen and protein synthesis, protection from apoptosis, oxidative stress and inflammation, and inhibition of lipolysis. Regarding the non-metabolic properties, they comprise proliferative, mitogenic, pro-inflammatory and pro-atherogenic effects. In an insulin resistance setting, PI3K-dependent signalling is impaired while the MAPK (mitogen activated protein kinase) pathway is unaffected, which triggers a compensatory hyperinsulinemia to keep euglycemia. As a consequence, the mitogenic arm is overstimulated, which has a negative impact on the cardiovascular and endothelial tissue contributing to its dysfunction (55) (Figure 1).

4.2. Hyperglycaemia

As mentioned above, insulin-signalling impairment comprises failures of the hormone to reduce liver and kidney gluconeogenesis, or promote glucose uptake and metabolism in insulin sensitive tissues (ie, muscle and adipose tissue). It also induces compensatory mechanisms in the pancreatic beta-cells to normalize circulating glucose levels that consist on greater secretion of insulin by an increase in beta-cell function and mass (hyperinsulinemic state) (56). In the long term, pancreatic beta-cells become dysfunctional, both for the hyperactivity to try to maintain normoglycemia and for the lipotoxic effect of free fatty acids, which leads to accumulation of long chains of Acyl-Coa, then to apoptosis and, finally, hyperglycemia (57). Moreover, as free fatty acids (FFA) can trigger insulin secretion, the continued exposure to elevated concentrations reduces it (58).

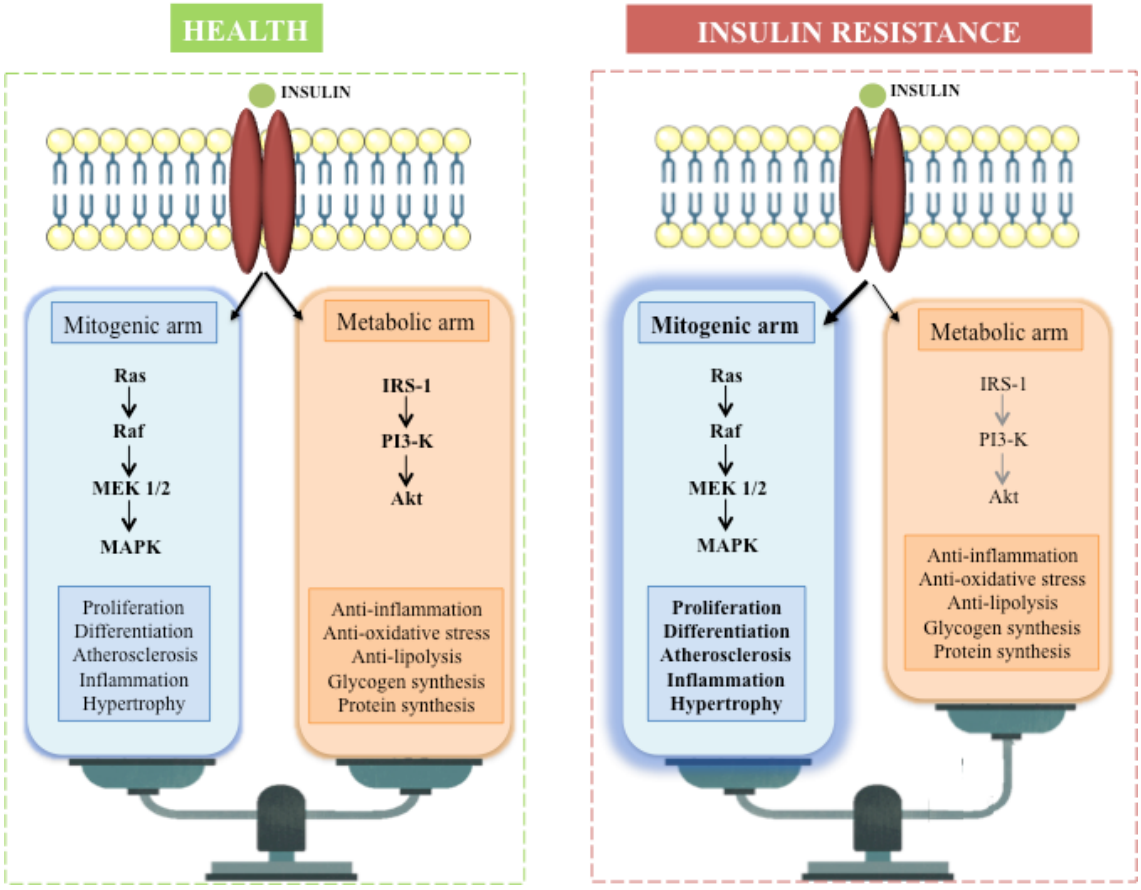

Figure 1: Insulin signalling pathway, and its impairment in insulin resistance. Normally, insulin receptor is activated by insulin, which results in parallel and balanced activation of PI3K-Akt (metabolic arm) and Ras-MAPK (mitogenic arm) pathways **(Left side)**. "Metabolic" effects are related to glucose transport inside the cell, protein and glycogen synthesis, and anti-apoptotic, anti-oxidative, anti-inflammation, and anti-lipolysis effects. On the contrary, "mitogenic" refers to proliferative, pro-atherogenic and pro-inflammatory effects of insulin. When insulin resistance appears, there is impairment in insulin signalling, specifically in PI3K-dependent signalling (metabolic arm), while the other is unaffected. The compensatory hyperinsulinemia leads excessive stimulation of the unaffected mitogenic arm, which contributes to CV and endothelial injury and dysfunction **(Right side)**. Adapted from (55).

4.3. Visceral adiposity

Visceral adiposity has been long included in different definitions of MetS (27-29). The abnormal high deposition of visceral adiposity is clearly related to insulin resistance, since normal weight patients with high visceral adiposity can also show insulin resistance (59). Furthermore, visceral adiposity has been distinctly linked to other pathological conditions, like increased risk of colon, breast or prostate cancers (60). It is well known that fat tissue is a hormonally active component of the human body, by involving the altered production and release of adipokines, which can also determine a greater cardiovascular risk profile and thus increasing the susceptibility to ischaemic heart disease and arterial hypertension (61). In fact, MetS has been associated with increased production of tumour necrosis factor-α (TNF-α) and interleukin (IL)-6 from

adipose tissue, two pro-inflammatory cytokines that contribute to insulin resistance and vascular dysfunction (62, 63). Moreover, the renin-angiotensin system is also stimulated in adipose tissue, which produces hypertension and insulin resistance (64). However, the levels of adiponectin, a protective adipokine that couples insulin sensitivity with energy metabolism, are decreased (65). In addition to these adipokines, FFAs, which are released from visceral fat, harm the PI3K-Akt pathway and elevate oxidative stress (66).

4.4. Dyslipidemia

Insulin resistance and visceral adiposity go usually together with high plasma TG levels, low HDL-cholesterol levels and an increase in small dense low density lipoprotein (LDL)-cholesterol, which are common features of atherogenic dyslipidemia (67).

Insulin has a complex effect on the lipid metabolism. In normal conditions, insulin suppresses lipolysis in adipocytes, so insulin resistance would result in increased FFA levels. The augmented flux of FFAs to liver enhances the production of ApoB containing triglyceride-rich very low-density lipoproteins (VLDL), which are metabolized to remnant lipoproteins and small dense LDLs, able to promote atheroma formation. Moreover, insulin impairs ApoB through PI3K-dependent pathways in physiological conditions, whereas insulin resistance directly increases VLDL production, as well as reduces the concentrations of lipoprotein lipase in peripheral tissues, which is the major mediator of VLDL clearance (50) (Figure 2).

The other main lipoprotein alteration in the MetS is the decrease in HDL-cholesterol. In normal conditions, HDL captures phospholipids and cholesterol for its clearance in the liver. Cholesterol ester transport protein (CETP), redistributes cholesteryl esters, triglycerides and to a lesser extent, phospholipids between plasma lipoproteins. When a dysfunctional adipose tissue causes hypertriglyceridemia, the TGs from VLDL are transferred to HDL particles in exchange for cholesterol esters, to equilibrate lipids between lipoprotein fractions. This leads to an increase in TG-enriched HDL particles, and an increase in cholesterol-enriched VLDL particles. Then, the TGs on both VLDL and HDL particles are hydrolysed by hepatic lipase and lipoprotein lipase, producing smaller dense lipoprotein particles (68) (Figure 2).

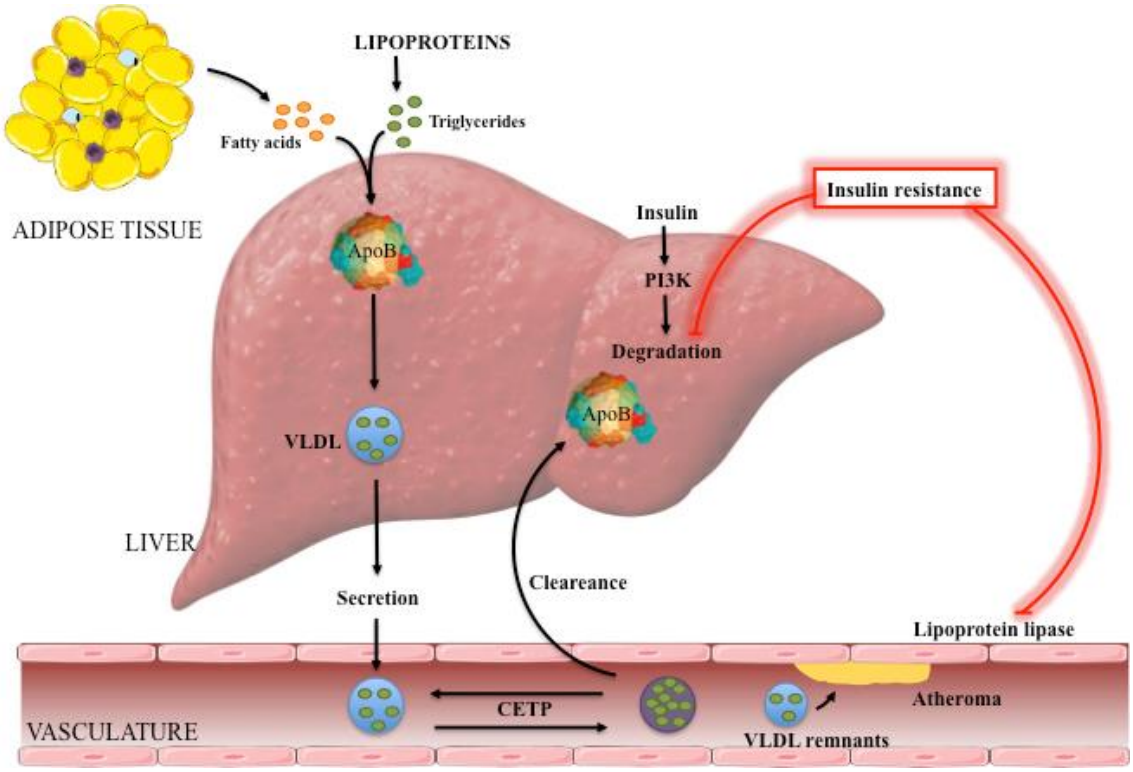

Figure 2: In an insulin resistance state, there is an increase in FFA levels, as the suppressive effect of insulin on lipolysis in adipocytes is lost. Thus, FFAs are transported to the liver. The high availability of lipids in liver, along with the impaired insulin signalling that normally degrades ApoB, increases the production of VLDL particles (rich in TGs). In insulin resistance, lipoprotein lipase activity is also decreased, that is found in the endothelium of peripheral capillaries and essential for the clearance of TG-rich lipoproteins. VLDL is metabolized to remnant VLDL and LDL, rich in cholesterol, which can promote atheroma formation. Moreover, increased VLDL particles affects HDL metabolism, as triglycerides from VLDL are transferred to HDL through cholesteryl ester transfer protein (CETP), and backwards, the cholesterol from HDL to VLDL. Thus, there are more cholesterol-rich small density lipoproteins (VLDL and LDL), and TG rich HDL. Furthermore, TG-rich HDL particles are more rapidly hydrolysed, leaving fewer HDL particles to clear cholesterol, further contributing to atheroma plates. Adapted from (68).

4.5. Endothelial dysfunction

Endothelial dysfunction is defined as a failure of the endothelium to perform its physiological and protective roles. The functionality of the endothelium depends on the activation of the PI3K-Akt pathway (metabolic arm) and the Ras-MAPK pathway (mitogenic arm), which stimulate cardiovascular and endothelial growth, cell metabolism and healthy vascular function. The metabolic arm may be compromised by different factors, including oxidative stress, hyperglycemia, increased levels of FFAs and inflammatory cytokines or adipokines (69).

A key element of endothelial dysfunction is limited bioavailability of Nitric oxide (NO) in the vasculature, the major responsible of vascular tone, which can occur in insulin resistance, when the PI3K-Akt pathway is inhibited, resulting in diminished

endothelium Nitric oxide Synthase (eNOS) phosphorylation and activity (70). By contrast, the MAPK pathway is unaffected, so insulin mediated endothelin-1 (ET-1) expression, a potent vasoconstrictor peptide, and vascular smooth muscle mitogenic effects are not compromised, further contributing to endothelial dysfunction (71, 72).

Visceral adiposity promotes endothelial dysfunction through a signalling pathway mediated by resistin, IL-6 and TNF-α that phosporylate eNOS. Moreover, TNF-α activates NADPH oxidase by blocking IRS-1 activation (73), promoting superoxide generation (74); and also boots lipolysis and, thus, FFA release. Regarding to the two main hormones produced by adipose tissue, adiponectin, which facilitates eNOS phosphorylation, is reduced in MetS, and leptin, which is increased as a consequence of the leptin resistance that occurs in obesity, upregulates endothelial eNOS expression, increasing the generation of NO by promoting the secretion of pro-inflammatory cytokines. Leptin has also been reported to increase the production of vasoconstrictor ET-1, primarily in endothelial cells, causing a rise of the blood pressure (75).

4.6. Pro-inflammatory state

It is well documented the association between MetS and a chronic inflammatory state (3). This inflammatory state does not fit into the classical definition of inflammation because there is no massive tissue injury, but there are increased levels of inflammatory molecules, like C-reactive protein, TNF-α, plasma resistin or IL-6 (76).

The inflammation in obesity is linked to macrophage infiltration in the adipose tissue, thus resulting in the release of cytokines and promoting systemic inflammation (77). These cytokines can prompt insulin resistance in different target organs (78), impair the pituitary-adrenal axis and rush the loss of pancreatic beta-cells (79). This insulin resistance effects boost inflammation by increasing FFA levels that hampers the insulin anti-inflammatory effects (80). Furthermore, the low-grade inflammation in the atherosclerotic lesions can elevate the probability of plaque rupture that can lead to acute CVD (81).

5. Disorders associated with MetS

Several conditions that accompany MetS should be considered in detail. Some of them are directly related to the intrinsic excess of adiposity and insulin resistance linked to MetS. Obviously, T2DM and CVD are directly linked to the MetS, since the risk of developing them is directly related to the central features of the metabolic syndrome.

5.1. Non-alcoholic fatty liver disease (NAFLD)

NAFLD is characterized by a collection of pathological features that go from mild steatosis, to non-alcoholic steatosis, hepatitis and cirrhosis. It has been proposed that 95% of obese people (82) and more than 70% of T2DM patients suffer from some kind of NAFLD (83). Worryingly, the prevalence of NAFLD is also elevated in children with obesity and insulin resistance (84). Moreover, NAFLD is a robust predictor of MetS (85), and liver fat is associated with all the components of MetS (86). Thus, in MetS patients liver fat content is significantly augmented (up to 4-fold) compared to healthy individuals (87).

5.2. Obstructive sleep apnoea (OSA)

Obstructive sleep apnoea (OSA) could be a dangerous consequence of obesity and is related to elevated body mass index (BMI). Additionally, there is a link between OSA and insulin resistance (88), systemic inflammation (89) and reduced adiponectin concentrations (90, 91). These patients with OSA have more probabilities to display features of MetS than those without OSA, even after adjusting for obesity (92). Furthermore, sleeping disorders are generally linked to weight gain and insulin resistance (93); and some authors have also proposed that OSA should be taken as a symptom of MetS (94, 95).

5.3. Polycystic ovarian syndrome (PCOS)

POS is a clinical syndrome related to anovulation, androgen excess and insulin resistance. PCOS and MetS often co-occur, especially in obese women. Insulin resistance and obesity are regularly described in women with PCOS and could increase their risk for CVD and metabolic disorders (96). More than 60% of women with PCOS show glucose intolerance and have, consequently, an elevated risk for diabetes (97, 98) and CVD risk factors (99).

5.4. Hypogonadism

Male gonadal and erectile dysfunctions have been associated with greater risk for MetS, as well as women with PCOS. Thus, men with MetS show higher prevalence of hypogonadism while hypogonadism is a risk factor for the development of MetS and T2DM (100). Additionally, MetS has been reported to be independently linked to a higher prevalence of erectile dysfunction (101).

6. Obesity, inflammation and MetS

As commented above, one of the main components of MetS is obesity, especially visceral adiposity. It is well known that overweight and obese patients show a chronic low-grade inflammatory status. Besides, studies in mice and humans have evidenced that consumption of specific nutrients may induce an inflammatory response. In consequence, the starting signal in obesity-associated inflammation may be continuous overfeeding, which affect the functionality of different tissues involved in metabolism, like adipose tissue, liver and muscle (102, 103). Then, the high circulating concentrations of pro-inflammatory cytokines seem to have a central function promoting insulin resistance (104). In fact, the degree of inflammation is linked to the severity of insulin resistance and the onset of T2DM (105, 106). All these evidences suggest that the better understanding of the inflammatory response that occurs in this condition could drive the development of novel approaches for treating obesity, MetS and the associated metabolic disorders.

Chronic inflammation typically presents three stages (105): an initial trigger, usually some kind of tissue stressor, followed by an acute, adaptive inflammatory response, and finally a long-term maladaptive phase, which triggers the typically associated complications. In obesity, the starter could be the homeostatic stress derived from a positive energy balance and a hyperanabolic state, principally in adipocytes, which react liberating chemokines. They launch an adaptive inflammatory response that enables a healthy expansion of adipocytes and reduces energy storage, which compromises homeostasis. Thus, with time, the homeostasis cannot be maintained and only improving weight, blood levels of glucose, hormones and lipids, and the sympathetic tone could help. In addition, other changes have deleterious effects, including reduced metabolic flexibility, long-term insulin resistance, abnormal tissue remodelling and fibrosis (107).

7. Adipose tissue

Mammals have two very well distinguished types of adipose tissue, the white adipose tissue (WAT) and the brown adipose tissue (BAT). When considering its location, adipose tissue is present either as visceral or subcutaneous fat. While BAT is specialized in heat production (thermogenesis), WAT adipocytes store energy as triglycerides. BAT adipocytes contain numerous small lipid droplets in the cytoplasm

that make them multilocular and the triglycerides are easily accessible for hydrolysis and oxidation of fatty acids. However, WAT adipocytes are unilocular and enclose one single lipid droplet that occupies almost all the cell, dislocating the cytoplasm, nucleus and other organelles to the perimeter (108). Lipids constitute an efficient form of energy storage due to two reasons: first, the substantial greater caloric value of lipids in comparison with carbohydrates and, second, TGs can be stored with little associated water, in contrast to carbohydrates. Structurally, BAT adipocytes have many big mitochondria that contain high amounts of thermogenic uncoupling protein 1 (UCP1), which participates in fatty acid oxidation and heat production (109). This type of non-shivering thermogenesis is the result of adaptation to cold climates in many homeotherms, but in humans, BAT can only be found in foetus and children until adolescence, when the major amount of BAT converts to WAT (110). Therefore, WAT is the major adipose tissue type present in humans.

7.1. Adipose tissue as an endocrine organ

The adipose tissue can be considered as an endocrine organ since the adipocytes produce numerous adipokines that affect the function of the central nervous system and other metabolic tissues that maintain the body energy homeostasis (111). These adipokines are considered hormones that can act both locally, in autocrine and paracrine ways, and also in an endocrine form, affecting the rest of the body (112). Interestingly, different adipokines have been associated with insulin resistance and MetS, which are commented below (Table 2).

7.1.1 Leptin

Leptin is a protein mainly produced by adipocytes proportionally to the adipose tissue mass (113). When it is secreted, it circulates in plasma and enters by diffusion into the central nervous system, where acts as satiety signal on hypothalamus, thus reducing energy intake and increasing energy expenditure (114). The main determinant of leptin secretion is glucose metabolism, as its circulating levels are reduced under fasting or caloric restriction, and raises after food intake (115). But leptin secretion is also regulated by other different factors, as glucocorticoids, TNF-α, estrogens, and declines by androgens, FFAs, growth hormone and peroxisome proliferator activated receptors (PPAR)γ agonists (116).

In order to reduce energy intake and induce energy expenditure, leptin inhibits lipogenesis and prompts lipolysis (117), decreasing intracellular lipids in skeletal muscle, liver and pancreatic beta-cells, thus enhancing insulin sensitivity (118). On the other hand, obesity is associated with increased leptin levels and hyperleptinemia, which is a reflection of the leptin resistance linked to obesity (119). The mechanisms for leptin resistance are not well understood, but may be associated with defects in leptin signalling or transport through the blood-brain barrier (120).

Other important endocrine leptin-associated effect consists on regulation of the immune function. Actually, it is generally accepted that leptin functions as a pro-inflammatory adipokine; indeed, leptin boots monocyte TNF-α and IL-6 production (121), and also the production of reactive oxygen species (ROS) and cell proliferation and migratory responses (122). Also, as a loop, leptin secretion is increased by TNF-α and lipopolysaccharides (LPS) (123).

7.1.2. Adiponectin

Adiponectin is a protein hormone with antiatherogenic, anti-inflammatory and insulin sensitizing properties mainly produced by adipocytes (124). It is inversely related to obesity, diabetes and other states that cause metabolic dysfunction (65). Thus, differently from leptin, its concentration is decreased in obese people. Moreover, the same is observed in patients with coronary artery disease, T2DM and essential hypertension (125). Adiponectin receptors have been described in skeletal muscle, liver and endothelial cells. In liver, it suppresses the expression of several gluconeogenic enzymes and decreases endogenous glucose production, resulting in lower fasting plasma glucose levels (126, 127). In muscle, adiponectin increases muscle fat oxidation and glucose transport via Amp-activated protein kinase (AMPK) pathway (65). Adiponectin has also vasculoprotective properties, reducing adhesion molecules (VCAM-1; ICAM-1; E-selectin) expression in endothelial cells in the presence of inflammatory stimuli like TNF-α (128). Thus, low plasma concentrations of adiponectin may be a key mechanism that relates obesity to hypertension and atherosclerosis.

7.1.3. IL-6

IL-6 is one of the principal pro-inflammatory mediators mainly secreted by immune cells. Of note, 20-30% of the circulating IL-6 is generated by the adipose tissue, and in obese individuals its participation is even greater (129). IL-6 adipose tissue expression

and circulating levels are positively linked to obesity, impaired glucose tolerance and insulin resistance (130). Furthermore, plasma IL-6 levels can be used as a predictor of T2DM and CVD (131), as its peripheral administration promotes hyperlipidemia, hyperglycemia and insulin resistance both in animal models and humans (132). Moreover, it should be pinpointed that IL-6 induces fibrinogen production and platelet activity, which augments clot formation risk, and thus the cardiovascular risk (133).

7.1.4. TNF-α

TNF-α is mainly produced by the macrophages that infiltrate the fat tissue (134). Several mechanisms for the metabolic effects exerted by TNF-α have been described. For instance, in adipose tissue, TNF-α inhibits different genes responsible for the uptake and storage of glucose and non-esterified fatty acids, transcription factors implicated in adipogenesis and lipogenesis, as well as modifies the expression of several adipocyte-secreted factors, including adiponectin and IL-6. In liver, it suppresses the expression of genes involved in glucose uptake and metabolism, as well as fatty acid oxidation, and increases the expression of genes participating in de novo synthesis of cholesterol and fatty acids. Moreover, TNF-α impairs insulin signalling by activating serine kinases that promote serine phosphorylation of IRS-1 and -2, reducing their affinity for insulin receptor kinases and raising their degradation. Additionally, it indirectly hampers insulin signalling by augmenting serum non-esterified fatty acids, which have been reported to prompt insulin resistance in different tissues (135).

7.1.5. IL-1β

IL-1β is another pro-inflammatory cytokine, which is involved in obesity, as its levels are increased in overweight and obese people (136). Moreover, a combined increase in levels of IL-1β and IL-6 is a predictive of developing T2DM (137). It has been shown to increase the expression of IL-6 in adipose tissue, and also the release of the chemokine monocytes chemoattractant protein-1 (MCP-1) (138).

7.1.6 MCP-1

MCP-1 is also released by hypertrophied adipocytes, promoting macrophage infiltration and monocyte influx to the obese adipose tissue (139).

Table 2: Adipocytokines and their effects.

Adipokine	Production site	Effect on inflammation	Function
Leptin	Adipose tissue	Pro-inflammatory	Regulation of energy intake and expenditure. Regulation of fat storage and insulin signalling
Adiponectin	Adipose tissue	Anti-inflammatory	Insulin sensitizing. Improvement of glucose metabolism
TNF-α	Adipocytes and macrophages	Pro-inflammatory	Increases insulin resistance, impairing glucose metabolism. Stimulates lipolysis.
IL-6	Adipocytes and macrophages	Pro-inflammatory	Increases insulin resistance, impairing glucose metabolism.
IL-1β	Adipocytes and macrophages	Pro-inflammatory	Impairs insulin signalling in combination with IL-6
MCP-1	Adipocytes and macrophages	Pro-inflammatory	Increases macrophage recruitment, and thus inflammation. Also increases insulin resistance

7.2. WAT hypertrophy and hyperplasia

It is well known that obesity results from a situation of energy imbalance (more calories consumed than expended), causing fat mass growth to store the excess energy (140). This is invariably associated with greater adipocyte size (hypertrophy) and number (hyperplasia). Initially, the excess energy is stored as fatty acids in the adipose tissue, which are used as a source of energy when there is a negative energy balance, by inhibiting lipid storage and releasing fatty acids. In consequence, adipocyte lipid uptake, esterification, and TG storage favour adipocyte expansion (hypertrophy), which is an initial positive adaptive response to over nutrition that limit ectopic lipid deposition and lipotoxicity (141). Moreover, the lipid droplet actively maintains systemic energy homeostasis occupying most of the space of the adipocyte and favouring the contact with the endoplasmic reticulum and the mitochondria where the TG are esterified and hydrolysed respectively (141). If the positive energy imbalance continues, the adipose tissue mass keeps expanding (hyperplasia), and the lipid droplets become hypoxic (142). The limited availability of oxygen, especially for those cells that are distant from the capillaries, leads to derangements in lipid metabolism: FAs are redirected to the

liver causing raise of plasma FFAs, TGs and small dense low-density lipoprotein, as well as decrement of high density lipoproteins (143). According to *in-vitro* studies, hypertrophic adipocytes release factors, such as TNF-α and Insulin-like growth factor (IGF)-1, stimulating adipocyte hyperplasia in a paracrine fashion (144). Various transcription factors have also a role in hyperplasia, influencing differentiation of preadipocytes to functional mature adipocytes, including PPARγ, a key nuclear receptor that favours adipocyte hyperplasia, and contribute to fat redistribution and decrement of adipose size (145). On the other hand, PPARγ has also an important role as a whole-body insulin sensitizer (146). Actually, thiazolidinediones are PPARγ agonists used in diabetes treatment that clearly improve insulin sensitivity as well as enhance adipocyte differentiation, although they can induce weight increase (147). Thus, the development of partial agonists of PPARγ, or agonists that stimulate the insulin sensitizer role but not the adipogenic role of PPARγ is an important goal of the antidiabetic therapy. Moreover, it seems that the activation of PPARγ has beneficial effects in metabolic parameters when a metabolic disease occurs, but in healthy subjects the inhibition of PPARγ seems to have antiobesity effects (146).

As mentioned before, hypertrophy precedes hyperplasia to handle the excessive energy intake, but when it is exceeded, hyperplasia occurs (148). It must be noted that several studies have pointed out that adipocyte number is determined during childhood, i.e., number of adipocytes in childhood and adulthood is maintained, which underlines the importance of preventing childhood obesity (149). Interestingly, when weight loss occurs, there is a reduction in adipocyte volume (hypertrophy), but not in adipocyte number (hyperplasia), which remains the same (150). Thus, adipose tissue expanded by hyperplasia will be maintained, making difficult to sustain weight loss with time, and thus worsening the prognosis for the treatment. That is the reason of the importance of preventing adult childhood.

7.2.1. Adipose Tissue Macrophages: The Main Source of Obesity-Associated Inflammation

It is well accepted that the adipose tissue generates large amounts of inflammatory cytokines and chemokines, all termed adipokines, including TNF-α, IL-1β, IL-6, and MCP-1, and being the principal contributor to the raise in circulating TNF-α in obesity (151). Interestingly, in obesity, there are more signs of inflammation in the visceral

adipose tissue than the in subcutaneous adipose tissue depots (142), consistent with the negative impact of visceral adipose tissue (VAT) expansion on insulin sensitivity (152).

Of note, macrophages are the major source of inflammatory mediators within murine and human adipose tissue, despite the contribution of other cells types, such as adipocytes, preadipocytes, vascular endothelial cells, T-lymphocytes, and the mesothelial cells (4). Indeed, an important breakthrough for the understanding of obesity-associated inflammation was the observation that the higher pro-inflammatory adipokine levels in obesity was linked to more macrophage infiltration in the adipose tissue (153). Macrophages constitute 40% of the adipose tissue cells in obese mice, but 10% in lean mice, which shows that the ratio macrophages: adipocyte is altered in obesity (153-155). Moreover, these macrophages display different localization and inflammatory potential in obese and lean animals (156). In lean animals, adipose tissue macrophages present an alternatively activated (M2) profile, which are less inflammatory than classical (M1) phenotype, and they are evenly scattered throughout the adipose tissue. However, in obese mice macrophages are mainly M1 type and usually form crown-like structures around dying adipocytes (157). Actually, it has been described that the appearance of necrotic adipocytes is linked to macrophage infiltration in the adipose tissue and the onset of insulin resistance in obese mice (158) (Figure 3).

7.2.1.1 Classically activated macrophages (M1)

It is well described that, in obesity, macrophages are recruited to the adipose tissue, promoting inflammation as well as insulin resistance (154). Dietary saturated fatty acids are able to trigger Toll-like receptor (TLR)-2 and TLR-4 in these macrophages, which stimulate different inflammatory signalling cascades, including interferon regulatory factor 4 (IRF4), activator protein 1 (AP1) and nuclear factor-κB (NF-κB), that promote the release of TNF-α, IL-1β and other pro-inflammatory cytokines. These prevent adipocytes insulin response by inhibitor IKKB and c-jun N-terminal kinase (JNK) signalling pathways activation. Then, the inflammatory response is maintained by upregulation of diverse chemokines and chemotactic factors derived from the interaction between inflamed adipocytes, M1 macrophages and T and B lymphocytes (159).

7.2.1.2 Alternatively activated macrophages (M2)

It is described that M2 macrophages lessen inflammation and protect from the detrimental effects of diet-induced obesity (160). They exhibit immunosuppressive properties, great phagocytic capacity, and produce anti-inflammatory cytokines, like IL-10 (161). Moreover, PPARδ and PPARγ are amply expressed in murine and human monocytes and macrophages, and their activation partially inhibits pro-inflammatory genes, which shows that PPARs may control M1 macrophage activation (162).

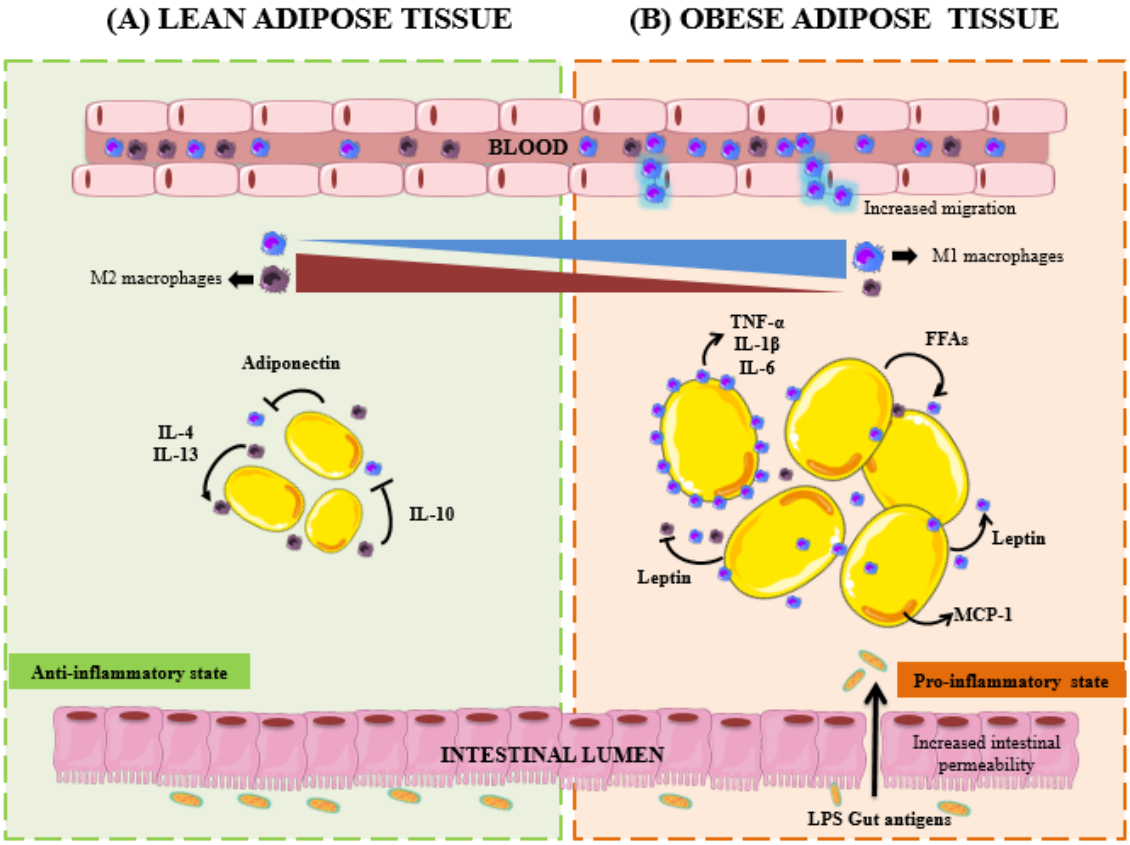

Figure 3: (**A)** Adipocytes in lean adipose tissue are of normal size, and produce adiponectin, with anti-inflammatory properties. In addition, it contains M2 macrophages, with immunosuppressive properties, secreting anti-inflammatory cytokines, such as IL-4, IL-10 and IL-13. **(B)** On the other hand, obese adipose tissue is infiltrated with pro-inflammatory immune cells, such as M1 macrophages, and secreting high amounts of pro-inflammatory cytokines (TNFα, Il-6, or IL1β) and chemokines (MCP-1). Moreover, M1 macrophages accumulate in crown-like structures around hypertrophic adipocytes. The increased secretion of FFAs further activates M1 macrophages to produce more pro-inflammatory mediators. Adipocytes in obese adipose tissue also increase leptin production, which also acts as a pro-inflammatory mediator. Moreover, in a obese state the gut barrier is also disrupted, increasing the translocation of bacterial products, such as LPS, to the circulation, thus stimulating systemic inflammation. Adapted from (163).

8. Inflammatory processes in liver and skeletal muscle

8.1. Liver

It is well known that inflammatory mediators alter liver function, including TNF-α that upregulates hepatic lipogenesis and sustains hyperlipidemia (164). In obesity, there is a low-grade inflammation in the liver mediated by NF-κB activation and generation of inflammatory cytokines (165, 166). In this regard, IL-6 interferes with insulin signalling in hepatocytes (167, 168), and TNF-α deficient mice have been reported to be protected from high-fat diet-induced hepatic steatosis (169). In addition, these cytokines upregulate genes implicated in ceramide biosynthesis, whose hepatocyte levels get increased (170), and can reduce insulin signalling by inhibiting Akt activation (171). In obesity, hepatocytes stress responses and inflammatory activation could also be mediated by fatty acid-mediated activation of TLR-4 (172). Additionally, M1/M2 polarization profile of Kupffer cells in the liver may hamper insulin sensitivity by the secretion of inflammatory factors (173).

8.2. Skeletal muscle

The skeletal muscle is the main organ for insulin-stimulated glucose clearance, so muscle insulin resistance is a primary component of the aetiology of the MetS and T2DM (174). As observed in other organs, obesity is also associated with an increased macrophage infiltration in the skeletal muscles that secrete different pro-inflammatory cytokines, such as TNF-α and IL-6 (175). This produces a low-grade chronic inflammation that, as well, worsens insulin resistance (176).

9. Obesity associated intestinal inflammation – intestinal permeability and metabolic endotoxemia

Significant increases in knowledge have been done of the role of gut microbiota, and in particular its imbalance (dysbiosis) in metabolic conditions. Although the main cause of obesity is excessive calorie intake compared with energy expenditure, it is linked to changes in gut microbiota, and this variation might contribute to the pathogenesis of obesity (177). Although there is a high diversity in the intestinal microbiota composition among healthy individuals, it is evident that obese patients have a lesser richness (178). In healthy individuals, the ratio *Bacteroidetes* to *Firmicutes* is high, while the prevalence of *Firmicutes* becomes higher in obese patients (179). Moreover,

there are also differences in two species that are correlated with insulin resistance: *Lactobacillus* and *Clostridium*, being *Lactobacillus* positively associated with fasting glucose levels, and *Clostridium* negatively (180). This indicates that particular bacterial phyla, class or species, or even bacterial metabolic activities, may prevent or contribute to the onset of obesity.

The evidences connecting gut microbiota with obesity have been mainly based on animal studies. For instance, germ-free mice are resistant to high-fat diet (HFD)-induced obesity, regardless of the higher calorie consumption (181). However, germ-free mice receiving a faecal transplant from obese women displayed body fat accumulation and metabolic disorders linked to obesity (182). Similarly, faecal transplant from obese mice to germ-free mice significantly increased total body fat (183). Thus, these observations indicate that variations in the intestinal microbiota could modify the metabolic profile of the host, although the mechanisms are not well understood. It has been proposed that the increased *Firmicutes*/*Bacteroidetes* ratio found in obese people leads to more effective hydrolysis of non-digestible carbohydrates in the intestinal lumen, thus extracting more calories from food than lean people (184). Actually, the pathways for short-chain fatty acids (SCFA) production are elevated in obese and overweight subjects, which evidences that there are more fermentable substrates and calories available in the host, thus increasing energy absorption (185). Another mechanism proposed is the ability of gut microbiota to reduce liver fatty acid oxidation by inhibiting AMPK (186). Interestingly, gut microbiota bacterial components, including LPS, pepditoglycan, lipoteichoic acid, flagellin and bacterial DNA can activate the immune response (187).

In this regard, the maintenance of the integrity of the intestinal mucosa is key to prevent LPS translocation. Different membrane proteins and cytoskeletal components form the intercellular tight junctions that constitute the structural framework that keeps the barrier (188). Of note, the decrease in *Bifidobacterium* results in reduced tight junction integrity (lower mRNA of junctional proteins Zonula occludens (ZO)-1, occludin, claudins and actin-myosin cytoskeletal proteins), and thus increased gut permeability (189). Increased amount of LPS can therefore be translocated to the circulation. Then, LPS infiltrates tissues, including liver and fat, eliciting an innate immune response by primarly acting as an agonist to TLR-4 in the surface of macrophages, which triggers a cascade of production and secretion of pro-inflammatory cytokines (190), via signalling

pathways that upregulate the expression of inflammatory mediators encoding genes, such as NFκB (191). On the other hand, LPS can also cross the gastrointestinal mucosa by infiltrating chylomicrons, which are responsible for dietary triglycerides and cholesterol the absorption. Chylomicrons' formation is stimulated by high-fat diets, so this route is intensified in obesity (192). In this context, it has been observed a two- to three-fold elevation of LPS serum levels, a threshold named "metabolic endotoxemia".

10. Treatment of obesity

The tendency to manage obesity is to treat the individual components of the MetS, to reduce the risk for CVD and T2DM. However, weight loss is well known to have a beneficial impact on many components of the MetS, such as excessive fat accumulation, dyslipidemia, hypertension, insulin resistance and hyperglycemia (193).

10.1 Lifestyle modification

The lifestyle modifications to reduce weight refer to a simultaneous implementation of dietary changes to reduce energy intake and an increase in physical activity.

Regarding to dietary changes, simple carbohydrates and high glycaemic index foods (those that are super-processed) must be avoided, as well as the average fat intake must be decreased (194). Of note, each type of fat might affect differently the components of the MetS, and it is recommended that the intake of unsaturated fats should be greater than saturated fats (195). In addition, excess consumption of sodium is associated with hypertension, and for this reason its intake should be restricted (196). On the contrary, higher potassium intake has been associated with better blood pressure levels (197).

Regarding to physical activity, high cardiorespiratory fitness has demonstrated to decrease visceral adipose tissue and enhance glucose homeostasis in skeletal muscle by improving insulin sensitivity (198, 199). Moreover, it increases HDL-cholesterol and decreases TGs (200).

10.2. Pharmacological therapy

Lifestyle interventions to reduce weight loss may produce significant clinical benefits but are too often associated with failure and frustration, so obese patients may require adjunctive therapies to achieve their weight loss and health goals. Drugs are prescribed, as adjunct to diet, exercise and behavioural changes for patients with BMI $\geq$ 30 kg/m or in patients with a BMI $\geq$ 27 kg/m^2 with one or more obesity related comorbid

conditions, including T2DM, dyslipidemia and/or hypertension (201). In any case, most of the drugs developed for obesity management have been withdrawn due to safety reasons, mostly related to augmented risk of cardiovascular and psychiatric conditions. Moreover, even though monotherapies that target a single protein or pathway involved in obesity have shown some efficacy, combination therapies provide better outcomes by synergistic mechanisms that prevent compensatory responses, as well as reduce adverse effects and increase tolerability since lower doses of the drugs are used (202).

Most of the weight loss medications available are appetite-suppressant, such as diethylpropion, bupropion, benzphetamine, phentermine and phendimetrazine. Although they were approved by the Food and Drug administration (FDA) in the late 1950s and early 1960s, their use is limited, since the studies investigating these noradrenergic drugs were limited in sample size, retention rates or study duration, and they are classified as controlled substances just recommended for short-term use only (203).

Nowadays, phentermine is the most frequently prescribed drug of this type (204). It is a noradrenergic drug that acts on the sympathetic nervous system promoting norepinephrine release, thus leading to appetite suppression and increase in energy expenditure. Its main side effects comprises constipation, insomnia, dizziness, dry mouth, as well as mood changes and irritability (201), but it also shows serious complications like palpitations, tachycardia and hypertension which made the International Endocrine Society Guidelines to strongly recommend against prescribing this drug to patients with uncontrolled hypertension and/or a history of CVD (205).

In 2012, the FDA approved the combination therapy of phentermine with topiramate, an antiepileptic drug able to produce sedative effects that also induce reduction of calorie intake and weight loss. The combination of both drugs has been properly evaluated in long-term clinical trials, showing a mean weight loss of 10% (206, 207). This therapy has also reported improvements in obesity-associated cardiovascular and metabolic conditions, including hypertension, hypercholesterolemia, hyperglycemia, hyperinsulinemia, and obtained significant reductions in waist circumference (208). As adverse reactions, it has been described paresthesia, dizziness, insomnia, constipation and dry mouth, as well as teratogenic effects that contraindicate its use during pregnancy (209). In Europe, this treatment has not been approved, principally forconsidering insufficient data for long-term treatments with phentermine on

cardiovascular effects (arrhythmia, ischemic heart disease, pulmonary hypertension, valvulopathy), potential abuse and the psychiatric and cognitive side effects of topiramate (80).

Orlistat is a drug against obesity whose mechanism of action consists on inhibition of pancreatic and gastrointestinal lipases that reduces TG hydrolysis and fatty acid absorption by the gut epithelium, resulting in the absorption of approximately just two-thirds of the dietary fatty acids consumed (210). After a 4-year trial, orlistat led to 2.4% total body weight loss, but more interestingly, the treatment significantly reduced the risk of T2DM, improving insulin sensitivity, glucose levels, cholesterol levels and blood pressure (211). The most commonly side effects ascribed to orlistat include oily stools, oily spotting, faecal urgency, faecal incontinence, hyper-defecations and flatus with discharge (212). Besides, several cases have reported acute kidney injury caused by the presence of orlistat-induced oxalate crystals in those patients predisposed to metabolic conditions (213), in others, the crystals have been found in renal parenchyma, and for this reason, the renal function should be controlled in orlistat-treated patients (214).

Lorcaserin is a selective agonist of the 5-hydroxytryptamine 2C (5HT-2C) receptor that reduces caloric intake without modifying energy expenditure in humans. Its mechanism consists on activation of pro-opiomelanocortin neurons in the hypothalamus (215). Two long-term trials, BLOOM and BLOSSOM, found that lorcaserin reduced total body weight loss approximately 3.3% and improved fasting glucose, insulin and hemoglobin A1c (HbA1c) levels (216). Its most common side effects comprise headache, dizziness, fatigue, nausea and dry mouth. This drug is used in the USA since 2012, but the European Medical Agency (EMA) has not approved it because of its psychiatric side effects that include depression, suicidal ideation and psychosis; and the risk of developing valvulopathy (217).

Naltrexone and bupropion combination is a drug approved by the FDA in 2014. The opioid antagonist naltrexone is used for drug and alcohol abuse, whereas bupropion, a selective inhibitor of neuronal reuptake of catecholamines (norepinephrine and dopamine), is used in the management of depression and smoking cessation. Both drugs showed weight loss as side effect so they were combined for its use against obesity. This combination has been evaluated in 4 long-term trials, where weight loss ranged from 5 to 9%, with improvements in high-density lipoprotein and triglycerides levels (218). Moderate and transient nausea, headache, constipation, dizziness, vomiting and

dry mouth have been reported as the most frequent side effects (204), but it has also been observed increased risk of suicidal behaviour and ideation, and neuropsychiatric symptomatology (219), and also cardiovascular events (220).

The newest weight loss medication is Liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, originally approved for the treatment of T2DM. GLP-1 is an incretin hormone that regulates hunger by upregulating insulin release and producing anorexigenic effects. It also delays gastric emptying and increases postprandial satiety and fullness, thus decreasing appetite and food consumption by acting in the hypothalamus (221). Three long-term (56 weeks) trials have evaluated the effect of Liraglutide in obese patients, and found a weight lost from 6 to 8%, with improvements in T2DM associated markers, including HbA1c, fasting plasma glucose levels and HOMA-IR (222-224). Nevertheless, it also has side effects like nausea, vomiting, diarrhoea, constipation, hypoglycemia and dyspepsia (225).

Apart from these pharmacological treatments, there are some candidate therapeutics in clinical development. These drugs have passed some pre-clinical and clinical studies, but long-term and safety studies are still needed for their approval, although the prospects are good.

10.3 Complementary and/or alternative therapy

As seen above, the anti-obesity drugs that are available nowadays are associated with serious adverse effects so there is a renewed interest in the search for safer non-conventional therapies. Among these, it is interesting to highlight plant-based medications that produce satiety, metabolism increase or weight loss, which have become very popular.

The anti-obesity properties of medicinal plants may be due to their bioactive metabolites, like the phenolic compounds (226). Phenolic compounds are very common in the plant kingdom, constituting one of the most abundant groups of plant secondary metabolites. They are mainly found in fruits, vegetables, cereals and legumes (227), and the increase in their consumption is associated with anti-obesity effects, improving inflammatory, glycaemic and oxidative status in humans, as well as regulating insulin sensitivity, glucose homeostasis and lipid metabolism (228).

Phenolic compounds have been investigated in pre-clinical studies for their anti-obesity actions by using both *in-vitro* cell cultures of adipogenesis (3T3-L1 cells) and *in-vivo*

models of obesity in rodents (genetic and diet-induced) (Table 3), as well as in human trials (Table 4). It is important to note that the bioactivity of each phenolic compound depends on the activity level and its pharmacokinetic properties. Thus, although a given phenolic compound shows interesting bioactivities *in-vitro*, it could have little or non-biological activity *in-vivo*. Considering this, both *in-vitro* and *in-vivo* studies suggest that phenolic compounds display different activities of potential interest against obesity and its related conditions, including inhibition of intestinal absorption of lipids, reduction of the differentiation from preadipocytes to adipocytes, induction of adipocyte apoptosis and increased uptake of glucose by skeletal muscles, among others (229). Moreover, phenolic compounds can enhance gene expression of endogenous antioxidant enzymes showing an anti-inflammatory effect (230).

10.3.1. Polyphenols and lipid metabolism

As mentioned above, phenolic compounds may interfere with lipid absorption. Thus, bean sprouts (*Phaseolus vulgaris* L.), rich in polyphenols, have been reported to increase triacylglycerol (TAG) faecal excretion and decrease TAG serum levels when rats were fed with high fat and fructose diet, which was associated to the inhibition of pancreatic lipase enzyme activity (231). Similarly, the tea polyphenol (-)-epigallocatechin- 3- gallate (EGCG) has also shown to increase the faecal excretion of cholesterol and total lipids in mice (232). Besides, a nutraceutical product made of Annurca apple polyphenolic extracts significantly increased faecal cholesterol excretion in a randomised, double blind, single centre, placebo-controlled, crossover study (233).

Regarding lipid metabolism, many rich phenolic extracts have shown to improve it in different pre-clinical obesity studies and clinical trials. Long-term diet-supplementation with quercetin, one of the best studied phenolic compound, in HFD-fed obese mice significantly decreased plasma TG levels and accumulation of hepatic lipid by regulating lipid metabolism, by reducing the expression of genes involved in de novo lipogenesis and decreasing hepatic lipid peroxidation and thus, oxidative stress (234). Rutin has also been reported to significantly decrease lipid mass in the liver in HFD-fed mice (235), as well as hepatic oxidative stress by reducing lipid peroxidation and improve the levels of antioxidant enzymes (236). Besides, Kaempferol, another flavonoid present in apples, grapes and berries, has been described to reduce TGs and LDL-cholesterol levels in HFD-fed obese mice (237), and lipid synthesis by decreasing the hepatic activation of the lipogenic enzymes, PPAR-γ, Sterol regulatory element-

binding protein (SREBP)-1c and fatty acid synthase (FAS) (238).

Furthermore, a meta-analysis with humans has evidenced that green tea consumption lowers total and LDL-cholesterol, with increasing HDL-cholesterol (239). High polyphenol intake has also been linked to rather lower levels of LDL-cholesterol and TGs, and higher serum concentration of HDL-cholesterol in a multicentre, cross-sectional, randomized study with T2DM patients (240). Also, greater consumption of polyphenols was correlated with lower total and LDL-cholesterol, lower TGs and higher levels of HDL-cholesterol in a Moli-sani cohort (241).

10.3.2. Polyphenols and glucose metabolism

Various dietary polyphenols have been reported to influence carbohydrate metabolism, by attenuating hyperglycaemia and improving insulin sensitivity (242). The proposed mechanisms include:

- Reduction of postprandial glycaemia by regulating Sodium glucose linked transporter (SGLT)-1 and Glucose transporter type (GLUT)-5 (243).
- Improvement of insulin stimulated glucose transport by GLUT-4 (73, 244).

In skeletal muscle cells, quercetin has exhibited the property of significantly induce glucose uptake, via AMPK and PI3K activation (245, 246), which has been linked an improvement of insulin resistance (234). Kaempferol has also been reported to increase glucose induced insulin secretion, by enhancing intracellular cAMP, Akt protein and ATP production in beta cells and in pancreatic human islets (247).

Rutin has been able to significantly decrease serum glucose and insulin concentrations in HFD-fed obese rats, compared to the control group (236). Moreover, it had a beneficial effect in diabetic rats improving their pancreatic beta-cell mass and insulin secretion, and restoring glycogen content in liver and muscles (248, 249). In addition, an extract of *Opuntia ficus-indica*, traditionally consumed by Mexican people, significantly ameliorated hyperglycemia, and augmented hepatic and muscle glucose usage in HFD-fed obese mice by raising tyrosine phosphorylation of IR and IRS-1; and activation of PI3K and Akt proteins (250). Kaempferol has, as well, reduced obesity-related complications in HFD-fed obese mice by decreasing plasma glucose levels, increasing glucose use of muscle and adipose tissues through GLUT-4 and AMPK activation (237). In addition, myricetin, a flavonoid present in berries, significantly

reduced hyperglycemia, by improving insulin action, as the phosphorylation of IR, IRS-1 and Akt increased with the treatment. Moreover, it also improved the translocation of GLUT-4 (251).

10.3.3. Polyphenols and adipogenesis

Recently, different plant-bioactive compounds have been reported to prevent obesity and its associated metabolic disorders by regulating adipocyte life cycle, including inhibition of proliferation and adipogenesis, and stimulation of lipolysis and apoptosis (252). *Solanum nigrum* polyphenols have been shown to reduce body weight and fat in HFD-fed mice, by reducing adipocytes lipid content and modulating lipid metabolism. They increased lipolysis rate by activating carnitine palmitoyltransferase-1 (CPT-1) and PPARα, and decreased lipogenesis rate through inactivation of FAS (253). Similarly, Canola meal phenolic-enriched extract has also shown to inhibit adipogenesis in mesenchymal stem cell line (C3H10T1/2), an effect that was correlated with inhibition of PPARγ and pancreatic lipase (254). Curcumin has shown to limit adipogenesis by downregulating the activation of PPARγ and CCAAT-enhancer-binding protein (C/EBP), and to impair adipogenesis by inducing adipocyte apoptosis in 3T3-L1 preadipocytes (255). In addition, rutin has also been able to inhibit adipogenesis in 3T3-L1 adipocytes by PPARγ and C/EPB-α downregulation (235). Besides, quercetin supplementation to HFD-fed rats has been described to reduce WAT mass by (256). The molecular mechanism of the inhibition of this adipogenesis was later confirmed in a study with 3T3-L1 adipocytes, suggesting that quercetin decreases the expression of adipogenic enzymes SREBP-1, CEBP/α AND PPARγ via AMPK activation (257). Moreover, also in 3T3-L1 adipocytes, quercetin induced apoptosis, by upregulating the expression levels of pro-apoptotic genes caspase-3 and 9 (258). In this regard, a red pepper seed water extract has also proved to promote adipocyte apoptosis in 3T3-L1 preadipocytes by increasing pro-apoptotic related proteins, like Bak, Bax and Bad, and inhibiting anti-apoptotic proteins, Bcl-2 and p-Bad (259).

Furthermore, the effect of polyphenol supplementation on adipocyte function has been studied in humans. In a randomized placebo-controlled study, treatment with a combination of the polyphenols EGCG and resveratrol downregulated pathways contributing to adipogenesis in obese and overweight patients in comparison with control group although no changes were observed in adipocyte size and distribution (260).

10.3.4. Polyphenols and adipose tissue inflammation

Dietary polyphenols are described to exert anti-inflammatory functions acting through AMPK, MAPK and NF-κB signalling pathways. Quercetin has shown to increase adiponectin and reduce TNF-α plasma levels and also diminish the expression of inducible Nitric oxide synthase (iNOS) in visceral adipose tissue in obese Zucker rats (261). Kobori et al., also reported suppression of plasma TNF-α in HFD-obese mice after quercetin treatment (262). In addition, quercetin is able to attenuate activation of NF-κB and MAPKs in human macrophages and adipocytes treated with macrophage-conditioned media, thus ameliorating insulin resistance parameters as well (263). Kaempferol was able to reduce hepatic inflammation in obese diabetic rats by lowering plasma levels of the pro-inflammatory cytokines TNF-α and IL-6 by inhibiting IKKB and NF-κB kinases activation, thus improving liver insulin resistance and systemic insulin resistance (264). Besides, gallic acid (GA) has also shown to downregulate the MAPK/NF-κB pathway in RAW 264 macrophages in a co-culture system with 3T3-L1 preadipocytes, suggesting that GA could have an anti-inflammatory effect (265). Actually, GA inhibited the expression of the inflammatory genes Tnf-α and Ccl-2, in the same experimental settings, and IL-6 in mice fed high fat, high sucrose diet, where it also improved insulin resistance (265).

The activation of AMPK, in addition to its effects improving insulin resistance, has been also described to decrease inflammation. In a recent study, artemisinin administration to HFD-fed ApoE–/– mice, an experimental model of atherosclerosis, showed reduced inflammation by upregulating AMPK activation and down-regulating NF-κB phosphorylation (266). A combination of quercetin and resveratrol has also revealed to ameliorate adipose tissue inflammation by activating AMPK pathway in HFD-fed obese rats (267). This was confirmed when a HFD supplemented with raspberry, which is rich in polyphenols, was given to wild type and AMPKα1-/- mice. Inflammation was ameliorated in the wild type mice while this effect was not observed in the knockouts (268).

Actually, in humans, berry supplementation has also been reported to reduce serum levels of pro-inflammatory cytokines and improve endothelial dysfunction in MetS (269). Moreover, a mediterranean style diet, rich in polyphenols, has been shown to lower C-reactive protein levels (inflammatory marker) and serum levels of IL-6, and increase endothelial function even 2 years after (270).

10.3.5. Polyphenols and endothelial dysfunction

There is strong evidence, in *in-vitro* and animal experimental models, that some flavonoids induce vasodilator effects and improve endothelial dysfunction, which supports their beneficial properties against cardiovascular diseases (271). In this regard, it has been reported the ability of different polyphenols to inhibit NADPH oxidase activity, the main source of ROS, thus reducing their production and increasing the availability of NO (272). Accordingly, a phenolic fraction of extra virgin olive oil has been found to modulate the angiogenic response by preventing NADPH oxidase activity and its expression in human cultured endothelial cells (273). Similarly, hypertensive rats treated with (-)-epicatachin prevented the increase in blood pressure, which was associated with a decrease in plasma ET-1 levels and NADPH activity, thus improving endothelium dependent relaxation response to acetylcholine (274). Moreover, oral administration of quercetin to spontaneously hypertensive rats reduced their increased systolic blood pressure, which was related to the improvement in endothelium-dependent relaxation and the decrease of vascular NADPH activity (275). More recently, acacia polyphenols have shown anti-hypertensive effects in hypertensive rats, including lowering systolic and diastolic blood pressures (276).

Interestingly, in a Mediterranean population with high cardiovascular risk, polyphenol intake was correlated with lower blood pressure levels and lower prevalence of hypertension (277). Similarly, green tea intake has been described to reduce systolic blood pressure (278), and high consumption of berries, blueberries and strawberries, which are rich in anthocyanins, has also been linked to lower risk of hypertension in women (279).

10.3.6. Polyphenols on gut microbiota and barrier function

Growing evidence is showing that the protective role of polyphenols against obesity can be exerted by modulating gut microbiota composition and functionality, together with their beneficial impact on intestinal inflammation and barrier integrity. It has been proposed that the principal benefit of polyphenols regarding their effects on the microbiota is due to their selective antimicrobial effect against potentially harmful bacteria (185). Such selective effect has been described in different studies with several phenolic compounds. For example, a review has shown that *in-vivo* and *in-vitro* studies with phenolic compounds derived from berries have shown inhibitory effects on both

growth and adhesion of possible human pathogenic bacteria, such as *Staphylococcus*, *Salmonella*, *Clostridium* or *Enterococcus*, while promoting the proliferation and adhesion of beneficial bacteria, including *Lactobacillus* and *Bifidobacterium* (280).

Indeed, a recent study has reported that HFD-fed mice transplanted with the microbiota of resveratrol treated mice increases the relative abundance of *Bacteroidetes* and the expression of key markers of tight-junction integrity, such as mucin (Muc)-2, Muc-3, Zo-1, occludin, claudin-1 and junctional adhesion molecule (JAM)-A in HFD-fed mice (281). Moreover, a reduction in five genera positively correlated with obesity, such as *Desulfovibrio*, *Ruminiclostridium*, *Anaerotrucus*, *Oscillibacter* and *Lachnospiraceae* was observed (281). Quercetin has also shown beneficial effects in gut microbiota, increasing short-chain fatty acid (SCFA) producing bacteria *Bacteroidia*, *Akkermansia*, *Proteobacteria*, and decreasing Gram-negative bacteria *Firmicutes* and *Helicobactoer*, which improve gut barrier function and thus reducing the LPS plasma levels (234). Several polyphenols, including those obtained from grape seed extracts as EGCG (282), (-)-epicatechin (283) or berberine (284) have also shown the capacity to increase the expression of *Zo-1*, *occludin*, and several claudins involved in the dynamics and functions of the tight junctions, and thus mucosal permeability.

In humans, although the number of intervention studies focusing on intestinal permeability has increased lately, only a few have explored the potential benefits of polyphenols, and are still ongoing (285). These studies differ in terms of population, foods administered, dose of bioactives, duration of intervention, and markers of intestinal permeability selected (NLM-1, 2 and 3 or BMC), but the results will be useful to understand the actual role of polyphenols in humans.

Regarding the microbiota, there are also few studies in humans that have evaluated the effect of polyphenols on gut microbiota in obese patients, as most of them are performed with healthy volunteers. Dueñas et al., in 2015 reviewed the effect of polyphenols in the modulation of intestinal microbiota in human intervention studies (286). These studies are also different in terms of dose of polyphenols, treatment duration or microbial techniques applied, and thus, it is difficult to compare the results obtained regarding to changes in the gut microbiota. There is a randomized crossover study with MetS patients that reports how polyphenols from red wine raised the number of faecal *Bifidobacteria*, *Lactobacillus*, *Faecalibacterium prausnitzii* and *Roseburia*, which are intestinal barrier protectors, and lessened LPS producing bacteria, including

Escherichia coli and *Enterobacter cloacae*, which was linked to an improvement in MetS markers, such as plasma TGs, cholesterol, HDL-cholesterol, and glucose (287). Furthermore, in a randomized double-blind study with overweight and obese men, the consumption of soy milk (riche in isoflavones) was associated with a reduction in the *Firmicutes* to *Bacteroidetes* ratio, which has positive effects (288).

11. *Hibiscus sabdariffa*

Hibiscus sabdariffa, ordinarily known as roselle, is a perennial herb belonging to the family of *Malvaceae*, originally native from India to Malaysia, although it is, as well, grown in Sudan, Egypt, Nigeria, Saudi Arabia, Taiwan, West Indies and Central America (289, 290). It is cultivated for industrial and medicinal applications, being all of its parts used (291). Actually, it has been commonly used as food, drink and medicine for colds, toothaches, urinary tract infections and hangovers (292). In Thailand, it is traditionally used to treat kidney and urinary bladder stones (293); in India, as a pain killer in urinary and digestive complaints, and in Mexico, as antihypertensive (8).

It has been reported that Roselle calyces contains different bioactive molecules with potent antioxidant, anti-inflammatory, antiobesity, antihyperlipidemic, antihypertensive, platelet antiaggregant, diuretic, antimicrobial, anticancer, hepatoprotective, renoprotective, antitumour and immunomodulatory properties (294-302). In fact, Roselle calyces are rich in organic acids, being the hibiscus acid the most representative compound. But there are also phenolic acids, such as chlorogenic acid, and flavonol derivatives, phenylpropanoids and anthocyanins (303). Furthermore, the biological activity of the extracts is attributed to the polyphenolic content, which enhances the nutritive value of Roselle (292).

When considering the impact of *H. sabdariffa* polyphenols on metabolic functions, it has been reported that these compounds show concurrent effects in mitochondrial activity, energy homeostasis, oxidative stress and inflammation, which can result in cardiovascular-protective effects (304). Moreover, several preclinical studies in obese mice have demonstrated the capacity of its polyphenols to decrease body-weight by inhibiting fat accumulation and improving glucose tolerance, thus normalizing the glycemic index (305, 306). In addition, *in-vitro* and *in-vivo* studies have revealed the inhibitory effects exerted by these polyphenols on digestive enzymes such as α-amylase,

thus blocking the absorption of sugars and starch (307), or pancreatic lipase, impairing fat absorption (308), which can clearly facilitate promote weight loss.

In human studies, the oral administration of various aqueous extracts of *H. sabdariffa* significantly decreased body weight and abdominal fat deposits, and these effects were associated with an improvement of the modified glucose levels, liver steatosis and lipid profiles in patients with obesity or metabolic syndrome (309).

Different studies in obese mice have pointed out potential mechanisms and active compounds that may be involved in the therapeutic effects. It has been proposed that the ability of the aqueous extract of *H. sabdariffa* to control weight gain and display hepatoprotective properties in diet-induced obese mice was linked to the downregulation of liver PPAR-γ and SREBP-1c transcriptional activity (305). In fact, the overexpression of both genes is correlated with upregulation of lipogenic proteins involved in the development of hepatic steatosis in obesity (310). Of note, *H. sabdariffa* extracts have been described to inhibit adipocyte differentiation in 3T3-L1 preadipocytes, an effect associated with a downregulation of the expression of the major adipogenic transcription factors, PPARγ and CEBP/α (311, 312). In addition, *H. sabdariffa* polyphenols have been reported to exert anti-inflammatory activity in 3T3-L1 adipocytes by inhibiting the secretion of pro-inflammatory adipokines, including TNF-α, IL-6 and interferon (IFN)-γ, as well as the activation of the transcription factor NF-κB (311, 313).

12. *Lippia citriodora*

Lippia citriodora Paláu (or *Aloysia citriodora* Kunth), popularly referred as lemon verbena, is a deciduous shrub belonging to the family of *Verbenaceae*, originally from South America, although nowadays it is cultivated in any temperate zone, including Southern Europe and North Africa (303). Traditionally, leaves of *L. citriodora* are used as tea, refreshing beverage, food or spice, for the treatment of insomnia and anxiety in South-America (314-317). In Mexico, it is employed for stomach conditions, depression and anxiety (318). In Brazil, the plant is commonly used as a bactericide and for dermatologic conditions (319); In Argentina, it is used as an antispasmodic, diuretic, digestive, cardiotonic and tranquilizer drug (314, 320).

L. citriodora extracts contain polyphenols as bioactive compounds, especially phenylpropanoids such as verbascoside (or acteoside), the most abundant compound,

but also iridoids like gargoside and flavonoids such as luteolin-7-diglucoronide, all of them with antioxidant properties that contribute to its reported biological effects (321, 322). Verbascoside has been described to be the primary responsible for the pharmacological activities of *L. citriodora*, however, other metabolites, like vitexin, can also contribute (323).

As expected, preclinical studies have shown the antioxidant properties of *L. citriodora* when administered to rats, which enhanced the antioxidant activity of glutathione peroxidase and glutathione reductase, and reduced myeloperoxydase activity (9, 323). Supporting this, the administration of an aqueous extract of *L. citriodora* to female Wistar rats prevented lipid peroxidation and protein carbonylation (324). Moreover, *L. citriodora* has been reported to exert beneficial effects in obesity, facilitating sustainable weight loss modulating appetite biomarkers (325). Interestingly, these phenolic derivatives have also shown to modulate AMPK activity in hypertrophied 3T3-L1 adipocytes (326), similarly to that reported for metformin as well, an antidiabetic drug able to promote weight loss in obese patients (327). The administration of an extract of *L. citriodora* to KK-Ay mice, which spontaneously develop diabetes and obesity, significantly improved hepatic lipid metabolism by decreasing serum and hepatic lipid content, via activation of AMPK to regulate lipid synthesis and degradation (10). Moreover, *L. citriodora* extract has shown to significantly improve fat metabolism in an *in-vitro* model of insulin resistant hypertrophic 3T3-L1 adipocytes, which resembles obesity-induced metabolic disturbances, being this effect evidenced by decreased TG accumulation and production of reactive oxygen species (ROS), thus confirming its antioxidant ability (328).

Table 3: Anti-obesity effects of polyphenols in *in-vitro* cell culture and *in-vivo* models of obesity in rodents

Treatment	Target	Type of study	Model	Duration	Finding
Artemisinin	Atherogenesis Inflammation	Animal model	HFD-fed ApoE–/– mice	8 weeks	Amelioration of atherosclerotic Suppression of inflammation b Decreased serum IL-6, IL-12 a
Berberine	Intestinal barrier	Animal model	Endotoxemic mice (LPS inyection)	-	Increased expression of *Zo-1*, C
Curcumin	Adipogenesis	Cell culture	3T3-L1 preadipocytes	1h	Decreased adipogenesis by dec And *Cebp/α* expression Increased Adipocyte apoptosis
EGCG	Intestinal barrier Inflammation	Animal model	Ulcerative colitis induced male CF-1 mice	3 days	Mitigated colon shortening and Decreased IL-1β, IL-6 and TNI Decreased Intestinal permeabil
EGCG	Lipid metabolism	Animal model	Male C57BL/6J fed high fat western style diet	33 weeks	Increased faecal excretion of to Decreased bile acid and lipid al Decreased serum cholesterol le Decreased severity of fatty live
(-)-epicatechin	Endothelial dysfunction	Animal model	Spontaneously hypertensive rats	5 weeks	Prevented increase in systolic b Increased plasma ET-1 Attenuation of NADPH activity Improved endothelium depende
(-)-epicatechin	Intestinal barrier	Animal model	Male C57BL/6J mice fed HFD	15 weeks	Decreased Intestinal permeabil junction proteins and reduced e
Gallic acid	Lipid metabolism Glucose metabolism Inflammation	Cell culture	3T3-L1 and RAW 264 co-culture	4 h	Decreased activation of MAPK Decreased MCP-1 levels, incre
		Animal model	Male C57BL7/6J mice fed HFHS diet	9 weeks	Decreased cholesterol, adipocy Decreased IL-6, iNOS and CCI Decreased macrophage infiltrat
Kaempferol	Glucose metabolism	Cell culture	INS-1E beta-cells and pancreatic human islets	4 days	Increased secretion of insulin in Increased cAMP, Akt activatio

Kaempferol	Lipid metabolism Glucose metabolism	Animal model	Male C57BL/6J fed HFD	5 months	Improvements is total cholester hyperinsulinemia Improvement in *Glut-4* and *Am* tissue
		Animal model	Diabetic male C57BL/6J fed HFD	6 weeks	Improvements in hyperglycemi levels
		Cell culture	C2C12 myoblasts	24 h	Increased lipolysis, improved g *Ampk* and *Glut-4* expression
Kaempferol	Lipid metabolism Glucose metabolism Inflammation	Animal model	Spargue dawley diabetic rats	10 weeks	Decreased serum lipid and insu resistance and increased glucos Decreased activation of IKKB Decreased TNF-α and IL-6 lev
Kaempferol	Lipid metabolism Glucose metabolism Adipogenesis	Animal model	Male C57BL/6J fed HFD	92 days	Decreased body weight, adipos HbA_{1c} and insulin resistance Decreased *Pparγ* and *Srebp-1* e
Myiricetin	Glucose metabolism	Animal model	Male wistar rats fed fructose-chow diet	14 days	Decreased plasma glucose leve increased IRS-1 and PI3K phos translocation of GLUT-4 in mu
Resveratrol	Gut microbiota and intestinal barrier	Animal model	Male C57BL7/6J mice fed HFD transplanted resveratrol-treated mice's microbiota	16 weeks	Increased abundance of bacter Decrease in generas associated Decreased expression of *Zo-1*, *A*
Rutin	Glucose metabolism	Animal model	Male diabetic wistar rats	45 days	Decreased fasting plasma gluco
Rutin	Lipid metabolism Adipogenesis	Cell culture	3T3-L1 pre-adipocytes	First 48 h of differentiatio n days (total 8)	Decreased adipogenesis by Decreased *Pparγ* and *Cebp/α* e
		Animal model	Male C57BL/6J fed HFD	4 weeks	Lesser body weight gain Decreased blood cholesterol Decreased adipogenesis in hepa *Cebp/α* expression

Rutin	Lipid metabolism Glucose metabolism Oxidative stress	Animal model	Male wistar rats fed HFD.	8 weeks, 4 weeks after pre-feeding with HFD	Decreased fat pads Decreased hepatic TGs and cho Decreased oxidative stress Decreased serum glucose and i
Rutin	Glucose metabolism	Tissue culture	Soleous muscle from wistar rats	90 minutes	Increased glucose uptake via ac Increased synthesis and translo
Quercetin	Adipogenesis	Cell culture	3T3-L1 preadipocytes	48 h	Decreased adipogenesis: Decre expression Increased apoptosis: increased
Quercetin	Lipid metabolism Glucose metabolism Inflammation	Animal model	Obese Zucker rats	10 weeks	Reduced plasma TG, cholestero Increased Adiponectin and eNO iNOS levels
Quercetin	Adipogenesis Oxidative stress	Animal model	Male wistar rats fed HFD	4 weeks	Increased adiponectin levels Decreased *Pparγ* expression in Reduced oxidative stress
Quercetin	Lipid metabolism Oxidative stress Inflammation	Animal model	C57BL/6J mice fed High-fat, sucrose and cholesterol diet	20 weeks	Decreased TGs, oxidative stres Improvement in hyperglycemia Increased adiponectin and decr
Quercetin	Endothelial dysfunction	Animal model	Spontaneously hypertensive rats	5 weeks	Restored endothelium depende Reduced systolic blood pressur Attenuation of NADPH activity
Quercetin	Glucose metabolism	Cell culture	L6 myoblasts	Pre-incubation of 24 h before assay	Increased glucose uptake by in Increased translocation of GLU
Quercetin	Lipid metabolism Glucose metabolism Gut microbiota	Animal model	Male C57BL/6J fed HFD	16 weeks	Decreased NAFLD Decreased plasma TGs Decreased Insulin resistance Increased SCFA producing bac Decreased Gam (-) bacteria Decreased plasma LPS levels (
Quercetin + resveratrol	Adipogenesis Inflammation	Animal model	Male wistar rats fed HFD	11 weeks	Lower body weight, adipose tis Decreased leptin, TNF-α, IL-6, Decreased recruitment of mast
Acacia polyphenols	Endothelial dysfunction	Animal model	Spontaneously hypertensive rats	4 weeks	Decreased systolic and diastoli

Blueberry peel extract, rich in quercetin	Adipogenesis	Cell culture	3T3-L1 preadipocytes	4 or 7 days	Decreased adipogenesis: Decre expression
Canola meal extract	Adipogenesis Lipid metabolism	Cell culture	C3H10T1/2 mesenchimal stem cell line	24 hours	Decreased adipogenesis by dec Decreased pancreatic lipase act
Extra virgin olive oil phenolic extract, rich in ligstroside	Endothelial dysfunction	Cell culture	Human umbilical vein endothelial cells	1 h	Decreased NADPH oxidase act Decreased expression of NADI
Grape powder extract, rich in quercetin	Inflammation	Cell culture	Human macrophages (U937 cell line)	5 h	Decreased inflammatory marke Decreased activation of JNK ar
***Phaseolus vulgaris* L (common bean) sprouts Rich in hesperidin Soysaponin-I**	Lipid metabolism	Animal model	Male wistar rats fed high fat and fructose diet	12 weeks	Hypolipidemic effect (decrease Increased TAG faecal excretion enzyme activity
Raspberry extract	Lipid metabolism Glucose metabolism Inflammation	Animal model	AMPKα1 -/- mice fed a HFD	10 weeks	Reduced ectopic lipid storage, sensitivity in wild type (Thus, A
Red peper seed water extract	Adipogenesis	Cell culture	3T3-L1 preadipocytes	24 h	Decreased adipogenesis adipog and *Srebp-1*, *FAS* and *Acc* expr Increased apoptosis: Increased decreased *Bcl2* and *p-Bad* expr
***Solanum nigrum* extract,**	Lipid metabolism	Animal model	Male C57BL/6J mice fed HFD	10 weeks	Decreased plasma TG, choleste Increased hepatic lipolysis and
***S. nigrum* extract derived polyphenols**	Lipid metabolism	Cell culture	3T3-L1 preadipocytes	24-48 h	Decreased lipid content of adip Promotion of lipolysis and inhi

Table 4: Anti-obesity effects of polyphenols in human trials

Treatment	TARGET	Study design	Population	Duration	Finding
Green tea, rich in (-)-epigallocatechin-3-gallate (EGCG)	Lipid metabolism	Cross sectional study	Men over 40	-	Decreased p TG levels, a Decreased a
Mediterranean style diet, rich in polyphenols	Inflammation	Randomized, diet-controlled study	Individuals with MetS	2 years	Decreased b Decreased s Decreased i Improveme
Flavonoid intake	Endothelial dysfunction	*Review	Healthy individuals	-	Increased co hypertensio
Total polyphenol intake	Endothelial dysfunction	Large, parallel- group, multicentre, randomized study	High CV risk individuals	4.8 years	Decreased i Decreased E
Soy milk rich in isoflavones	Gut microbiota	Randomized, double-blind study	Overweight and obese men	3 months	Decreased *E*
Bilberries	Inflammation	Randomized, diet-controlled study	Individuals with MetS	8 weeks	Decreased s
Green tea intake	Endothelial dysfunction	*Systematic review and meta-analysis of clinical trials	-	-	Reduction i Decreased t
Dietary flavonoids and phenolic acids	Lipid metabolism	Cross sectional study	People with T2DM aged 50-75 years	-	Decreased p HDL levels Decreased b
Red wine polyphenols	Gut microbiota	Randomized, crossover study	MetS individuals	30 days	Increase in i Increase in l Decrease in
EGCG + Resveratrol	Adipogenesis	Randomized, placebo-controlled study	Obese and overweight humans	12 weeks	Decreased a decreased p oxidative st
Annurca apple, rich in (+)-catechin -()-epicatechin	Lipid metabolism	A randomized, placebo-controlled, crossover study	Healthy subjects fed a high cholesterol diet	35 days	Increased fa

AIMS

Metabolic syndrome (MetS) is one of the major public health problems of our time. Its worldwide prevalence is constantly increasing, and it is estimated that 25% of the worldwide adult population has MetS (1). The associated metabolic dysfunctions such as obesity, hypertension, insulin resistance, glucose intolerance, dyslipidemia and atherosclerosis that characterize MetS are risk factors for developing T2DM, cancer and CVD (2).

Of note, obesity is considered the central axis of MetS, and arises due to an energy imbalance. Lately, it has been observed that obese individuals have a subclinical chronic inflammatory state, as a result of an enlargement of the adipose tissue mass, which leads to an overproduction of pro-inflammatory mediators (3). Adipose tissue is composed by fibroblast, preadipocytes, adipocytes and macrophages, and these last are the ones contributing significantly to the systemic inflammatory process (4). Thus, there is an intimate, highly coordinated connection between inflammatory and metabolic pathways (3).

Moreover, recent attention has been paid to gut microbiota, which is considered to significantly contribute to the development of obesity and its associated disorders (329). In fact, there is a link between obesity and an altered intestinal microbiota composition, characterized by an elevated ratio of *Firmicutes*/*Bacteroidetes*. This leads to an impaired intestinal barrier function, allowing the translocation of bacterial endotoxins, like LPS, into the circulation and the subsequent systemic endotoxemia that further contributes to the development and progression of inflammation (5).

Currently, the treatment for obesity is mainly focused on body weight reduction by dietary and lifestyle interventions. However, they are difficult to implement in these patients, so anti-obesity drugs are frequently necessary. Unfortunately, many of them have serious adverse effects (6). Therefore, investigation of novel therapeutic strategies combining efficacy and safety has become essential. Recently, plant-based medicines have shown to be effective for the management of obesity and associated disorders, including a decrease of the inflammatory response, or an improvement of the intestinal barrier function. In fact, their therapeutic properties are attributed to a range of biologically active compounds, mainly phenolic derivatives (7).

Among plants with potential therapeutic use in obese patients, roselle (*H. sabdariffa*) and lemon verbena (*L. citriodora*) deserve special attention.

H. sabdariffa has been traditionally used as a folk remedy, and it has shown antibacterial, diuretic, antioxidant, anticholesterolemic, antihypertensive and antidiabetic properties, among

others (8). Furthermore, the efficacy of different aqueous extracts of *H. sabdariffa* has been demonstrated in several studies in obese and MetS patients, reporting reduction in body weight and liver and abdominal fat accumulation, and an improvement of the hyperglycemia and hyperlipidemia (309, 330). In addition, its beneficial activity has been ascribed to the ability of polyphenols to induce insulin secretion in an experimental model of obesity, thus improving glucose metabolism and preventing liver, pancreas and kidney harm (331).

Regarding to *L. citriodora*, it has been traditionally used as a beverage, food or spice against dyspepsia. The extracts of *L. citriodora* are also characterized by the presence of polyphenolic compounds. Among them, verbascoside is the most abundant, and it has shown potent antioxidant (9, 332, 333), antimicrobial (334) and antitumor (335) properties. Moreover, *L. citriodora* extracts have been reported to modulate appetite (325) and to reduce obesity-related complications, such lipid (10, 328) and glucose (326) metabolism.

Several studies have suggested that gut microbiota composition can be regulated by phenolic substrates from plant extracts with particular prebiotic and antimicrobial effects (336, 337). Therefore, it would be interesting to investigate the effects of phenolic extracts on gut microbiota composition and if they may protect against obesity-induced MetS.

With this purpose, the present study aims to study the potential beneficial effects of well-characterized extracts of *L. citriodora* (LCE) and *H. sabdariffa* (HSE) in an experimental model of metabolic syndrome in mice.

The specific objectives are:

1. To evaluate the effect of the extracts on body weight, plasma biochemical profile and glucose tolerance.
2. To determine the anti-inflammatory effects of the extracts on target metabolic tissues (liver and fat) and on intestine epithelial barrier function.
3. To assess the effects of the extracts on the composition of gut microbiota.
4. To study the effects of the extracts on vascular endothelial dysfunction.

MATERIALS AND METHODS

1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (Madrid, Spain).

2. Preparation of plant extracts

2.1 *Hibiscus sabdariffa* extract (HSE)

2.1.1. Chemicals and reagents

All chemicals used for the preparation of the plant extract were of analytical HPLC-MS grade. Water was obtained by purification with a Milli-Q system from Millipore (Bedford, MA, USA). Formic acid was purchased from Sigma-Aldrich (Steinheim, Germany) and acetonitrile for mobile from and Fisher Scientific (Madrid, Spain). Finally, the standards used for the quantification were acquired from Sigma-Aldrich, (Steinhemin, Germany): gallic acid, citric acid, chlorogenic acid, rutin, p-coumaric acid, quercetin, myricetin, quercetin-glycoside and apigenin (internal standard), except quercitrin, which was supplied by Extrasynthese (Genay Cedex, France).

2.1.2. Extraction of phytochemicals from *H.sabdariffa* calyces

The extraction of phytochemicals was performed from commercial dried calyces of *H.sabdariffa* provided by Monteloeder Inc. (Elche, Alicante, Spain). The sample was grounded into fine uniform powder with an Ultra Centrifugal Mill ZM 200 (Retsch GmbH, Haan, Germany) equipped with 12-tooth rotor and ring sieve with 2 mm aperture size. After that, bioactive compounds were recovered by performing a conventional solid–liquid extraction (SLE), using water. Three replicates of *H. sabdariffa* extract were prepared by shaking 8 g with 160 mL of water for 15 min. Then, extracts were centrifuged at 10,000 rpm in a Sorvall 16 R provided by Thermo Scientific (Leicestershire, UK). The supernatants were filtered through 0.45 μm filters and solvent was removed under vacuum conditions in a Savant™ Speed Vac Concentrator SC250 EXP, supported by Thermo Scientific (Sunnyvale, CA, USA).

2.1.3. Identification and quantification of phenolic profile of *Hibiscus sabdariffa* by HPLC-ESI-TOF-MS

The chemical profile was determined by HPLC-ESI-QTOF-MS (MS) analysis. The instrumentation used was an Agilent 1260 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 6540 Ultra High Definition (UHD) Accurate Mass Q-

TOF equipped with a Jet Stream dual ESI interface. The components were separated by chromatography with a reversed-phase C18 analytical column (Agilent Zorbax Eclipse Plus, 1.8μm, 4.6 × 150 mm). The compounds were eluted with two polar mobile phases consisted of phase A (water-acetonitrile, 90:10 v/v plus 0.1% of formic acid) and phase B (acetonitrile). Thus, the elution program was a multi-step linear gradient at a flow of 0.3 mL/min, beginning at 0 min with 5% of mobile phase B, followed by 20% phase B at 34 min, at 45 min increasing until 95% phase B, at 55 min back to 5% of phase B. Finally, the initial conditions were maintained for 5 min. The sample injection volume was 10 μL, whereas the column and auto-sampler compartments temperatures were set at 25 °C and 4 °C, respectively. The MS detection was performed in negative ionization mode with a mass range of 100–1700m/z, the detection window was set to 100 ppm and data acquisition (2.5 Hz) was performed in centroid mode. The capillary voltage was set +4000 V, nebulizer pressure 20 psi, fragmentor 130 V, nozzle voltage 500 V, skimmer 45 V and octopole 1 RF Vpp 750 V. Ultrahigh pure nitrogen was used as drying and nebulizer gas at temperatures of 325 and 350 °C and flows of 10 and 12 L/min, respectively. The MS data were processed through the software Qualitative Analysis of MassHunter workstation version B.06.00 (Agilent Technologies, Palo Alto, CA, USA). In order to quantify the individual content of the identified compounds in the HSE extract, nine calibration curves were prepared using commercial standards. Seven concentration levels were prepared using citric acid, chlorogenic acid, gallic acid, p-coumaric acid, quercetin, rutin, quercetin-3-glucoside, quercitrin and myricetin. Apigenin was used as internal standard at a concentration of 20 mg/L. Concentrations were determined using the corrected area of each individual compound (area standard/area internal standard) and by interpolation in the corresponding calibration curve. The remaining compounds were tentatively quantified on the basis of calibration curves from other compounds with structural similarities. It should be taken into account that the response of the standards can differ from that of the analytes found in the extracts, and consequently the quantification of these compounds is only an estimation of their actual concentrations, although it can be considered a useful approximation.

2.2. *Lippia citriodora* extract (LCE)

2.2.1. Chemicals and reagents

Double-deionized water (conductivity of <18.0 MΩ) used for extraction and analysis (HPLC mobile phase) was obtained by a Milli-Q system acquired from Millipore (Bedford, MA,

USA). Conversely, analytical grade ethanol was provided by VWR chemicals (Radnor, PA, USA). In addition, to prepare the mobile phase, LC-MS grade acetonitrile was acquired from Fisher chemicals (Waltham, MA, USA) and formic acid from Sigma-Aldrich (Steinheim, Germany). Standards (verbascoside, loganic acid, quercetin, kaempferol-3-glucoside and apigenin) to prepare calibration curves were provided by Fluka, Sigma-Aldrich (Steinheim, Germany) or Extrasynthese (GenayCedex, France).

2.2.2. Extraction of phytochemicals from *L. citriodora* leaves

Leaves of *L. citriodora*, provided by Monteloeder (Alicante, Spain), were grounded applying an ultra-centrifugal mill ZM200 (Restch GmbH, Haan, Germany). Conventional solid-liquid extraction (SLE) using ethanol-water (25% v/v) was performed to retrieve bioactive compounds from *L. citriodora* leaves. Three replicates were prepared shaking 1.5 g of leaves with 50 mL of hydro-alcoholic mixture during 90 minutes. Then, extracts were centrifuged at 13000 RPM in a Sorvall 16 R provided by Thermo Scientific (Leicestershire, UK). The supernatants were filtered through 0.45 µm filters and the solvent was removed under vacuum conditions in a Savant™ Speed Vac Concentrator SC250 EXP, supported by Thermo Scientific (Sunnyvale, CA, USA).

2.2.3. Identification and quantification of phenolic the profile of *L. citriodora by* HPLC-ESI-TOF-MS.

Separation of phenolic and non-phenolic compounds from *L. citriodora* leaves was carried out using a RRLC 1200 series Rapid Resolution LC (Agilent Technologies, Santa Clara, CA), comprised by a vacuum degasser, an autosampler, a binary pump and a diode array detector. The separation was performed by a Zorbax Eclipse Plus C18 column (Agilent Technologies,Palo Alto, CA, USA) whose dimensions were 150mm x 4.6mm id, 1.8µm. A validated multi step gradient was applied in order to separate the chemical compounds in *L. citriodora* leaves where water:acetonitrile (90:10) acidified with 0.1% of formic acid and acetonitrile were used as mobile phases. On the other hand, 10 µL of sample with a concentration of 5000 mg/L were injected in MS and the separation flow was 0.5 mL/minand was carried out at room temperature.

The HPLC system was coupled to a time-of-flight mass spectrometer (micrOTOF, BrukerDaltonik GmbH, Bremen, Germany), which comprised an electrospray interface (ESI) model G1607 from Agilent Technologies (Palo Alto, CA, USA) operating in negative ionization mode. The detection range was 50-1000 *m/z* and source parameters were selected

according to a previous work (338). In addition, quantification of polar compounds was performed preparing calibration curves of commercial verbascoside, quercetin, loganic acid and kaempferol-3-glucoside standards. Moreover, in order to avoid systematic and random errors, apigenin was added as internal standard. Individual concentrations were calculated by interpolating the ratio of target compound peak area / internal standard peak area in the pertaining calibration curve.

3. Animals, diets and experimental designs

This study was carried out in accordance with the 'Guide of the Care and Use of Laboratory animals' as promulgated by the National Institute of Health and the protocols used were approved by the Ethic committee of Laboratory Animal of the University of Granada (Spain) (Ref. No. 94-CEEA-OH 2015). Male C57BL/6J mice (7–9 weeks old) obtained from Janvier labs (St. Berthevin, Cedex, France) were housed in makrolon cages, maintained in an air-conditioned atmosphere (22 ± 1 °C,55 ± 10% relative humidity) with a 12 h light/dark cycle, and were provided free access to tap water. Mice were fed with either a standard chow diet (13% calories from fat, 20% calories from protein and 67% calories from carbohydrate (Global diet 2014; Harlan Laboratories, Barcelona, Spain)) or a HFD with 60% of its caloric content derived from fat (Purified diet 230 HF; Scientific Animal Food & Engineering, Augy, France.

For the experiment with HSE, 60 mice were randomly assigned to different groups (n = 10): control diet and control diet + HSE that received the standard chow diet, HFD and three HFD + HSE groups that were fed the HFD. Each HFD + HSE group was administered daily a different dose of HSE (1, 10 and 25 mg/kg) dissolved in water (vehicle) by oral gavage, and the control diet + HSE group was administered HSE at 25 mg/kg/day. The control diet and control + HFD groups were daily gavaged with the vehicle. The treatment was followed for 6 weeks.

For the experiment with LCE, 60 mice were randomly assigned to different groups (n = 10): control diet and control diet + LCE that received the standard chow diet, and 4 groups fed a HFD, of which three were administered daily a different dose of LCE (1, 10 and 25 mg/kg) dissolved in water (vehicle) by oral gavage. The control diet + LCE group was administered LCE at 25 mg/kg/day. The control diet and control + HFD groups were daily gavaged with the vehicle. The treatment stopped after 6 weeks.

For both experiments, the body weight of animals as well as the intake of food and water was regularly measured. Energy intake was calculated by multiplying the quantity of diet ingested (g/day/animal) by the energy density of each diet and expressed in kcal/g mouse weight/day. In addition, energy efficiency was determined by calculating the ratio between the final weight gain and the total energy intake during the period of the experiment (g/kcal) (Figure 4).

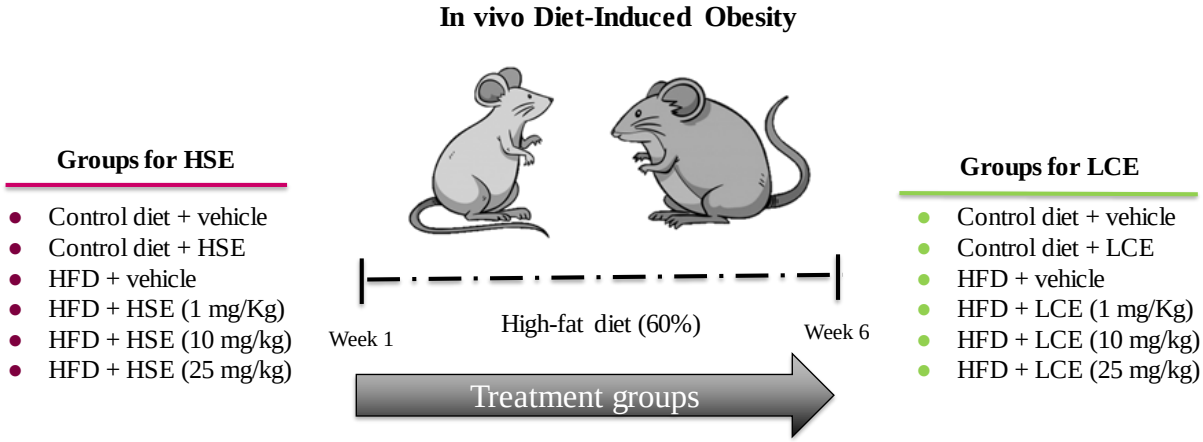

Figure 4: Experimental design in the mouse model of Diet-Induced Obesity HFD (High-fat diet); HSE (*Hibiscus sabdariffa* extract); LCE (*Lippia citriodora* extract).

4. Glucose tolerance test

One week before mice were sacrificed, a glucose-tolerance test was carried out on mice that were food deprived for 18 h (247). They received a 50% glucose solution in water at a dose of 2 g/kg of body weight by intraperitoneal injection and glucose tolerance test was performed. Tail vein blood glucose was measured just before (time 0) IP injection of glucose and 15, 30, 60 and 120 min post injection. Blood glucose was measured using a handheld glucometer (Contour XT, Ascensia Diabetes Care, S.L., Barcelona, Spain).

5. Plasma determinations

Mice were sacrificed under isoflurane anaesthesia when the treatment ended. In tubes containing heparin, blood samples were collected and centrifuged for 20 min at 5000g at 4°C, and the plasma frozen at −80 °C. Plasma glucose, TGs, LDL-cholesterol and HDL-cholesterol concentrations were measured by colorimetric methods using Spinreact kits (Spinreact, S.A., Girona, Spain). Plasma insulin levels were quantified using a mouse insulin ELISA kit (Alpco Diagnosis, Salem, NH, USA). HOMA-IR was calculated using the formula: fasting glucose (mM) fasting insulin (µU/mL)/22.

6. Morphological variables

Once mice were sacrificed, liver, colon, abdominal and epididymal fat were dissected, cleaned and weighed. Liver and fat weight indices were calculated by dividing their weights by tibia length. Then, all tissue samples were frozen in liquid nitrogen and stored at −80 °C until they were processed.

7. Analysis of gene expression by RT-qPCR

Total RNA from liver, colon and epididymal fat samples was extracted using NucleoZOL (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. RNA was reverse transcribed using oligo(dT) primers (Promega, Southampton, UK), and the resulting cDNA (20 ng) was amplified on optical grade 48-well plates in an EcoTM Real time PCR system (Illumina Inc., San Diego, CA, USA) using KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems, Wilmington, MA,USA). The specific primers used for each gene are shown in Table 5. To normalize mRNA expression, the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) was measured for comparative reference. The relative gene expression was calculated using the ΔΔCt method.

Table 5. qPCR primer sequences

Gene	Organism	Sequence 5'- 3'	Annealing T (°C)
Gapdh	Mouse	FW: CCATCACCATCTTCCAGGAG RV: CCTGCTTCACCACCTTCTTG	60
Ampk	Mouse	FW: GACTTCCTTCACAGCCTCATC RV: CGCGCGACTATCAAAGACATACG	60
Glut-4	Mouse	FW: GAGAATACAGCTAGGACCAGTG RV: TCTTATTGCAGCAGCGCCTGAG	62
Il-1β	Mouse	FW: TGATGAGAATGACCTCTTCT RV: CTTCTTCAAAGATGAAGGAAA	60
Il-6	Mouse	FW: TAGTCCTTCCTACCCCAATTTCC RV: TTGGTCCTTAGCCACTCCTTCC	60
Jnk-1	Mouse	FW: GATTTTGGACTGGCGAGGACT RV: TAGCCCATGCCGAGAATGA	60
Leptin	Mouse	FW: AGATCCCAGGGAGGAAAATG RV: TGAAGCCCAGGAATGAAGT	60
Leptin-R	Mouse	FW: GCTATTTTGGGAAGATGT RV: TGCCTGGGCCTCTATCTC	60
Mcp-1	Mouse	FW: AGCCAACTCTCACTGAAG	55

		RV: TCTCCAGCCTACTCATTG	
Muc-1	Mouse	FW: GCAGTCCTCAGTGGCACCTC RV: CACCGTGGGCTACTGGAGAG	60
Muc-2	Mouse	FW: GCAGTCCTCAGTGGCACCTC RV: CACCGTGGGGCTACTGGAGAG	60
Muc-3	Mouse	FW: CGTGGTCAACTGCGAGAATGG RV: CGGCTCTATCTCTACGCTCTCC	60
Occludin	Mouse	FW: ACGGACCCTGACCACTATGA RV: TCAGCAGCAGCCATGTACTC	56
Tff-3	Mouse	FW: CCTGGTTGCTGGGTCCTCTG RV: GCCACGGTTGTTACACTGCTC	60
Tlr-4	Mouse	FW: GCCTTTCAGGGAATTAAGCTCC RV: AGATCAACCGATGGACGTGTAA	60
Tnf-α	Mouse	FW: AACTAGTGGTGCCAGCCGAT RV: CTTCACAGAGCAATGACTCC	60
Zo-1	Mouse	FW: GGGGCCTACACTGATCAAGA RV: TGGAGATGAGGCTTCTGCTT	56

8. DNA extraction and sequencing analysis

Colonic luminal contents were collected from all mice at the end of the treatment and kept immediately at −80 °C until DNA extraction. Total DNA was isolated following the procedure described by Rodriguez-Nogales et al.(339). Amplicon fragments were PCR-amplified from the DNA in duplicate using separate template dilutions (1:10) with the high-fidelity Phusion polymerase. A single round of PCR was performed using “fusion primers”, targeting 16S V4-V5 regions with multiplexing on the Illumina MiSeq machine (Illumina Inc., San Diego, CA, USA). PCR products were verified visually by running a high-throughput Invitrogen 96-well E-gel. Any samples with failed PCRs (or spurious bands) were re-amplified by optimizing PCR conditions to produce correct bands to complete the sample plate before continuing. The PCR reactions from the same samples were pooled in one plate, then cleaned and normalized using the high-throughput Invitrogen SequalPrep 96-well Plate Kit. The samples were then pooled to make one library to be quantified fluorometrically before sequencing. After the sequencing was completed, all reads were scored for quality, and any poor quality and short reads were removed.

9. Taxonomic classification and statistical analysis

Sequences were selected to estimate the total bacterial diversity of the DNA samples in a comparable manner and were trimmed to remove barcodes, primers, chimeras, plasmids, mitochondrial DNA and any non-16S bacterial reads and sequences <150 bp. MG-RAST (metagenomic analysis server) (340) with the Ribosomal Database Project (RDP) were used for analyses of all sequences. The pipeline takes in bar coded sequence reads, separates them into individual communities by bar-code, utilizes a suite of external programs to make taxonomic assignments RDP database (341) and estimates phylogenetic diversity, with minimum e-value of 10^{-5}, minimum identity of 60% and a minimum alignment length of 15 measured in base pairs for RNA databases. Each value obtained indicated the percentage (percent relative frequency) of reads with predicted proteins and rRNA genes annotated to the indicated taxonomic level. The output file was further analysed using SPSS Statistics 17.0 Software Package (SPSS Inc., Chicago, IL, USA) and Statistical Analysis of Metagenomic Profiles (STAMP) software package version 2.1.3 (342).

10. Vascular reactivity studies

Descending thoracic aortic rings were dissected from mice and suspended in a wire myograph (model 610M, Danish Myo Technology, Aarhus, Denmark) for isometric tension measurement as previously described (21). The organ chamber was filled with Krebs solution (composition in mM: NaCl 118, KCl 4.75, NaHCO3 25, MgSO4 1.2, CaCl 2 2, KH 2PO4 1.2 and glucose 11) at 37ºC and gassed with 95% O2 and 5% CO2 (pH ~7.4). Length-tension characteristics were obtained via the myograph software (Myodaq 2.01) and the aortae were loaded to a tension of 5mN. After 90 min of stabilization period, cumulative concentration-response curves to acetylcholine (10-9 M-10-5 M) were performed in intact rings pre-contracted by U46619 (10-8 M). Relaxant responses to acetylcholine were expressed as a percentage of pre-contraction.

11. NADPH oxidase activity

To determine NADPH oxidase activity in intact aortic rings, the lucigenin-enhanced chemiluminescence assay was used, as previously described (343). Aortic rings from all experimental groups were incubated for 30 minutes at 37ºC in HEPES-containing physiological salt solution (pH 7.4) of the following composition (in mmol/L): NaCl 119, HEPES 20, KCl 4.6, MgSO4 1, Na2HPO4 0.15, KH2PO4 0.4, NaHCO3 1, CaCl2 1.2 and glucose 5.5. Aortic production of O_2 was stimulated by addition of NADPH (100 μmol/L).

Rings were then placed in tubes containing physiological salt solution, with or without NADPH and lucigenin was injected automatically at a final concentration of 5 μmol/L to avoid known artefacts when used at higher concentrations. NADPH oxidase activity were determined by measuring luminescence over 200 s in a scintillation counter (Lumat LB 9507, Berthold, Germany) in 5s intervals and was calculated by subtracting the basal values from those in the presence of NADPH. Vessels were then dried, and dry weight was determined. NADPH oxidase activity is expressed as relative luminescence units (RLU)/min/mg dry aortic ring.

12. Statistics

All results are expressed as the mean ± SEM. Statistical significance between means were tested using a one-way analysis of variance (ANOVA) and post-hoc least significance tests. Chi-squared test was used for the analysis of the Differences between proportions. All statistical analyses were carried out with the GraphPad 5.0 software package (GraphPad Software, Inc., La Jolla, CA, USA), with statistical significance set at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. Effects of *Hibiscus sabdariffa* extract (HSE) in diet-induced obesity in mice

1.1. Quantitative and chemical profile of HSE

27 compounds were identified and quantified by MS in the extract. They were classified attending to their chemical structure (Table 6). The total polar content was 373.2 ± 7.2 mg of compounds/g of HSE. Organic acids were the main constituents, being hibiscus acid the most abundant with 251 ± 3 mg/g extract, followed by the family of phenolic acids (53 ± 2 mg/g extract), with the chlorogenic acid and its derivatives as the most abundant. 13 of the identified compounds were recognized as flavonoids, ranging from 0,1027 ± 0,0003 to 3,071 ± 0,006 mg/g. The group of flavonoids was composed by derivatives of quercetin, kaempferol and myricetin, including anthocyanins like cyanidin 3-sambubioside and prodelphinidin B3.

Table 6: Quantitative results expressed in mg of compound/ g of extract. *Value* = *X* ± SD

Phytochemicals	**mg compound/g extract**
ORGANIC ACIDS	**308 ± 5**
Quinic acid	0.8471 ± 0.0002
Hydroxycitric acid	50 ± 1
Hibiscus acid	251 ± 3
Hibiscus acid hydroxyethylesther	0.974 ±0.003
Protocathechuic acid glucoside	5.85 ± 0.02
Protocatechuic acid	0.7883 ± 0.0001
PHENOLIC ACIDS	**53 ± 2**
Neochlorogenic acid	17.41 ± 0.01
Chlorogenic acid	14.95 ± 0.01
Methylchlorogenate	0.416 ± 0.001
Methyldigallate	9.575 ± 0.004
Coumaroylquinic acid	5.797 ± 0.002
Dihydroferulic acid-4-O-glucuronide	2.367 ± 0.005
Caffeoylshikimic acid isomer I	2.398 ± 0.001

Caffeoylshikimic acid isomer II	0.7216 ± 0.0002
FLAVONOIDS	***12.2 ± 0.2***
Quercetin-3-sambubioside	3.071 ± 0.006
Cyanidin-3-sambubioside	1.8777 ± 0.0003
Myricetin-3-arabinogalactoside	1.237 ± 0.002
Quercetin 3, 7, di-O-glucoside	0.1093 ± 0.0001
Myricetin-3-glucoside	0.6344 ± 0.0002
Kaempferol-3-O-sambubioside	0.1511 ±0.0001
Quercetin-3-glucoside	1.7453 ± 0.0007
Kaempferol-3-O-rutinoside	0.5713 ± 0.0005
Kaempferol-3-O-glucoside	0.2481 ± 0.0002
Myricetin	0.9116 ± 0.0001
Prodelphinidin B3	0.2239 ± 0.0002
Kaempferol 3-(p-coumarylglucoside)	0.1027 ± 0.0003
Quercetin	1.3514 ± 0.0004
Total polar content	**373.2 ± 7.2**

1.2. Effects of HSE on body weight, plasma biochemical profile and glucose tolerance test

Changes in body weight were measured twice a week in all groups during the treatment. As expected, untreated HFD-fed mice progressively increased more in body weight in comparison to the control groups that were fed the standard diet. The administration of the different doses of HSE to HFD-fed mice reduced significantly this weight gain, while it had no effect in standard diet-fed mice (Figure 4A). This reduction in body weight gain was not related to a reduction on energy intake in mice treated with HSE, as the energy intake was the same in all HFD-fed groups (Figure 4B). Nevertheless, there was a reduction in the ratio weight gain/energy intake in treated groups compared to HFD-fed mice without treatment, which suggests that HSE has probably an effect in reducing energy efficiency (Figure 4C).

Moreover, the reduction in weight gain is also evidenced in the amount of adipose tissue mass (Figure 4D and E) of HFD-fed treated groups, as it was significantly lower than untreated HFD-fed mice, although these were still higher than in those groups fed the standard diet.

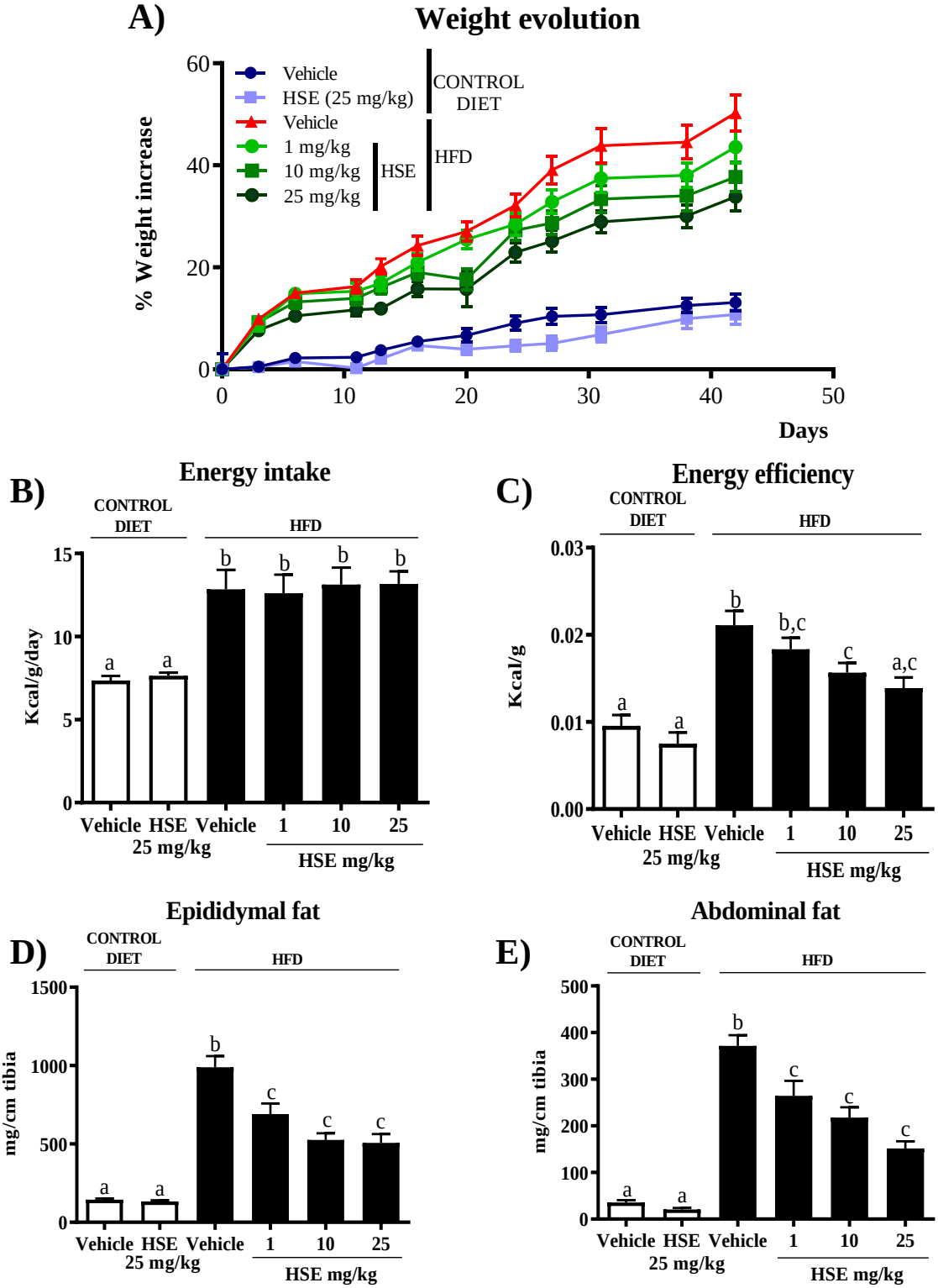

Figure 4: Effects of *Hibiscus sabdariffa* extract (HSE) (1-25 mg/kg) administration on morphological changes. **(A)** Body weight evolution, **(B)** energy intake **(C)** energy efficiency, and changes in epididymal **(D)** and abdominal **(E)** fat mass in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Obesity is also associated with an altered plasma cholesterol profile. Untreated HFD-fed mice had elevated cholesterol levels, showing higher values of both LDL-cholesterol and HDL-cholesterol than standard diet-fed mice, with increased LDL/HDL ratio. The administration of HSE at all doses to HFD-fed mice resulted in a reduced LDL/HDL ratio, even though there were no statistical differences compared to other groups (Figure 5).

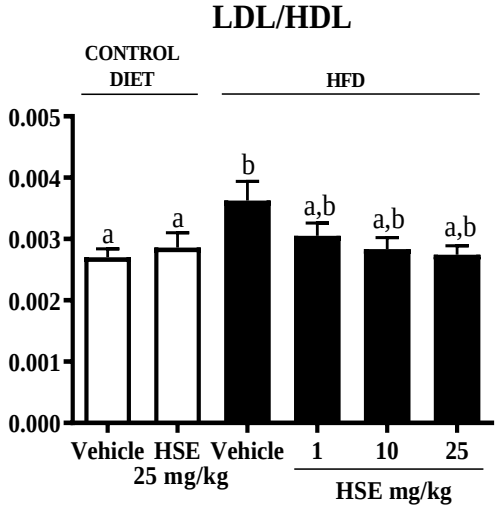

Figure 5: Effects of *Hibiscus sabdariffa* extract (HSE) (1-25 mg/kg) on LDL/HDL cholesterol plasma ratio in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Besides, the glucose metabolism was also improved in the obese mice treated with HSE. As mentioned, the week before mice were sacrificed, they were subjected to a glucose tolerance test. Blood glucose levels of all groups peaked 15 min after glucose administration (2 g/kg, IP), and decreased gradually to pre-prandial levels (Figure 6A). The glucose level peaks of HFD-fed non-treated mice were higher than the standard diet fed mice. Interestingly, administration of HSE, at all doses, reduced glucose levels from 15 min onwards in HFD-fed mice, compared to non-treated HFD-fed mice, thus reducing the area under the curve (AUC), being the values similar to those in mice receiving standard diet (Figure 6B).

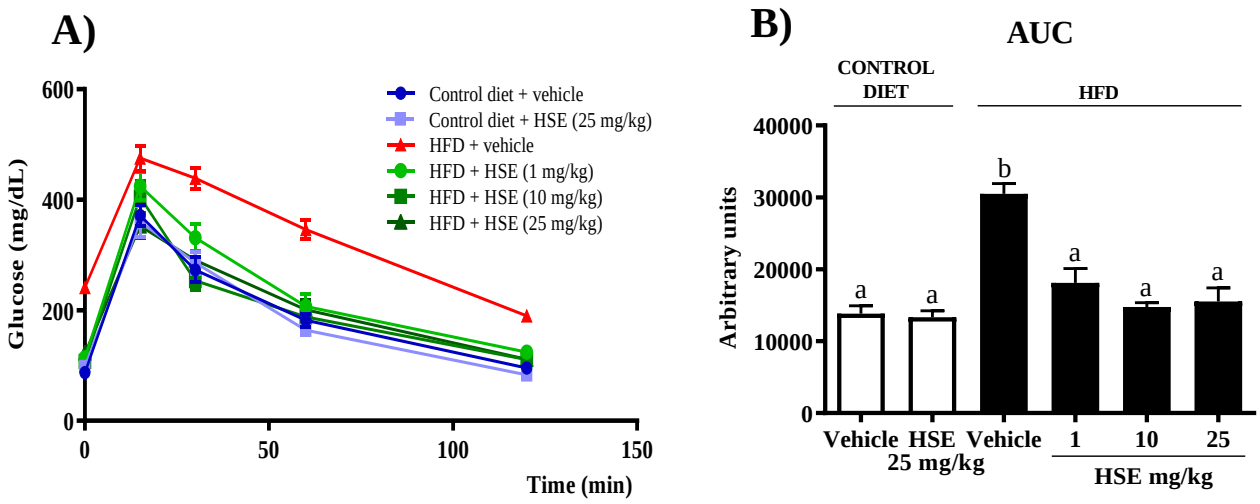

Figure 6: (A) Glucose tolerance test and **(B)** area under the curve (AUC) mass in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Accordingly, there was an improvement in fasting glycaemia (Figure 7A) and plasma insulin levels (Figure 7B) in HSE-treated HFD-fed mice in comparison with non-treated HFD-fed mice, in which both levels were increased. This is correlated with an improvement in insulin sensitivity and corroborated by a significant lower HOMA-IR index, with values close to the standard-diet fed groups (Figure 7C).

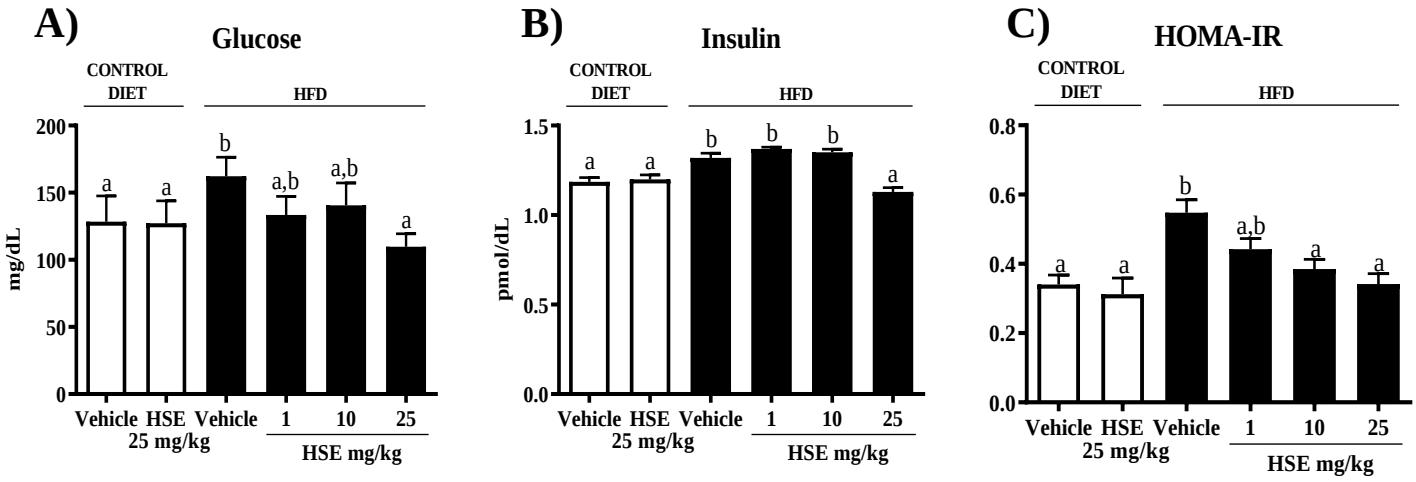

Figure 7: Effects of olive leaf extract (OLE) (1-25 mg/kg) on **(A)** glucose and **(B)** insulin levels, and **(C)** HOMA-IR index in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($P < 0.05$).

1.3. Effects of HSE on systemic inflammatory response

As mentioned before, obesity is associated with a systemic inflammatory process, which is probably related to impairment in the signalling of insulin in target tissues, including liver and fat. Actually, the expression of pro-inflammatory cytokines like *Tnf-α*, *Il-1β*, *Il-6* and *Mcp-1*

increased in liver (Figure 8A) and/or fat tissue (Figure 8B) in non-treated HFD-fed mice compared to groups fed a standard diet. The expression of these inflammatory markers were reduced by the treatment with HSE, at all doses, in liver, but only the highest dose evaluated had an effect in reducing *Tnf-α* and *Il-6* expression in fat.

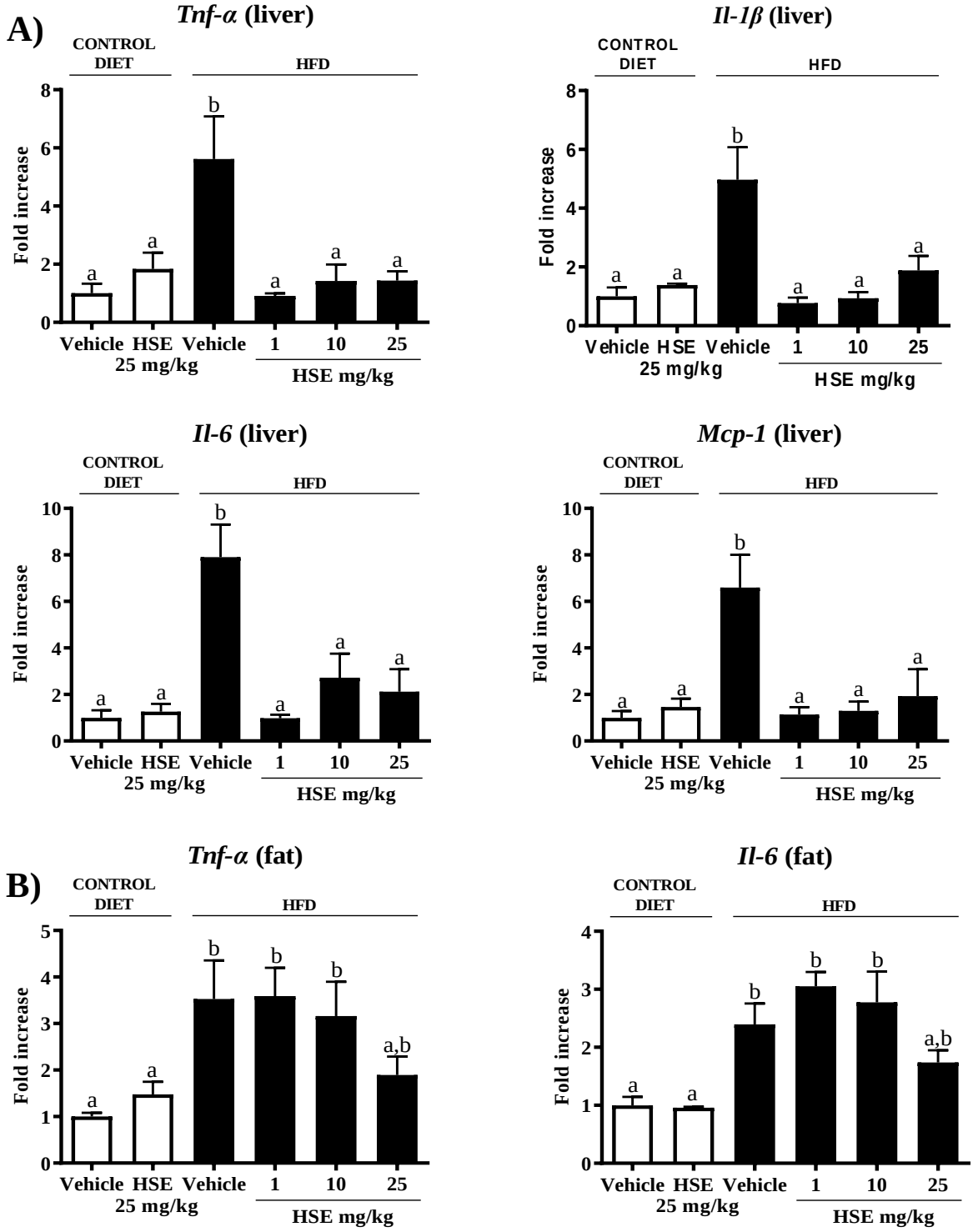

Figure 8: Effects of *Hibiscus sabdariffa* extract (HSE) on **(A)** liver gene expression of *Tnf-α*, *Il-1β*, *Il-6* and *Mcp-1*and **(B)** fat gene expression of *Tnf-α* and *Il-6* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Previous investigations on inflammatory cytokine expression have pointed the critical role of JNK in the production of inflammation in metabolic tissues. Actually, the hepatic expression of *Jnk-1* was significantly increased in non-treated HFD-fed mice in comparison with standard diet fed mice, which was reduced by the administration of HSE to similar values as standard diet fed mice (Figure 9).

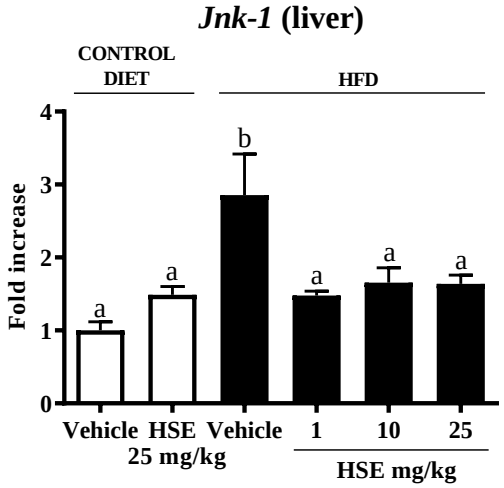

Figure 9: Effects of *Hibiscus sabdariffa* extract (HSE) (1-25 mg/kg) on expression of *Jnk-1* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Moreover, leptin, an adipokine secreted by the adipose tissue, has been reported to display a pro-inflammatory role, stimulating macrophage activation and production of pro-inflammatory cytokines, and later promoting insulin resistance. As expected, the expression of leptin (Figure 10A) and its receptors were impaired in fat (Figure 10B) and liver (Figure 10C) of HFD-fed mice, which evidences leptin resistance associated to obesity. The administration of HSE significantly improved the expression of the receptors in liver, but not in fat tissue, although the expression of the adipokine was significantly reduced.

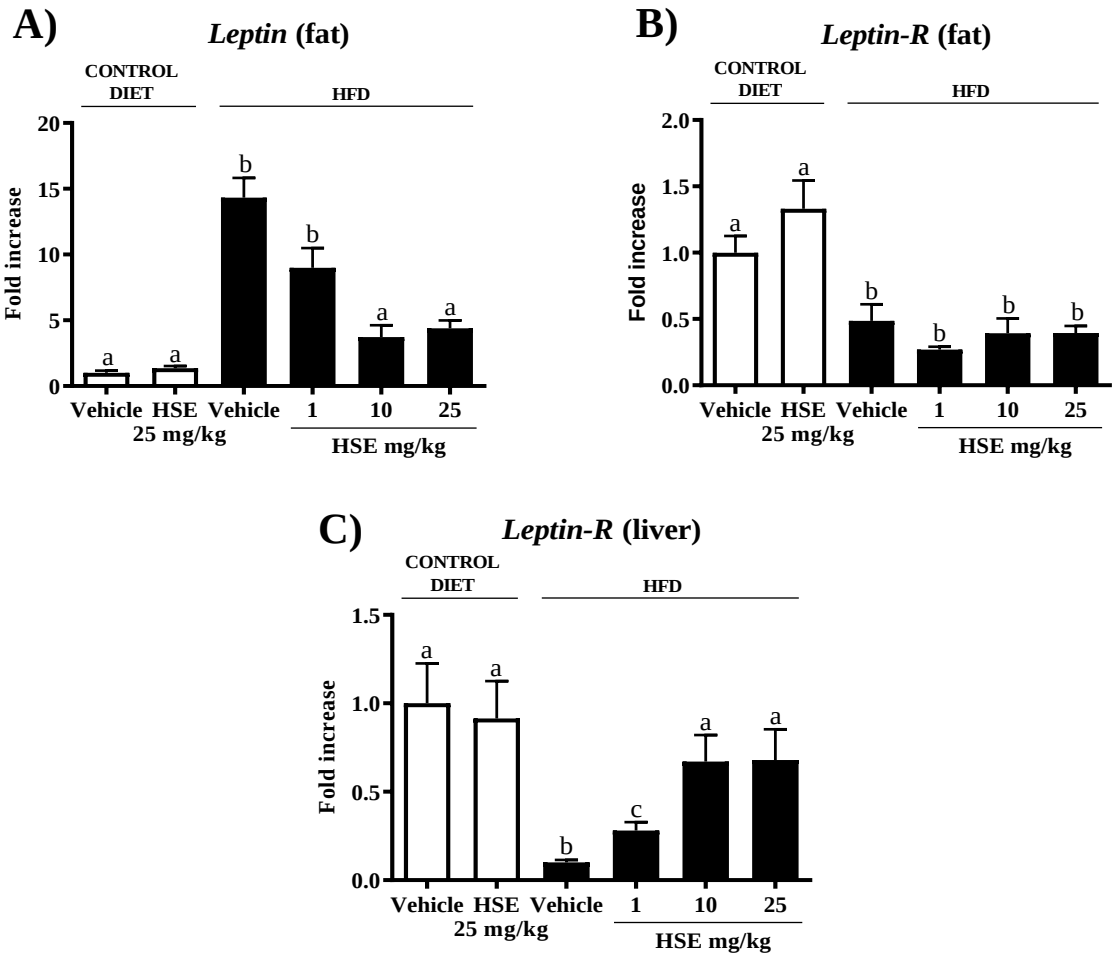

Figure 10: Effects of *Hibiscus sabdariffa* extract (HSE) on fat gene expression of **(A)** *Leptin* and **(B)** *Leptin R* and **(C)** liver gene expression of *Leptin-R* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

A decrease in the expression of the glucose transporter *Glut-4* is associated with the impairment in glucose metabolism and insulin resistance observed in obese mice. In fact, untreated HFD-fed mice showed a reduced expression of *Glut-4* in fat, while HSE-treated mice presented a normal expression in the case of the two highest doses (Figure 11A). Moreover, AMPK has a key role in the translocation of GLUT-4 to the plasma membrane, but it has also been suggested to suppress the activation of the NF-κB pathway, thus suppressing the expression of different pro-inflammatory cytokines. As expected, the expression of *Ampk* was decreased in non-treated HFD-fed mice, both in liver and in adipose tissue (Figure 11B and C). However, the treatment with HSE ameliorated the hepatic expression of *Ampk* in HFD mice, having no effect in adipose tissue.

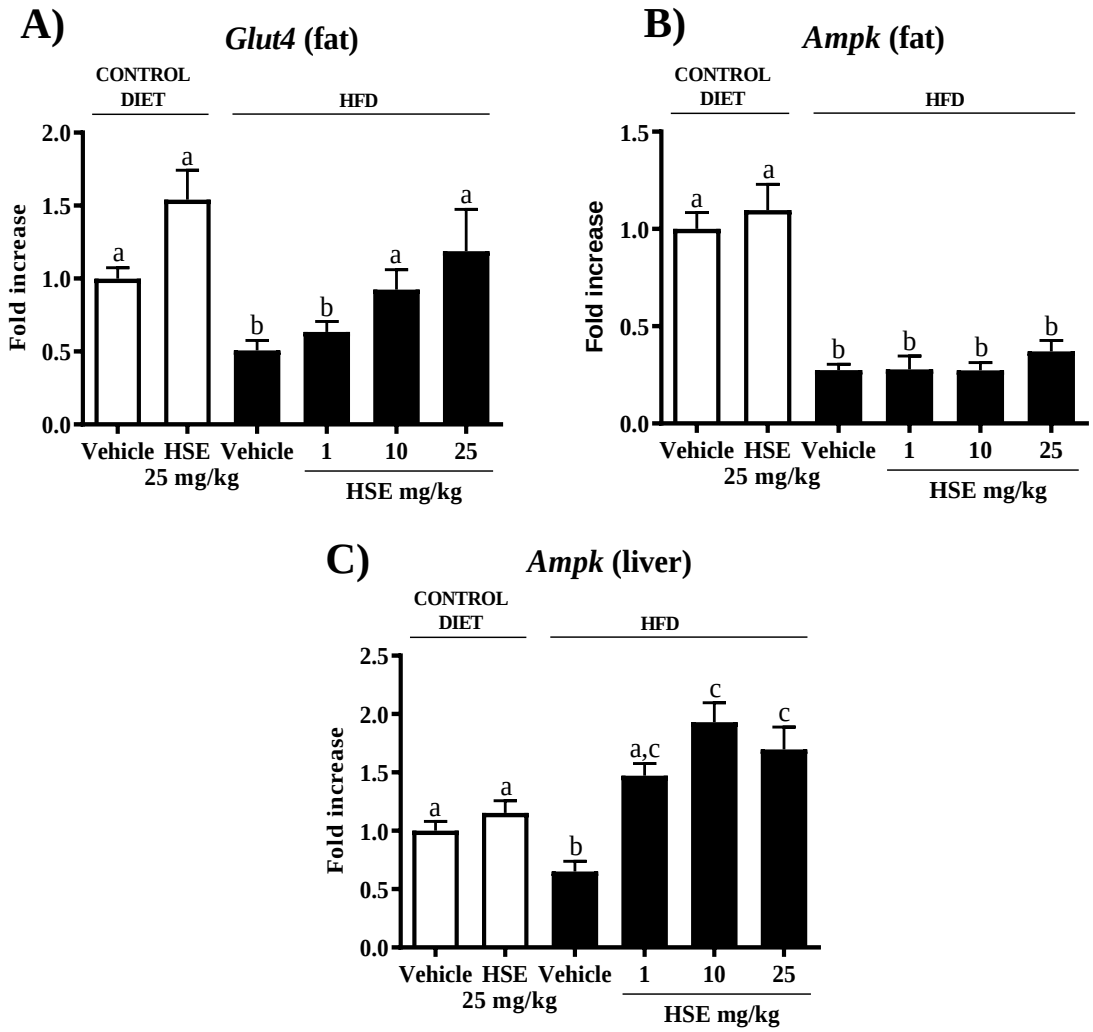

Figure 11: Effects of *Hibiscus sabdariffa* extract (HSE) on fat gene expression of **(A)** *Glut-4* and **(B)** *Ampk*, and **(C)** liver gene expression of *Ampk* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Furthermore, different studies have reported an altered intestinal permeability in obesity, allowing the systemic access of bacterial products, such as LPS, that enhance the systemic inflammatory response. This was confirmed in this study, since the expression of some epithelial integrity markers, such as the mucins *Muc-1*, *Muc-2* and *Muc-3*, *Occludin*, *Zo-1* or *Tff-3*, were downregulated in comparison to lean mice. HSE treatment also improved the expression of most of these proteins (Figure 12).

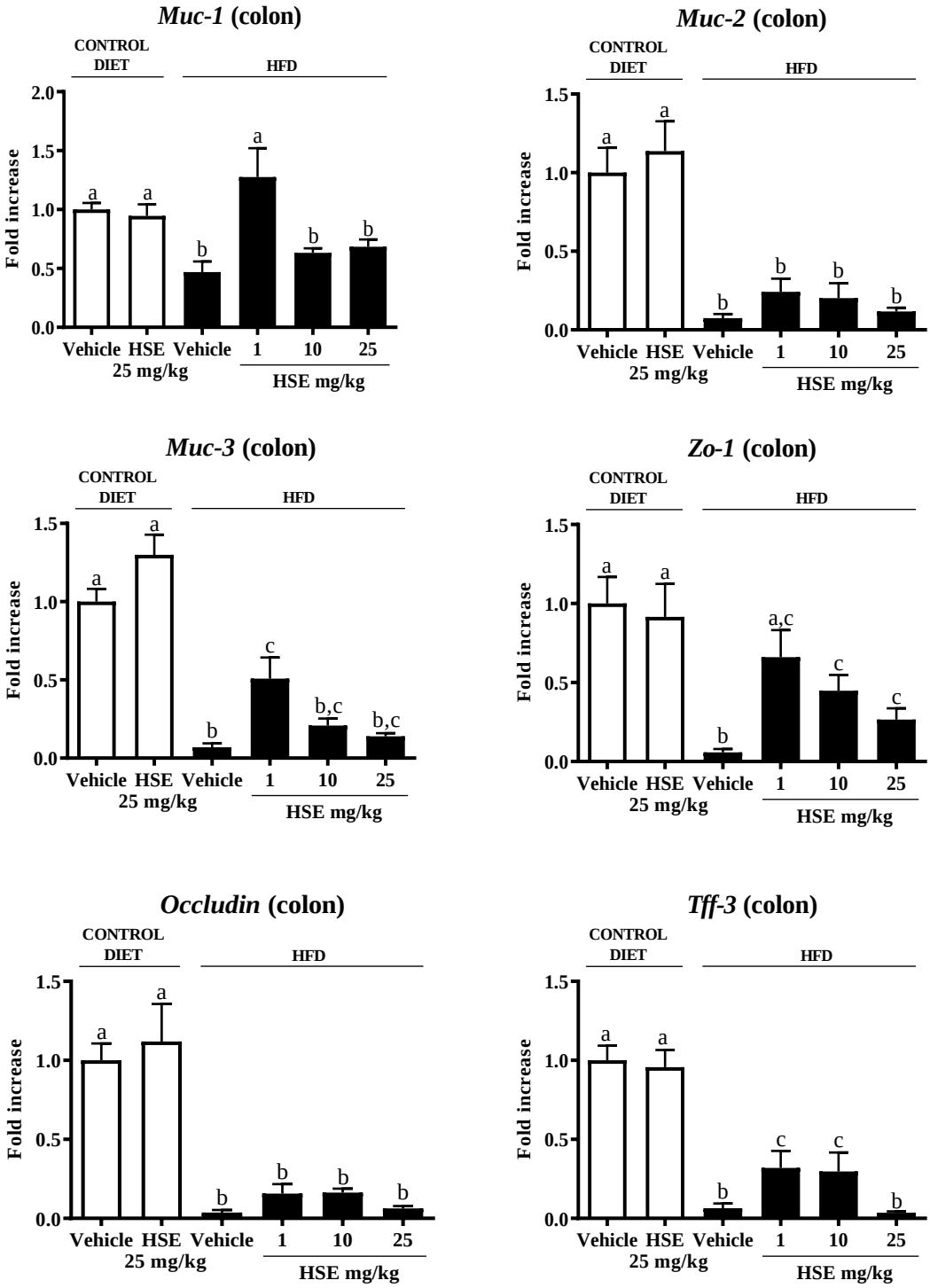

Figure 12: Effects of *Hibiscus sabdariffa* extract (HSE) on colonic gene expression of *Muc-1*, *Muc-2*, *Muc-3*, *Zo-1*, *Occludin* and *Tff-3* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

In fact, hepatic expression of *Tlr-4* increased significantly in non-treated obese mice, while it was reduced by the treatment (Figure 13).

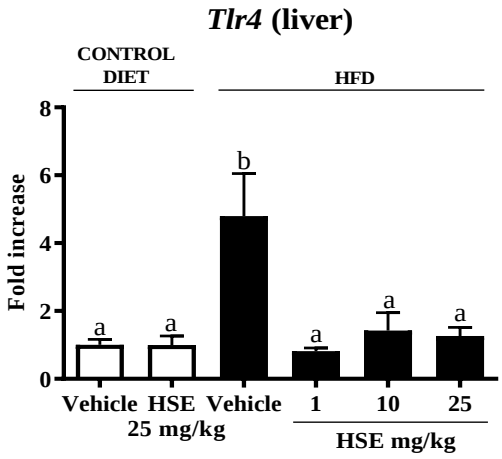

Figure 13: Effects of *Hibiscus sabdariffa* extract (HSE) on hepatic gene expression of *Tlr-4*in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

1.4. Effects of HSE on gut microbiota dysbiosis

The ability of phenolic compounds to exert prebiotic effects has been reported by different studies. They have shown to be able to modulate the gut microbiota composition, enhancing the intestinal barrier function, and therefore limiting the associated endotoxemia. When faecal microbiota was evaluated by 16S ribosomal DNA sequencing, the principal component analysis (PCoA) based on Bray-Curtis separation revealed two separated bacterial communities for control-diet and non-treated HFD-fed mice. The bacterial communities for the HSE treated HFD-fed groups were closer to control diet-fed groups than to the non-treated HFD-fed mice (Figure 14). Moreover, the analysis revealed that *Bacteroidetes* and *Firmicutes* were the most abundant phyla found in the microbiota. As previously reported, the HFD altered gut microbiota composition, significantly increasing *Firmicutes* (from 38.385 to 62.99%) and reducing *Bacteroidetes* (from 38.34% to 3.90%) in non-treated HFD-fed mice, in comparison to the standard diet-fed mice. The administration of HSE at the highest doses, 10 and 25 mg/kg, significantly improved this shift to values similar to the standard-diet fed mice (Figure 15A). The *Firmicutes*/*Bacteroidetes* ratio, which is used as a biomarker for health status, was also calculated. The F/B ratio in non-treated HFD-fed mice significantly increased in comparison to standard diet-fed mice. As expected, there was a decrease in the ratio of HFD-fed treated mice (Figure 15B).

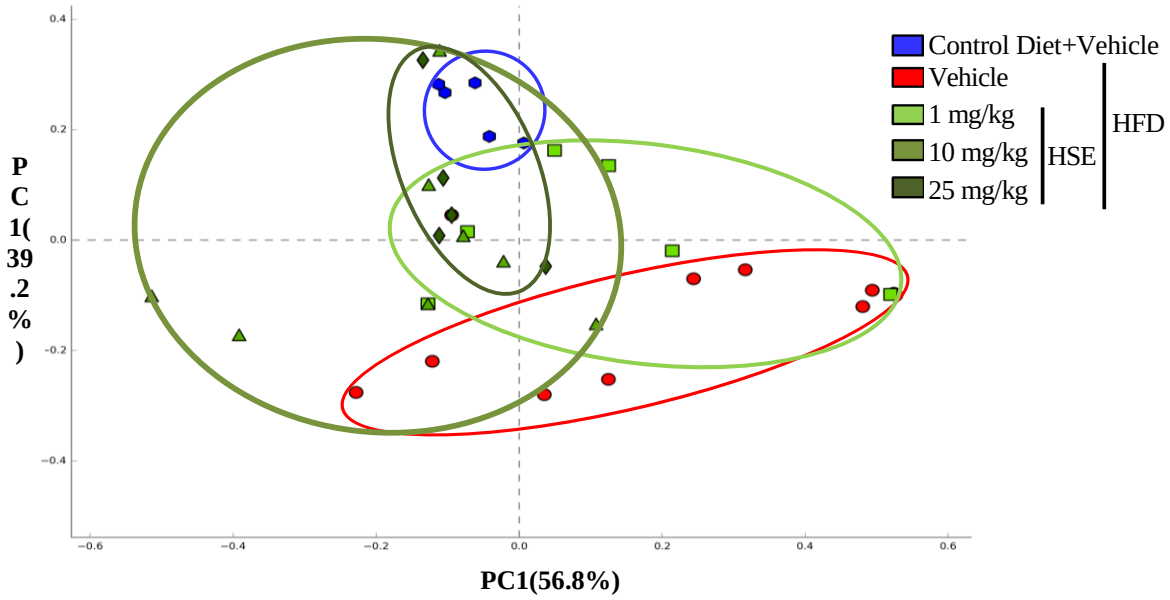

Figure 14: Comparison of faecal microbiota composition between control and high-fat diet (HFD)-fed mice: Principal component analysis plot based on Bray–Curtis distances, calculated on the metagenomic table of faecal samples of the different groups; Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p \leq 0.05$).

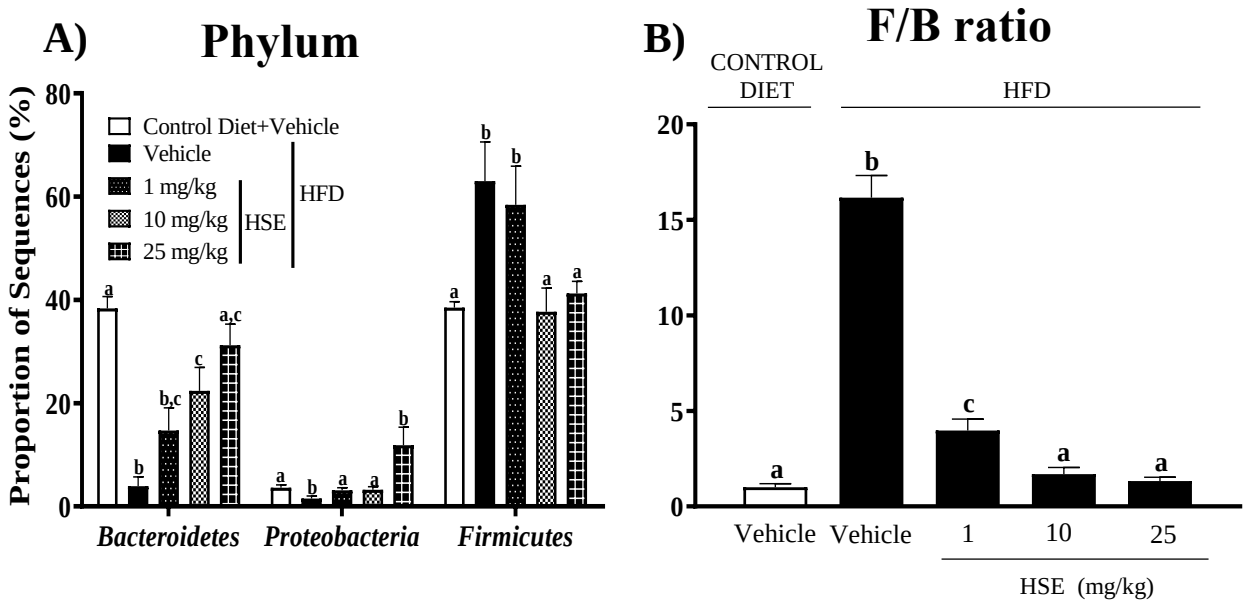

Figure 15: Comparison of faecal microbiota composition between control and high-fat diet (HFD)-fed mice: **(A)** Phylum breakdown of the most abundant bacterial communities in the different groups. **(B)** The *Firmicutes*/*Bacteriodetes* ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p \leq 0.05$).

At genus level, some differences were also observed. Non-treated HFD-fed mice had decreased richness of *Leuconostoc*, *Syntrophomonas*, *Halomonas* and *Phyllobacterium*. In HFD-fed mice treated with HSE there was an increase in *Caulobacter*, *Syntrophomonas*, *Halomonas* and *Phyllobacterium*, and the lowest dose of HSE (1 mg/kg) was also able to increase the abundance of *Leuconostoc* (Figure 16A). At order level, the abundance of *Actinomycetales*, *Lactobacillales* and *Rhizobiales* was increased, and the abundance of *Bifidobacteriales*, *Flavobacteriales*, *Bacillales*, *Burkholderiales* and *Rickettsiales* was decreased in non-treated HFD-fed mice compared to standard diet-fed mice. The administration of HSE at all doses reversed this situation in all these genera, except the *Bifidobacteriales* by the highest dose (Figure 16B).

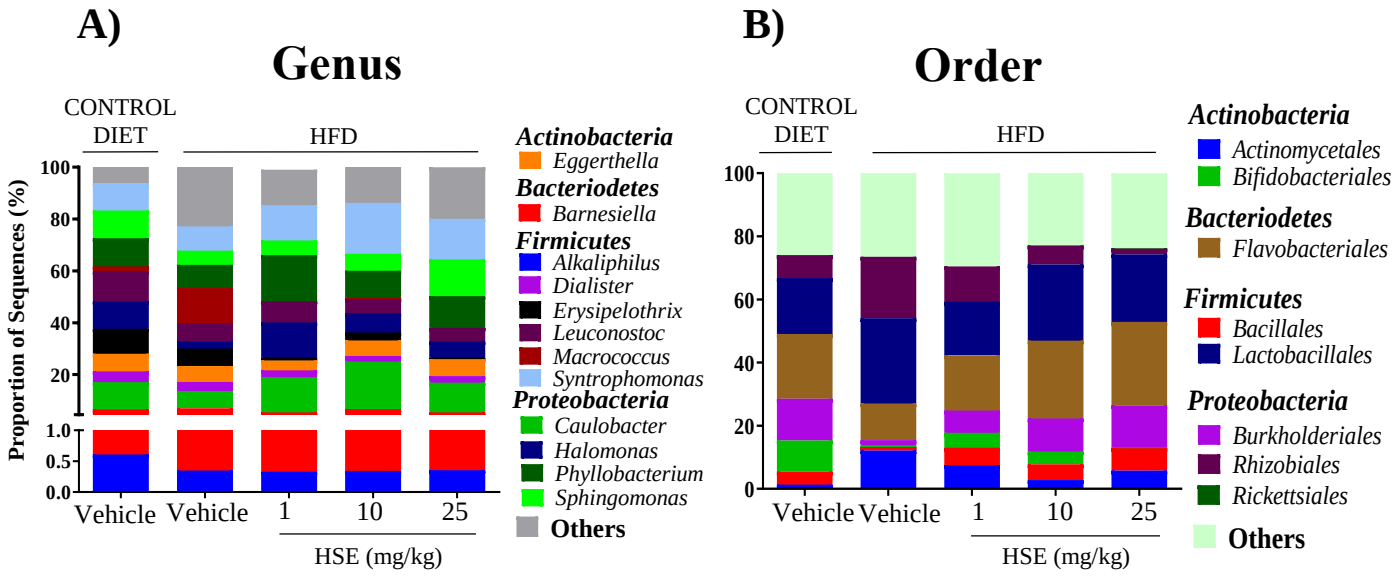

Figure 16: Comparison of faecal microbiota composition between control and high-fat diet (HFD)-fed mice: Phylum breakdown of the most abundant bacterial communities in the different groups. Proportion of sequences: **(A)** genera and **(B)** order taxa. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p \leq 0.05$).

Discussion

The use of alternative and/or complementary therapies in obese patients has become very frequent in the absence of safe and effective pharmacological treatments. In this regard, the use of medicinal plants is rising, not just because they are considered safe remedies, but also because they have been proven to be effective in the treatment of obesity and related disorders, which is linked to the presence of different biologically active compounds.

In the first study, a well-characterized HSE has been assayed in HFD-fed mice, exploring its effects on obesity and other MetS related dysfunctions.

The administration of HSE at different doses, 1, 10 and 25 mg/kg, ameliorated body weight gain induced by the high-fat diet and, consequently, decreased the amount of fat pads, although the treatment was not able to normalize the values. This effect was not due to a lower energy intake, but to a decrease in energy efficiency. Consequently, this would mean that the reduction of complications related to the consumption of a high-fat diet, such as dyslipidemia and glucose intolerance, are no associated with an anorexigenic effect. Moreover, it is important to note that the administration of the highest dose of HSE to standard die-fed mice showed no effect.

Hence, the beneficial effects of the extract were also related to an improvement in the glucose homeostasis and insulin resistance that obese mice display, as evidenced by the reduction in glucose levels observed in the glucose tolerance test and the lower HOMA-IR index in HSE-treated HFD-fed mice. Similarly, HSE administration ameliorated obesity associated alterations in lipid metabolism since it decreased the LDL/HDL ratio in comparison with the non-treated HFD-fed group. This results support previous investigations that assess the effects of *H. sabdariffa* on impaired lipid and glucose metabolism both in human (309, 330) and in experimental models of obesity and MetS in rodents (305, 306, 331, 344).

The metabolic changes induced by HFD, which are associated with an impaired insulin signalling in insulin-dependent tissues, such as skeletal muscle, liver and adipose tissue, have been associated with a state of systemic inflammation (11, 12). Actually, obesity is characterized by a chronic inflammatory state characterized by an increase in the production of pro-inflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6 and MCP-1 (3). All doses of HSE administered were able to reduce the expression of these inflammatory markers in liver, but only the highest dose *Tnf-α* and *Il-6* had an effect on adipose tissue, only in. Indeed, these both cytokines could promote insulin resistance and increase inflammation, as they have been described to increase the oxidation of fatty acids and lipolysis (13). Moreover, *in-vitro* studies have shown the specific actions attributed to these cytokines, which can account for the pathogenesis of obesity: IL-6 reduces the expression of *Adiponectin*, *Glut-4* and *IRS-1*, whereas TNF-α increases secretion of MCP-1 and IL-6 from preadipocytes(345). Thus the extract can improve altered glucose and lipid metabolism of HFD fed mice by acting on the systemic inflammatory response. Of note, polyphenol derivatives of HSE, including anthocyanins and flavonols, are well known for their antioxidant properties, which can synergistically act through this anti-inflammatory effect (346, 347). Besides, HSE can exert beneficial effects in experimental obesity targeting other

proteins, both in a direct or indirect ways. For instance, quercetin, which is a flavonoid present in HSE, has an anti-adipogenic effect *in-vitro* in 3T3-L1 preadipocytes, through the modulation of AMPK and JNK pathways (258). Actually, the expression of *Ampk* and *Jnk-1* in liver after treatment with HFD-fed mice was reduced, although *Ampk* in the fat tissue was not positively affected. AMPK is a signalling protein considered a nutrient sensor, which is suggested to act linking nutrient metabolism and inflammation in different target tissues. AMPK regulates lipid and glucose metabolism associated metabolic pathways, integrating nutritional and hormonal signals in periphery and hypothalamus (348). Thus, the activation of AMPK results in stimulation of ATP production, by increasing muscle glucose transport, FAs oxidation, mitochondrial biogenesis and caloric intake, while inhibits ATP-consuming anabolic pathways, such as FA and cholesterol synthesis, as well as hepatic gluconeogenesis (349, 350). Moreover, AMPK regulates inflammation signalling in different target tissues and immune cells, like macrophages, which could contribute to the development of insulin resistance in obesity. Actually, the downregulation of AMPK signalling has been associated with the inflammatory response in macrophages, whereas its activation reduces it (351), thus preventing inflammation-induced insulin resistance. In fact, metformin, an insulin sensitizer drug for diabetic individuals, acts mainly in the liver by stimulating AMPK pathway (352), as it is seen in the present study. The fact that HSE did not alter the expression of *Ampk*, but ameliorated the expression of the pro-inflammatory cytokines evaluated in fat (*Tnf-α* and *Il-6*) and the insulin resistance, particularly at the higher doses, suggest that its beneficial effects imply other signalling pathways or mechanisms in this target tissue. In this sense, leptin is considered a pro-inflammatory adipokine that stimulates insulin resistance through the activation of different immune cells present in different target tissues, including liver and adipose tissue (353). Actually, the expression of *Leptin* and its receptor was impaired in HFD-fed mice in those tissues, as previously reported (354, 355). Nonetheless, the expression of *Leptin-R* was improved in liver but not in adipose tissue, and the expression of *Leptin* was decreased, which could be implicated in the improvement of the state of insulin resistance. On the other hand, the implication of the glucose transporter GLUT-4 in intracellular insulin-dependent glucose uptake in adipocytes is well known, and its expression is reduced in adipose tissue as an indication of insulin resistance status (356), which agrees with the results obtained in our study. Furthermore, we observed that the administration of HSE to obese mice increased the expression of *Glut-4* in fat tissue, which could be related to an increased glucose uptake by adipocytes, with the consequent decrease of its serum levels, and thus enhancing insulin sensitivity.

The chronic inflammatory state observed in obese subjects has also been related to an endotoxemic status, as a consequence of augmented LPS plasma levels (5). Moreover, studies in humans and in rodent models fed a fat-enriched diet have addressed the correlation of increased LPS plasma levels with impairment in insulin signalling, including increased *Tlr-4* expression, which would also increase the production of pro-inflammatory mediators (172). This has been also observed in our study, as HFD-fed mice showed increased expression of this receptor, while HSE administration decreased it, suggesting an improvement in the endotoxemia-associated inflammatory process. In addition, the activation of AMPK in liver, as occurs in HFD-fed mice treated with HSE, could prevent LPS-stimulated expression of pro-inflammatory cytokines and NF-κB signalling in several cell types (357).

In this regard, there is a close association between obesity and increased LPS plasma levels, augmented intestinal permeability, and gut dysbiosis (357). This study also showed the presence of impaired epithelial barrier function in obese mice, with downregulated expression of mucins *Muc-1* and *Muc-3*, ant the peptide *Tff-3*, which is expressed by goblet cells and synergizes with mucins to improve the protective barrier function of the mucus layer (358). In addition, *ZO-1* expression was downregulated. This is linker protein in tight junctions that acts in association with transmembrane protein occludin (359). Interestingly, HSE treatment improved the expression of these proteins, which suggests an enhancement in epithelial barrier functionality, which could reduce the obesity-associated endotoxemia.

Many metabolic conditions, which include obesity and diabetes, are linked to an altered gut microbiota composition, termed as dysbiosis (360), which is also observed in experimental models of obesity in mice (361). Of note, recent studies have demonstrated the capacity of different phenolic extracts to modulate the gut microbiota, rebalancing *Firmicutes/Bacteroidetes* ratio (362), which is considered as a parameter of health status (19), and increasing SCFAs producing bacteria (264), among others. This is in correlation with the results observed in our study, as HFD-fed mice have a shift in the F/B ratio, which was reversed after the HSE administration. This indicates that HSE has the capacity to modulate the intestinal microbiota composition, having a positive influence on diet induced gut dysbiosis, and thus revealing that is has prebiotic properties. Actually, it has been suggested that polyphenols and their metabolites repress the growth of *Firmicutes*, favouring the balance to *Bacteroidetes* in the gut (363), as is the case of inulin and fructooligosaccharides, galactooligosaccharides and cyclodextrins, that are able to modulate the microbiota of obese and diabetic mice (364).

In conclusion, *Hibiscus sabdariffa* extract exerts beneficial effects ameliorating diet-induced obesity, insulin resistance, and lipid profile in high-fat diet fed mice. These effects are associated with amelioration of systemic inflammatory response and improvement on the altered intestinal permeability that characterizes obesity. Moreover, HSE seems to have the ability to modulate gut microbiota composition, counteracting obesity-associated intestinal dysbiosis. Thus, HSE can be a potential candidate to use as nutritional complement with prebiotic effects for the management of MetS.

2. Effects of *Lippia citriodora* extract (LCE) in diet-induced obesity in mice

2.1. Quantitative and chemical profile of LCE

After MS analysis, a total of 36 phytochemicals were identified, quantified and classified by its chemical structure (Table 7). Thus, the total polar content was 147 mg of compounds/g of LCE. The most abundant chemical group belonged to the family of phenylpropanoids, with 91 mg/g of LCE, being verbascoside the most representative compound, being, approximately, 63% of the phenylpropanoids and 40% of total polar content. Iridoids were the second major family found in the extract, with 29 mg/g of LCE. In this family, the most abundant compound was the theveside. Finally, at a similar content as iridoids, the group of flavonoids contributed with 27 mg/g LCE. In this case, chrysoeriol 7 diglucuronide (13.3 mg/g of LCE) and luteolin 7 diglucuronide (6.2 mg/g of LCE) were the most abundant, chrysoeriol 7 diglucuronide being the second major compound found in the extract, after verbascoside.

Table 7: Quantitative results expressed in mg of compound/ g *L. citriodora* of extract used in *in-vitro* assay. *Value* = *X* ± SD

Phytochemicals	mg compound/g extract
PHENYLPROPANOIDS/PHENYLETHANOIDS	**91± 8**
Verbascoside	58 ± 5
Isoverbascoside	5.6 ± 0.8
β -Hydroxyverbascoside	4.5 ± 0.4
β - Hydroxyisoverbascoside	4.2 ± 0.6
Verbascoside A	3 ± 0.2
Martynoside	3 ± 0.1

Verbasoside	2.4 ± 0.1
Leucoseptoside A	2.1 ± 0.1
Campneoside I	1.97 ± 0.08
Forsythoside A	1.6 ± 0.4
Osmanthuside B	1.4 ± 0.1
Cistanoside F	1.41 ± 0.05
Lariciresinol	1.1 ± 0.1
Lipedoside A I	0.333 ± 0.009
Descaffeoylcrenatoside	0.16 ± 0.02
β - Hydroxyisoverbascoside derivative	0.10 ± 0.01
β - Hydroxyverbascoside derivative	0.02 ± 0.01
IRIDOIDS	**29 ± 2**
Theveside	6.8 ± 0.3
Gardoside	4.4 ± 0.4
Ixoside	4.0 ± 0.2
Durantoside I	2.2 ± 0.2
Lippioside I	1.98 ± 0.05
Lippioside II	1.9 ± 0.1
Lippianoside B	1.69 ± 0.09
Hydroxycampsiside	1.50± 0.07
Lippioside I derivative	1.40 ± 0.06
Teucardoside	1.18 ± 0.05
Manuleoside H	1.05 ± 0.03
Myxospyroside	0.86 ± 0.04
FLAVONOIDS	**27± 2**
Chrysoeriol 7 diglucoronide	13.3 ± 0.3
Luteolin 7 diglucuronide	6.2 ± 0.6
Acacetin 7 diglucoronide	5.0 ± 0.5
Apigenin 7 diglucoronide	1.10 ± 0.04
Dimethyl quercetin	1.0 ± 0.1

Dimethyl kaempferol	0.10 ± 0.06
Methyl quercetin	NQ
Total Polar Content	**147 ± 12**

*NQ: compound detected but below quantitation limit.

2.2. Effects of LCE on body weight, plasma biochemical profile and glucose tolerance test

The present study also evaluated body weight changes in all control groups, which showed an increase in untreated HFD-fed mice during the 6 weeks that lasted the experiment, in comparison to standard diet-fed mice. LCE administration significantly reduced body weight gain in HFD-fed mice, showing no effect in standard diet-fed mice (Figure 17A). Moreover, administration of LCE was not related to a reduction in energy intake (Figure 17B), as it was the same in all HFD-fed groups. However, the reduction in the ratio weight gain/energy intake in LCE-treated HFD-fed mice suggests that LCE probably produces a reduction in energy efficiency (Figure 17C).

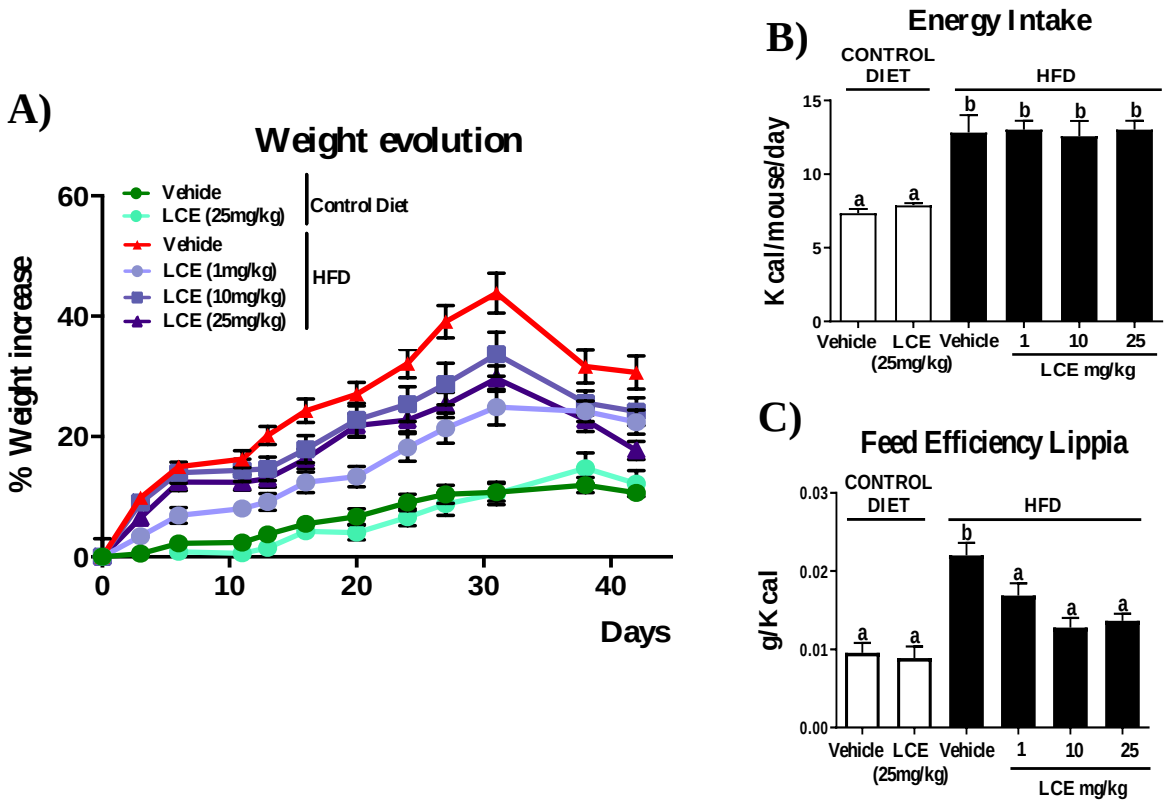

Figure 17. Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) administration on morphological changes. **(A)** Body weight evolution, **(B)** energy intake and **(C)** energy efficiency in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Consistent with body weight data, the adipose tissue mass, both epididymal and abdominal, was also increased in untreated HFD-fed mice in comparison with standard diet-fed mice (Figure 18). As expected, the LCE treatment significantly reduced the fat pads, although statistical differences were still found when comparing to standard diet-fed mice.

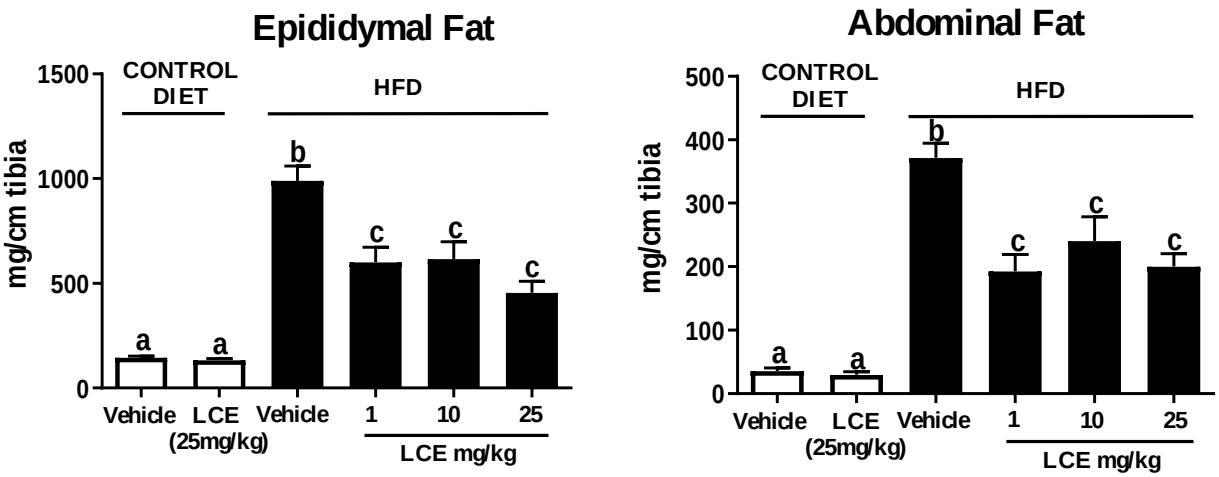

Figure 18: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) administration on epididymal and abdominal fat mass in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Obesity is also associated with hypercholesterolemia, which was also observed in our study. In non-treated HFD-fed mice, high values of both LDL- and HDL-cholesterol and, thus, an increased LDL/HDL ratio were seen. However, LCE reduced LDL-cholesterol levels without changing HDL-cholesterol, which remained high, thus reducing LDL/HDL ratio to similar levels observed in standard diet-fed mice (Figure 19).

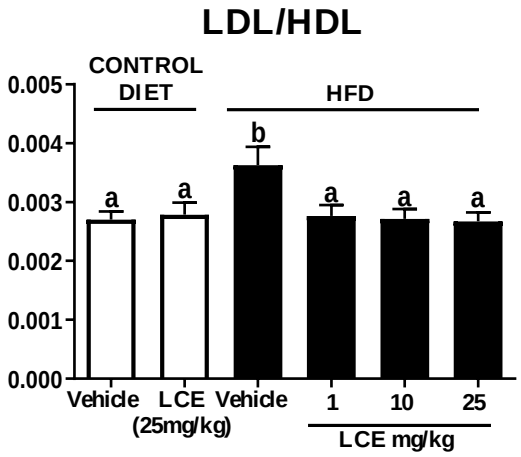

Figure 19: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) administration on LDL and HDL-cholesterol plasma levels in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($P < 0.05$).

Moreover, deficiencies in glucose metabolism were also improved with the treatment with LCE. When the glucose tolerance test was performed, plasma glucose levels at all-time points in mice treated with LCE where lower than untreated HFD-fed mice, which resulted in reduced AUC values, similar to the standard-diet group (Figure 20A and B).

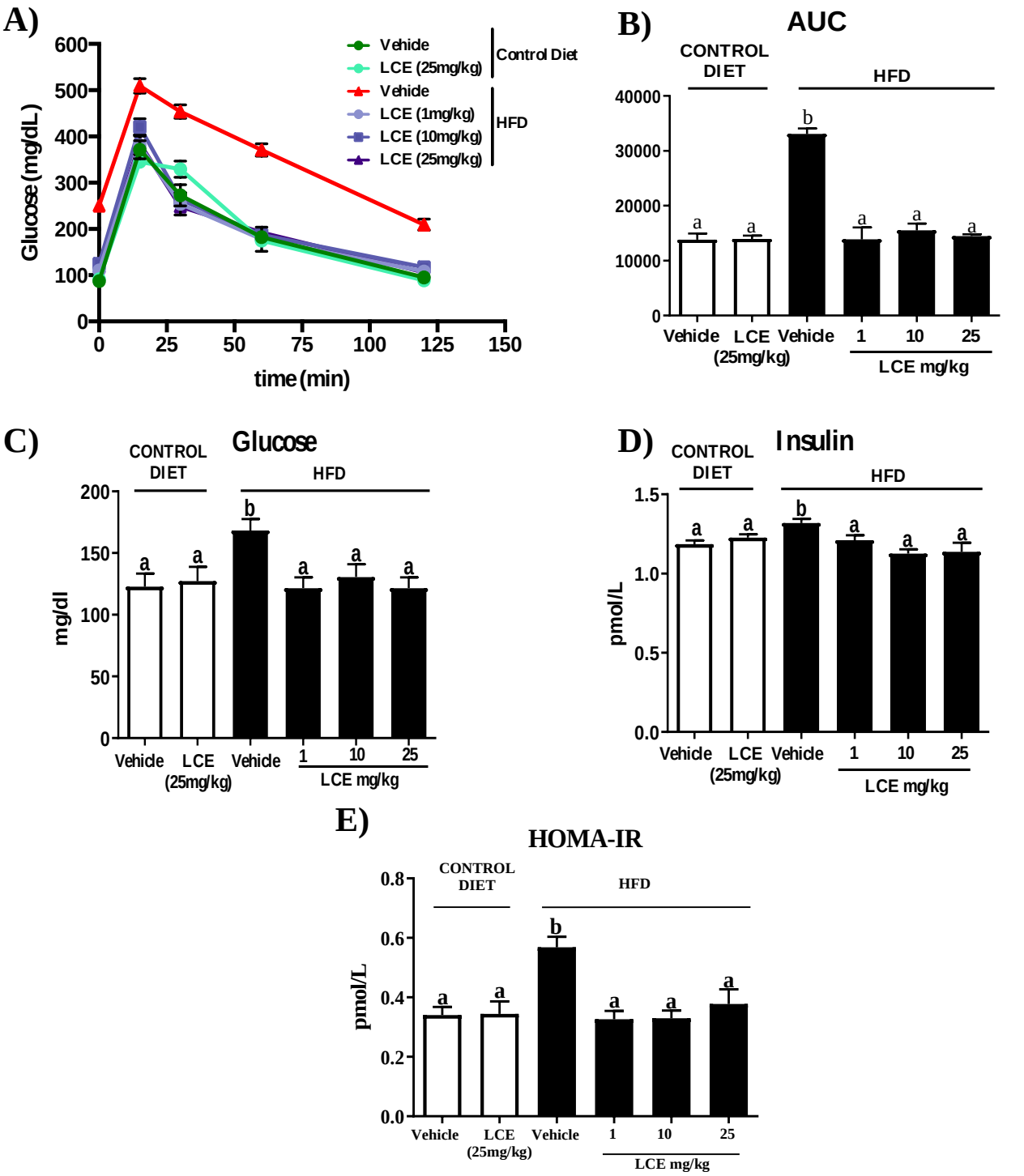

Figure 20: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) administration on **(A)** Glucose tolerance test and **(B)** area under the curve (AUC); **(C)** basal glucose, **(D)** insulin levels and **(E)** HOMA-IR index, and; Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ (P< 0.05).

When glucose homeostasis was evaluated at the end of the experiment, fasting glycaemia of untreated HFD-fed mice was significantly higher than in standard diet fed mice (Figure 20C). Moreover, the treatment with LCE reduced these values, which were similar to standard diet-fed mice. Accordingly, LCE treated mice showed reduced insulin levels, similar to standard-diet fed mice, in comparison to untreated HFD-fed mice, which showed hyperinsulinemia (Figure 20D). This resulted in lower HOMA-IR values in LCE treated HFD-fed mice, similar to standard diet-fed mice, with significant differences with non-treated HFD-fed mice (Figure 20E).

2.3. Effects of LCE on systemic inflammatory response

The subclinical inflammatory state in obesity is also clear in this study, as the untreated HFD-fed group has increased expression of *Tnf-α* and *Il-6* in both liver and adipose tissue (Figure 21). The treatment with LCE significantly reduced the expression of both cytokines in liver (Figure 21A). However, in fat, only *Tnf-α* was reduced by the lowest dose (1 mg/kg) (Figure 21B).

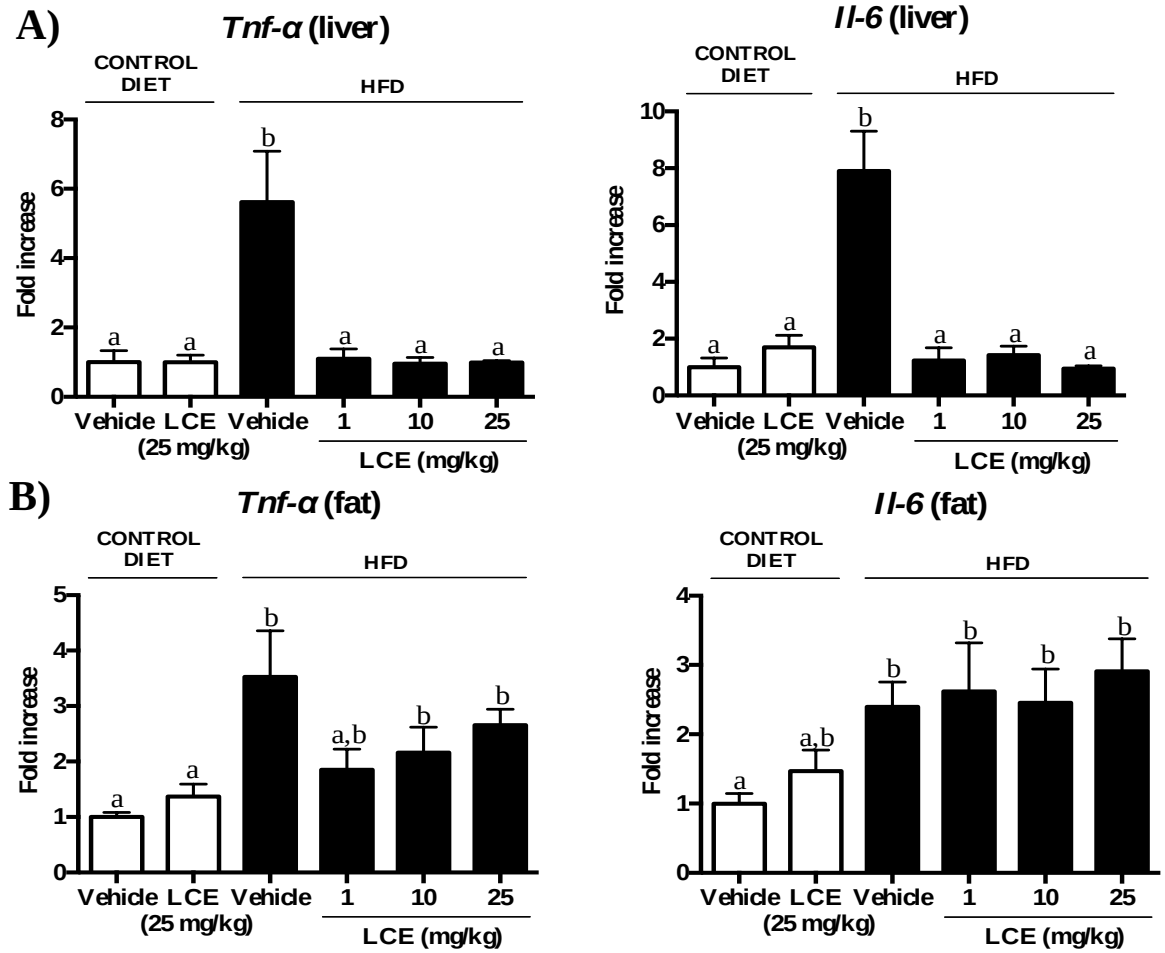

Figure 21: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) on **(A)** liver and fat **(B)** gene expression of *Tnf-α* and *Il-6*in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ (P< 0.05).

Besides, the expression of the inflammatory pathway *Jnk-1* was increased in HFD-fed mice in comparison to standard diet-fed mice, but LCE decreased it to levels similar to the ones in lean mice (Figure 22).

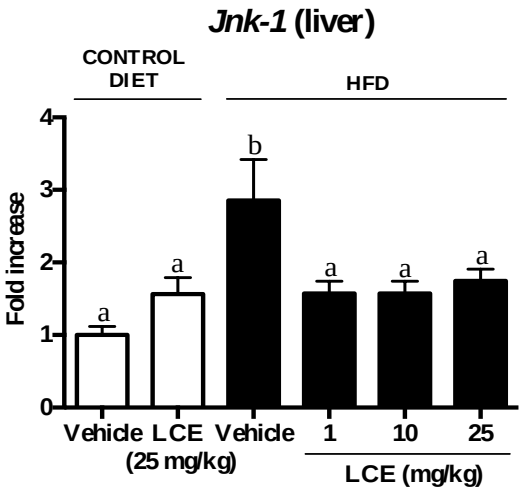

Figure 22: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) on hepatic expression of *Jnk-1*in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ (P< 0.05).

Leptin resistance, characterized by a hyperleptinemic state and decreased expression of its receptors, is common feature in obesity and it is also evident in this study. Obese mice presented high expression levels of *Leptin* (Figure 23A), and reduced expression of its receptor, both in liver and fat (Figure 23B and C). Of note, LCE treatment significantly ameliorated this state, reducing the expression of *Leptin* to normal values, and augmenting the expression of its receptor. However, the highest dose of LCE in fat did not increase the expression of the *Leptin* receptor in fat.

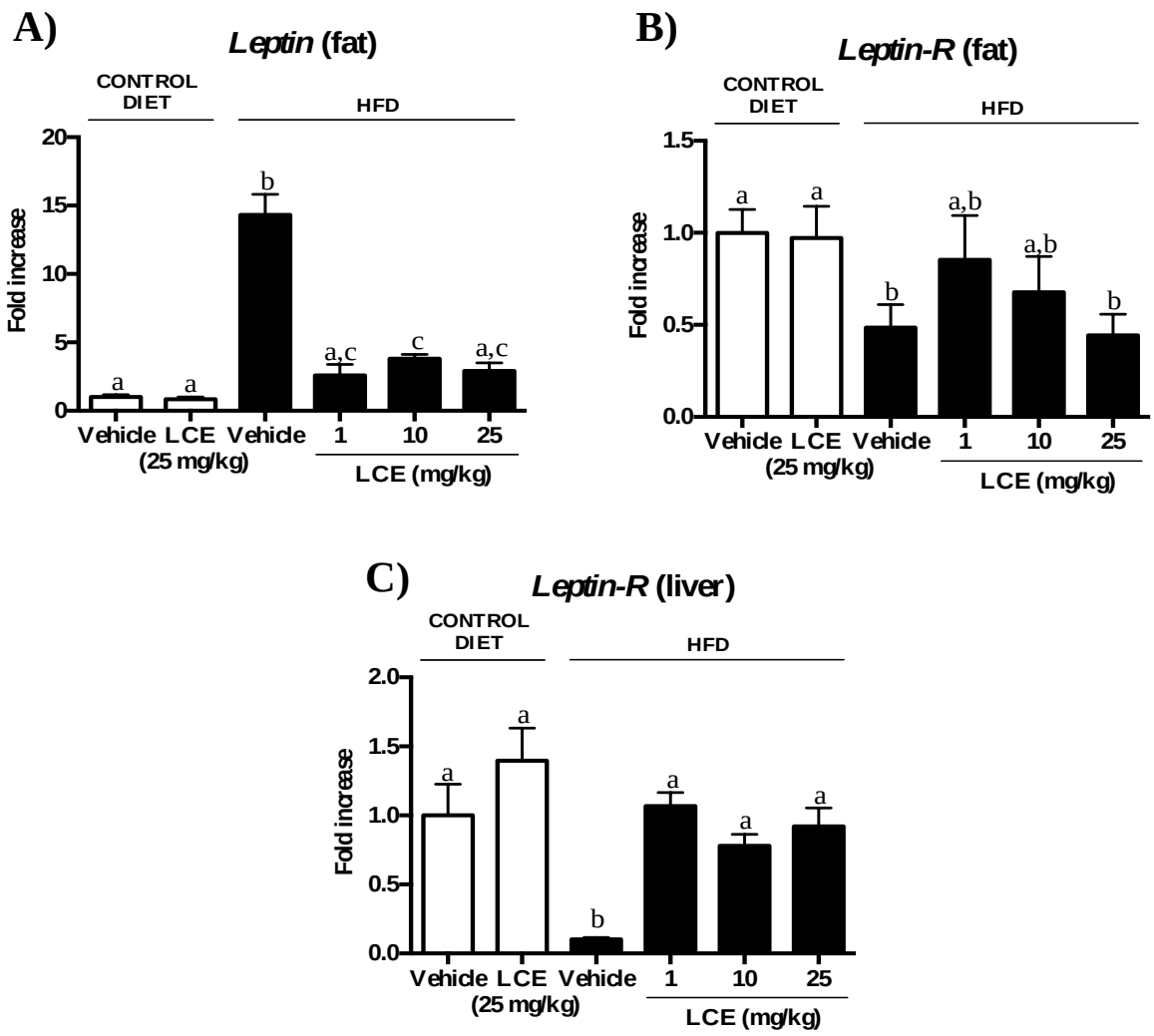

Figure 23: Effects of *Lippia citriodora* extract (LCE) on fat gene expression of **(A)** *Leptin* and **(B)** *Leptin R* and **(C)** liver gene expression of *Leptin-R* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($p \leq 0.05$).

Impairment in glucose metabolism is associated with decreased expression of *Glut-4*, which is reproduced in our experimental model, as HFD-fed mice have a reduced expression of this transporter, compared to standard diet-fed groups. However, the treatment with LCE significantly increased its expression, although in the case of the highest dose there was no difference with the control groups (Figure 24A). Related to GLUT-4, the expression of *Ampk* is also decreased in those mice receiving HFD, and it was reversed by the treatment, completely in the liver, and partially in fat, since the highest dose showed no effect (Figure 24 B and C).

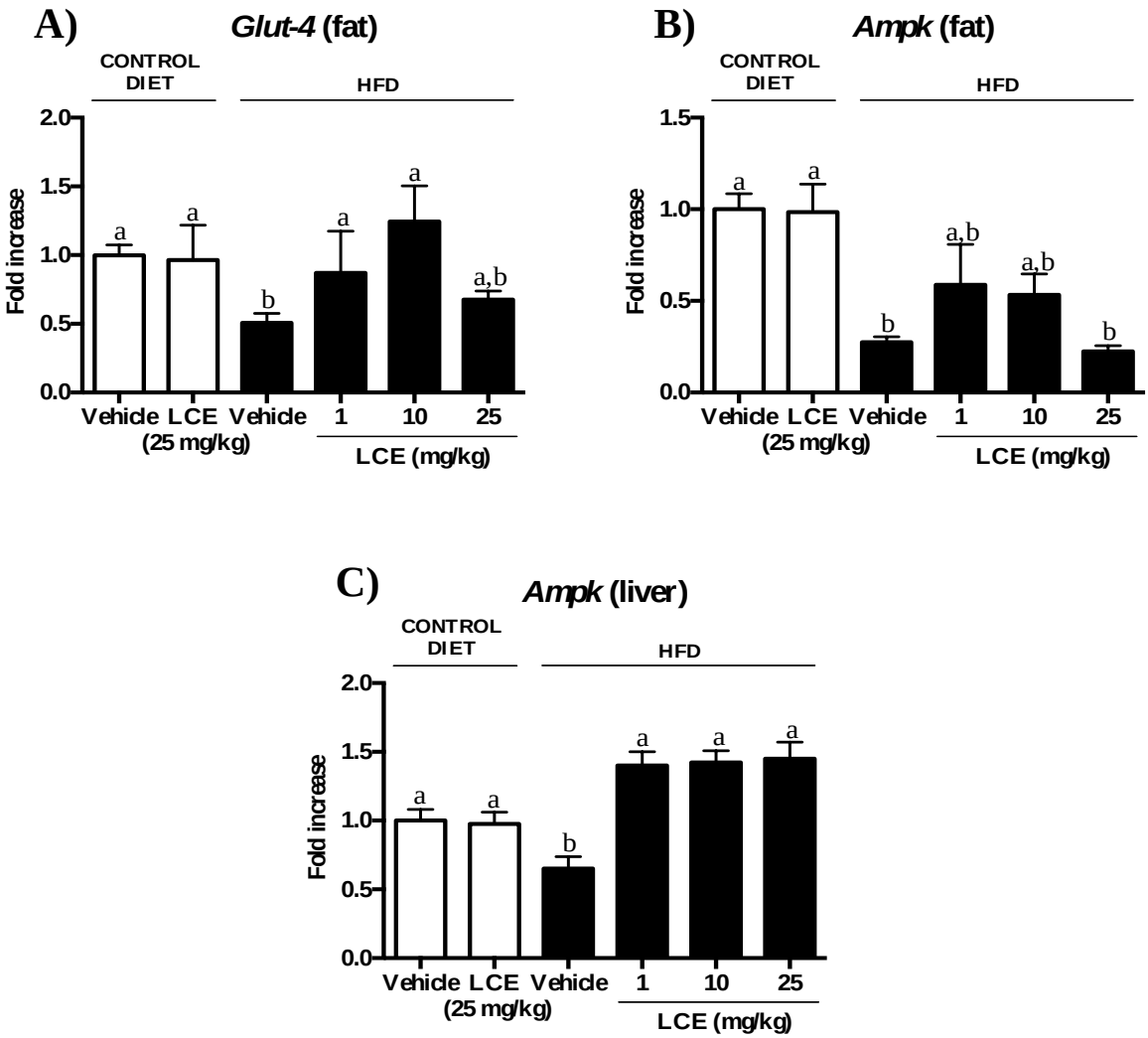

Figure 24: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) on fat gene expression of **(A)** *Glut-4* and **(B)** *Ampk*, and **(C)** hepatic expression of *Ampk* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ ($P < 0.05$).

When different parameters associated with intestinal permeability were evaluated, the link between obesity and an altered gut barrier function could be observed, as untreated obese mice had decreased expression of mucins *Muc-2* and *Muc-3*, *Occludin* and *Zo-1*. Interestingly, the administration of LCE to HFD-fed mice partially, but significatively restored it, thus limiting the translocation of bacterial components, such as LPS (Figure 25A). This was also evident when the expression of *Tlr-4* was analysed in liver, as LCE treated group had lower expression, with similar values as standard-diet fed groups, compared to non-treated HFD-fed group (Figure 25B).

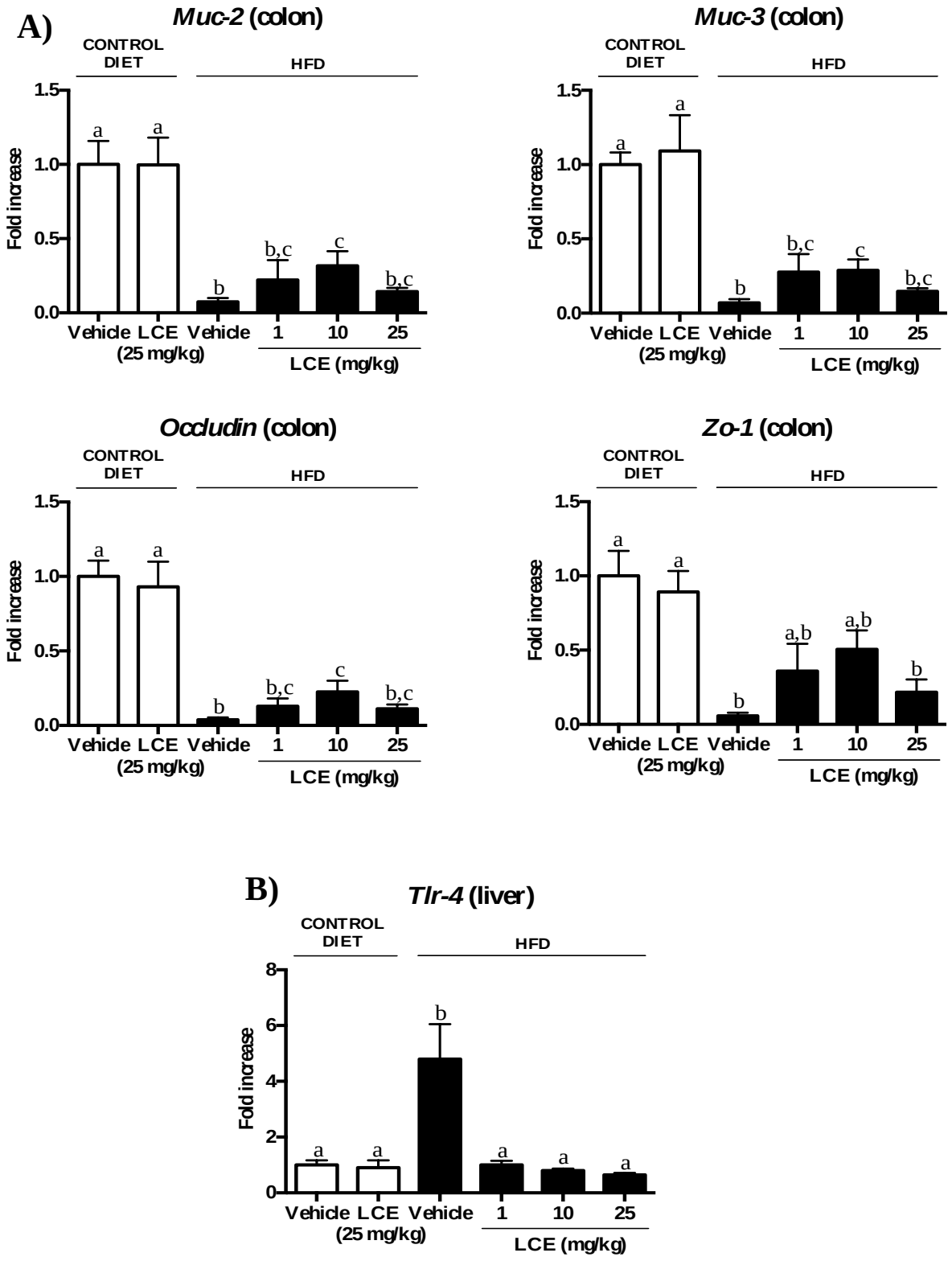

Figure 25: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) on **(A)** colonic gene expression of *Muc-2*, *Muc-3*, *Occludin* and *Zo-1*and **(B)** hepatic expression of *Tlr-4* in control and high-fat diet (HFD)-fed mice, analysed by real time qPCR. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ (P< 0.05).

2.4. Effects of LCE on gut microbiota dysbiosis

As altered intestinal permeability and associated endotoxemia are linked to an alteration in gut microbiota dysbiosis, its composition was analysed in all groups. Accordingly, PCoA plots showed a significant separation between standard diet and untreated HFD-fed groups. The treatment with LCE revealed a higher association with standard diet-fed groups at all doses, than with untreated HFD-fed mice (Figure 26).

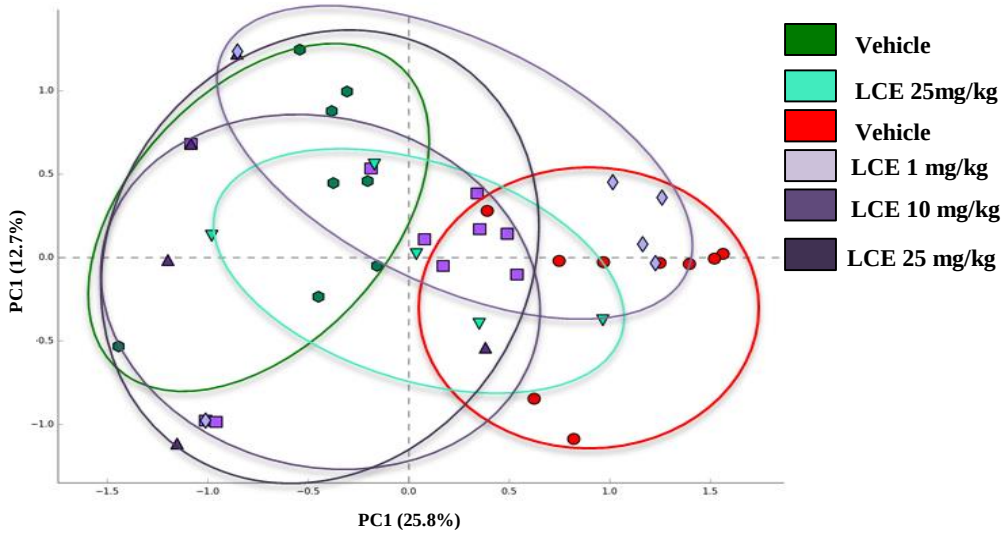

Figure 26: Comparison of faecal microbiota composition between control and high-fat diet (HFD)-fed mice: Principal component analysis plot based on Bray–Curtis distances, calculated on the metagenomic table of faecal samples of the different groups; Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p \leq 0.05$)

At phylum level, *Firmicutes* and *Bacteroidetes* were the most abundant bacteria, untreated HFD-fed mice showed a F/B ratio significantly higher than the other groups (up to 10 folds). LCE-treated HFD-fed mice presented a significantly reduced ratio, which was completely reversed by the two highest doses (Figure 27). At class level, untreated HFD-fed mice showed reduced abundance in *Bacteroidia* and *Erysipelotrichi*, and increased abundance of *Bacilli* compared to standard diet groups. Interestingly, LCE treatment reversed this situation in a dose-dependent manner. Moreover, at genus level, untreated HFD-fed mice had a reduced abundance in two genera, *Cytophaga* and *Akkermansia*, belonging to *Bacteroidetes* and *Verrumicrobia* families in comparison to standard diet-fed mice, respectively. The highest doses of the treatment with LCE significantly improved its abundance (Figure 28).

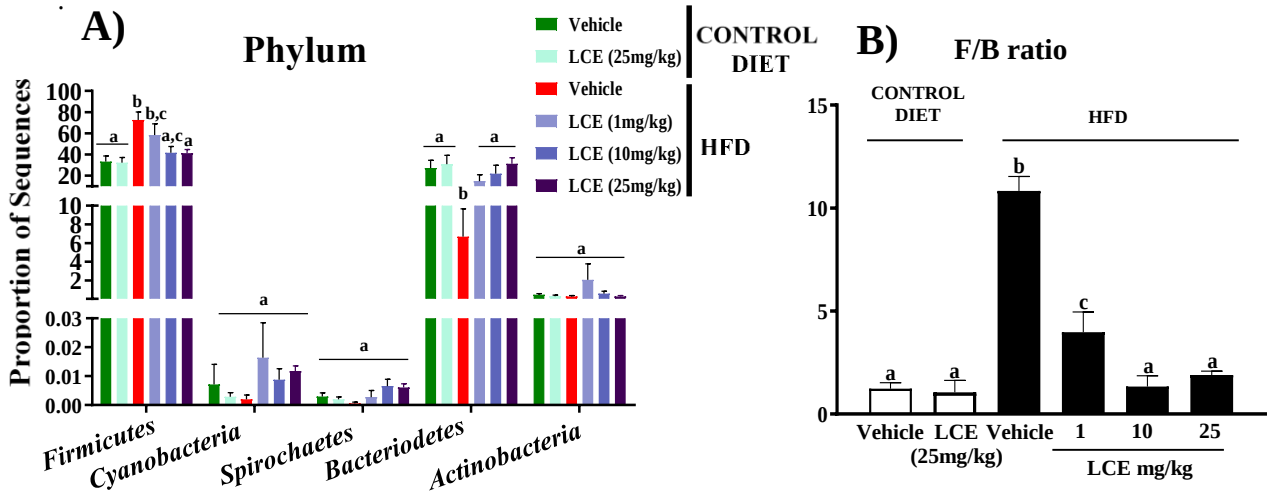

Figure 27: Comparison of faecal microbiota composition between control and high-fat diet (HFD)-fed mice: **(A)** Phylum breakdown of the most abundant bacterial communities in the different groups. **(B)** The *Firmicutes*/*Bacteriodetes* ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Groups with different letters statistically differ ($p \leq 0.05$).

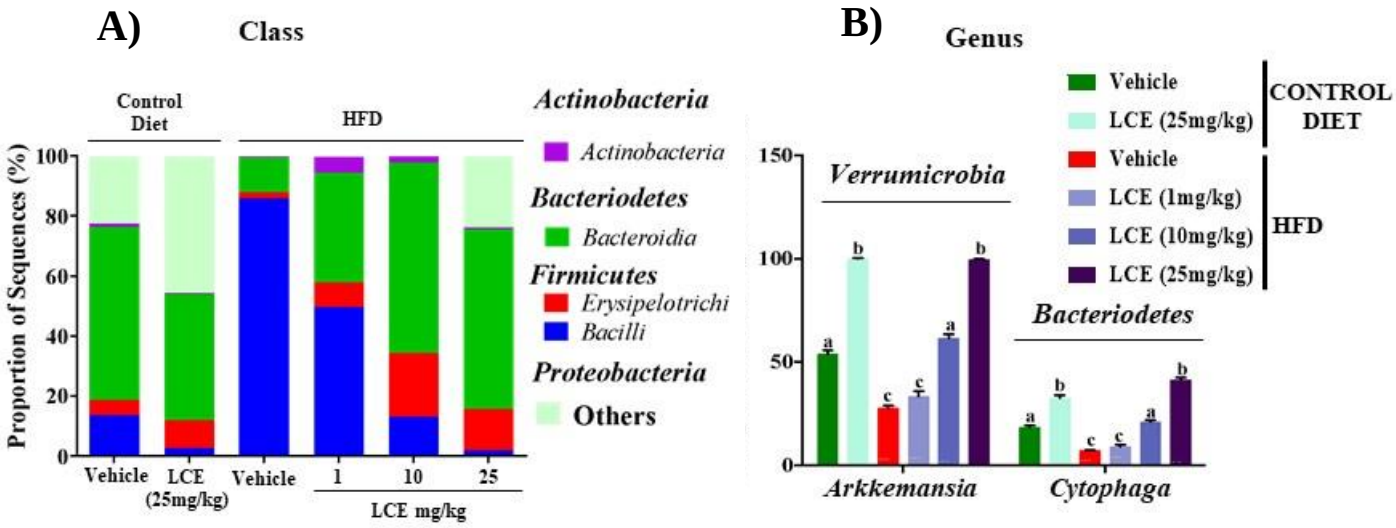

Figure 28: Effects of *Lippia citriodora* extract (LCE) (1-25 mg/kg) on faecal microbiota composition: Proportion of sequences of **(A)** class and **(B)** genus taxa. Statistical analysis was performed with one-way ANOVA followed by Tukey's test. Bars with different letters are significantly different ($p < 0.05$).

2.5. Effects of LCE on endothelial function

Obesity is associated with cardiovascular alterations, including endothelial dysfunction. Therefore, aortae from all mice were collected to examine the endothelium dependent vasodilator response to acetylcholine (Ach). As expected, HFD-fed mice had lower endothelium-dependent vasodilator responses to Ach compared to aortae from standard diet-fed groups, and a decrease in the maximal relaxant response (E_{max} values were 66.7±5.1% for control HFD-fed mice, and 81.8±2.0% for control diet-fed mice, respectively; $P<0.05$). In the case of treated mice, only the highest dose (25 mg/kg) was able to restore the endothelium

dependent relaxation induced by Ach (Figure 28A). Moreover, since endothelial dysfunction has been associated with production of reactive oxygen species (ROS), and NADPH oxidase is the major source of ROS, its activity was also evaluated. It was significantly increased in aortic rings of untreated HFD-fed mice, in comparison with standard diet-fed mice. The LCE treatment, at the highest doses, significantly reduced ROS production (Figure 28B).

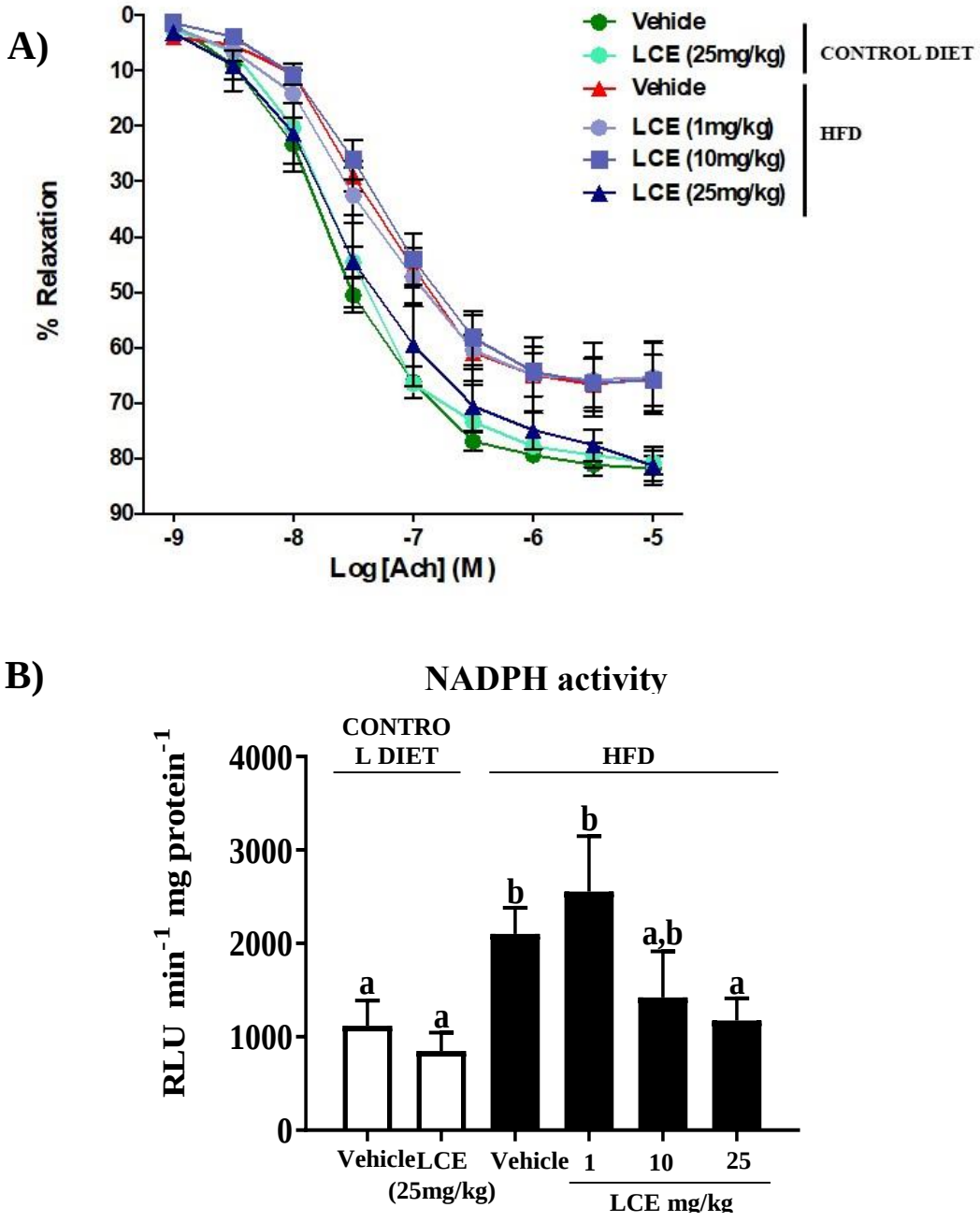

Figure 28. Effects of *Lippia citriodora* extract (LCE) (1–25 mg/kg) administration on endothelial function: **(A)** endothelium-dependent relaxation and **(B)** aortic NADPH activity in control and high-fat diet (HFD)-fed mice. Data are expressed as means ± SEM (n=10). Groups with different letter statistically differ (P< 0.05).

Discussion

Among medicinal plants showing potential properties against metabolic syndrome, *L. citriodora* is another plant that has been reported to have beneficial effects, likely associated to its bioactive components, as it has been reported to modulate glucose and lipid metabolism (10, 328), or to modulate appetite (325). In this second study, a well-characterized extract of the mentioned plant, *L. citriodora* (LCE) has been studied in an experimental model of obesity. Administration of LCE to HFD-fed mice reduced body weight gain, and thus the accumulation of fat pads, without modifying energy intake. Thus, it lowered energy efficiency but it did not have an anorexigenic effect, suggesting that other paths mediate its beneficial effects. This is in correlation with previous studies reporting the beneficial effect of LCE in a hyperlipidemic murine model (328).

The preventive beneficial effects exerted by the administration of LCE were associated with an improvement in systemic glucose intolerance and insulin resistance, evidenced by reduced plasma glucose and insulin levels, and reduced HOMA-IR index, which could suggest an improvement in insulin signalling pathway (365). Similarly, obesity-associated alterations related to lipid metabolism were also improved with the administration of the extract, as the LDL/HDL-cholesterol ratio was significantly reduced in LCE treated mice. As previously mentioned, these metabolic changes are associated with an abnormal accumulation of fat pads, and the presence of a chronic inflammatory state, with increased secretion of pro-inflammatory mediators in liver and fat tissue. This, as a loop, leads to the activation of other inflammatory pathways, as JNK-associated pathways, which also interferes with the insulin signalling pathway, and thus the glucose metabolism (14). In accordance with this, the expression of *Tnf-α* and *Il-6* in liver decreased in treated HFD-fed mice compared with non-treated obese mice, whereas LCE only was able to reduce the expression of *Tnf-α* in fat. Moreover, the expression of *Jnk-1* in liver also decreased after the treatment with LCE. Thus, LCE could have the capacity to improve insulin signalling, and therefore glucose metabolism, by decreasing the hepatic expression of *Tnf-α*, *Il-6*and *Jnk-1* in obese mice.

On the other hand, in insulin resistance states, like obesity, the expression of GLUT-4 is reduced. This is an insulin dependent glucose transporter that stimulates glucose entry into adipocytes and the synthesis of FAs and glycerol, while suppressing lipolysis (366). In our study, the treatment with LCE increased the expression of *Glut-4*, corroborating an improvement in insulin signalling, which agrees with the reduction of blood glucose levels.

Besides, leptin, an adipokine produced by adipose tissue in an endocrine manner, is involved on appetite and energy expenditure, but also, acts as a pro-inflammatory adipokine, opposing insulin action (16). In fact, in obesity, there is a hyperleptinemic state as a consequence of a decreased expression of the *Leptin-R* (367, 368), both in liver and fat, which is also confirmed in this study. However, leptin resistance was improved with the treatment with LCE, which could also contribute to the amelioration of the obesity-associated insulin resistance.

AMPK could be another therapeutic target for obesity, as it is a signalling protein that adapts cellular metabolism in response to nutritional changes (369). In this sense, the modulation of AMPK has been associated with decrease triglyceride accumulation and ROS generation in 3T3-L1 preadipocytes treated with polyphenols from *L. Citriodora* (328). In this second study, *Ampk* expression in the adipose tissue of obese mice was reduced, but restored with LCE treatment, which agrees with the improvement in lipid metabolism. Moreover, AMPK is reported to suppress activation of the NF-κB pathway, and thus, inhibit the expression of different pro-inflammatory cytokines, such as *Tnf-α, Il-1β* or *Il-6* (17), whose expression was also ameliorated in LCE-treated obese mice.

As previously mentioned, the chronic-inflammatory state associated with obesity is probably linked to an increased permeability in the gut, and the consequent development of metabolic endotoxemia (370, 371). In this sense, the expression of mucins *Muc-2, Muc-3* and of *Occludin* and *Zo-1* was decreased in non-treated HFD-fed mice, whereas its expression was significantly restored by the treatment with LCE, fact that have been previously described with other plant extracts (372, 373). Related to this, the expression of *Tlr-4*, which is considered indicative of obesity related endotoxemia (18, 374), was also decreased in LCE treated mice, while it was increased in those non-treated. This has also been observed in different experimental models of obesity with different phenolic compounds, quercetin among them (375), suggesting that the presence of flavonoids in LCE can be responsible for the beneficial effects on reducing intestinal permeability and thus the metabolic endotoxemia associated with obesity.

Gut microbiota composition has gain attention as could be a target in the management of inflammation-related conditions as it has been related to obesity, endotoxemia and increased intestinal permeability (357). Lately, the capacity of plant extracts rich in phenolic compounds has been explored (376). In the present study, the treatment with LCE improved the ratio *Firmicutes* to *Bacteroidetes*, which is a marker of gut dysbiosis (19). Actually, the ability to modulate gut microbiota by adjusting the F/B ratio has been described in different

phenolic extracts or single polyphenols in obesity (362, 372, 375). At genus level, it is important to note that the treatment with LCE increased the relative abundance of *Akkermansia*. *Akkermansia muciniphila* is reported to play an important role in the development of obesity. This bacteria colonizes the mucus layer and degrades the mucins. It counts for up to 5% of total microbiota present in healthy individuals (377), and its abundance is inversely related to body weight (20). Some studies have reported that treatments that are able to stimulate its growth are also able to mitigate metabolic disorders associated with HFDs (373). Thus, the beneficial effect of LCE on the growth of *Akkermansia* could imply the improvement of gut barrier function, by increasing the production of mucins in the colon, as they are its main energy source.

On the other hand, the chronic inflammatory state related to obesity also affects the proper endothelial function (372). Thus, in this experiment the aortae were examined to analyse the endothelium-dependent vasodilator response to acetylcholine, which is considered as an index of endothelial function (21). The treatment with the highest dose of LCE was able to increase the vasodilator response to acetylcholine. In aorta, NO is the major factor accounting for endothelium-dependent relaxation, and the diminished acetylcholine-induced relaxation indicates an impaired agonist-induced NO bioactivity (21, 378). In this sense, the major key mechanism for endothelial dysfunction is related to vascular production of reactive oxygen species, particularly superoxide anion, reacting rapidly with NO, inactivating it (379). NADPH oxidase is the main source of vascular reactive oxygen species, which are the major mediators impairing endothelium-dependent dilation in obesity associated cardiovascular conditions (21). In this study, the two highest doses of LCE were able to reduce the activity of NADPH oxidase, thus decreasing the superoxide production in vessels of obese mice.

In conclusion, administration of LCE to mice that were induced obesity by a HFD showed beneficial effects in body fat accumulation and improvements in plasma glucidic and lipidic profiles. These effects were associated with an amelioration of the systemic inflammatory status and the vascular dysfunction that characterizes of obesity. Moreover, different mechanism seems to participate, and include prebiotic properties, as LCE ameliorated obesity-associated dysbiosis, ameliorating intestinal barrier dysfunction, and thus improving altered immune response of obese mice.

CONCLUDING REMARKS

Two extracts obtained from *Lippia citriodora* and *Hibiscus sabdariffa* have been assayed in an experimental model of metabolic syndrome in mice. This model consisted in providing a high-fat diet (HFD) to mice, which reproduces the main features of this condition: obesity, metabolic alterations (glucose intolerance, dyslipidemia), systemic inflammation and vascular dysfunction (2, 3). Moreover, all these alterations were also associated with modifications in intestinal microbiota composition, or dysbiosis, typically reported in metabolic syndrome (5).

The selection of these two medicinal plants was based on their traditional use and on previous studies that reported their beneficial effects against obesity and/or its associated disorders (8-10). The results obtained in the present study revealed that both extracts were able to reduce body weight gain in those mice fed HFD, without showing any significant effect when standard diet was provided. The efficacy of both extracts was similar, although LCE had greater impact on energy efficiency since the values obtained did not significantly differ from those of the non-obese control group at all doses assayed. Both extracts also showed significant improvements in lipid and glucose metabolism, although these were more evident for LCE because all the biochemical markers analysed were similar to non-obese mice at all doses assayed.

Regarding the effects of the extracts on the obesity-associated systemic inflammatory state, both extracts downregulated the expression of all inflammatory markers in liver but, in adipose tissue, only the highest dose of HSE was able to decrease the expression of *Tnf-α* and *Il-6*, and the lowest dose of LCE significantly reduced de expression of *Tnf-α*. Moreover, both extracts also reduced significantly the expression of *Jnk-1* in liver. Of note, upregulated *Jnk-1* expression is closely related to increased expression of pro-inflammatory mediators involved in the impairment of insulin signalling (13); in consequence, this could mean that both extracts could improve insulin signalling in liver, and therefore, glucose metabolism. In fact, the reduced expression of *Glut-4* observed in obese mice was significantly increased with both HSE (at doses of 10 and 25 mg/kg) and LCE (at doses of 1 and 10 mg/kg). Moreover, whereas HSE did not show a significant effect on the expression of *Ampk*, LCE, at 1 and 10 mg/kg, did significantly increase the reduced expression observed in obese mice, which could be relevant since AMPK is involved in cellular glucose metabolism by promoting the translocation of GLUT-4 to the membrane.

Besides, obesity is associated with leptin resistance, as evidenced in this experimental model by increased expression of leptin in fat tissue together with reduced expression of its receptor in liver and fat tissue. Both extracts were able to reduce this leptin resistance status, although

LCE seemed to show a higher efficacy since the expressions of both *Leptin* and *Leptin-receptor* were improved with this extract in both target tissues, whereas HSE was devoid of any significant effect in increasing the expression of *Leptin-receptor* in fat tissue.

Obesity has been also associated with increased intestinal permeability that leads to the translocation of bacterial components, which in turn promotes metabolic endotoxemia and the systemic inflammatory status (18). The administration both extracts was able to significantly increase the expression of some of the evaluated markers involved in maintaining intestinal epithelial barrier function, being HSE (at doses of 1 mg/kg) the most efficient. This would result in a significant restoration of the protective barrier function of the intestine, which would prevent the access of microbial components, like LPS, to systemic circulation, thus ameliorating the obesity-associated inflammatory process. This was indirectly evidenced by the decreased expression of *Tlr-4* in the liver of obese mice treated with the extracts, since the expression of this receptor has been correlated with plasma LPS levels in experimental models of obesity (172).

Gut dysbiosis was also evidenced in this experimental model of metabolic syndrome, similarly to that previously reported in humans (5). Both extracts were able to modulate the altered intestinal microbiota composition, especially when considering the F/B ratio, being this obtained at all doses assayed. Additionally, LCE produced an increase in the relative abundance of *Akkermansia*, which may be very interesting since the higher abundance of *Akkermansia muciniphila* has been associated with the amelioration of metabolic disorders and gut barrier function (373).

Finally, obesity-associated inflammation is also characterized by vascular dysfunction, which can clearly contribute to the development of cardiovascular diseases in this condition (372). Vascular dysfunction has been shown in the present study, since the endothelium-dependent vasodilator response to acetylcholine was impaired in the aortae segments from control obese mice. This assay was only performed with LCE, and the results revealed that the highest dose of LCE (25 mg/kg) was able to increase the vasodilator response to acetylcholine. This effect may be due to the ability of LCE to reduce the activity of NADPH oxidase, thus decreasing superoxide production and preserving NO production, which is the major responsible of endothelium-dependent relaxation.

In conclusion, both HSE and LCE have shown beneficial effects in this experimental model of metabolic syndrome, induced by HFD intake to mice that results in obesity. This was evidenced through the amelioration of the different obesity-associated disorders evaluated,

including metabolic alterations, systemic inflammation, dysbiosis and vascular dysfunction (only evaluated with LCE). The efficacy showed by both extracts are due to the presence of active compounds, mainly polyphenols, which have been long reported to show biological properties with positive impact against the different obesity-associated disorders. The chemical characterization of the extracts used in the present study revealed one major compound in each extract, hibiscus acid in HSE and verbascoside in LCE. However, it is difficult to ascribe the beneficial effects observed with each extract to a single compound, and most probably, the synergic effect of all the compounds, including flavonoids, is essential for their biological activities.

This study confirms previous observations reported for these extracts in the treatment of metabolic syndrome in humans, providing additional information about the mechanisms involved in these beneficial effects, especially those involving the modulation of gut microbiota that ameliorates obesity-associated dysbiosis.

CONCLUSIONS

1. The daily administration of *Hibiscus sabdariffa* or *Lippia citriodora* extracts, at doses of 1, 10 and 25 mg/kg, showed beneficial effects in an experimental model of metabolic syndrome induced by high-fat diet in mice.
2. These effects were evidenced by a reduction in body weight gain in obese mice, which was associated with an improvement in the glycidic and lipidic metabolic profile, closely related to the amelioration of insulin resistance process that occurs in obesity.
3. The extracts ameliorated the obesity-associated subclinical chronic inflammatory state both in liver and in fat tissue, which could be related to the enhancement of the intestinal permeability, thus reducing the possibility of metabolic endotoxemia.
4. Both extracts were able to reduce the gut dysbiosis, thus demonstrating their prebiotic properties, including their capacity to promote the growth of beneficial bacteria, like *Akkermansia muciniphila* in the case of obese mice treated with *Lippia citriodora* extract.
5. The administration of *Lippia citriodora* improved vascular dysfunction in obese mice, which could definitely contribute to prevent the development of obesity-associated cardiovascular diseases.

REFERENCES

1. O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. Obes Rev. 2015 Jan;16(1):1-12. PubMed PMID: 25407540. Epub 2014/11/20. eng.
2. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018;20(2):12-. PubMed PMID: 29480368. eng.
3. Sur G, Floca E, Kudor-Szabadi L, Sur ML, Sur D, Samasca G. The relevance of inflammatory markers in metabolic syndrome. Maedica (Buchar). 2014;9(1):15-8. PubMed PMID: 25553120. eng.
4. Liu R, Nikolajczyk BS. Tissue Immune Cells Fuel Obesity-Associated Inflammation in Adipose Tissue and Beyond. Frontiers in immunology. 2019;10:1587. PubMed PMID: 31379820. Pubmed Central PMCID: PMC6653202. Epub 2019/08/06. eng.
5. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007 Jul;56(7):1761-72. PubMed PMID: 17456850. Epub 2007/04/26. eng.
6. Saunders KH, Umashanker D, Igel LI, Kumar RB, Aronne LJ. Obesity pharmacotherapy. Medical Clinics. 2018;102(1):135-48.
7. Cicero AFG, Colletti A. Role of phytochemicals in the management of metabolic syndrome. Phytomedicine. 2016;23(11):1134-44. PubMed PMID: 26778479. Epub 12/11. eng.
8. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. Hibiscus sabdariffa L. - a phytochemical and pharmacological review. Food Chem. 2014;165:424-43. PubMed PMID: 25038696. Epub 05/27. eng.
9. Funes L, Fernández-Arroyo S, Laporta O, Pons A, Roche E, Segura-Carretero A, et al. Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. Food Chem. 2009;117(4):589-98.
10. Zhang Y, Liu M, Chen Q, Wang T, Yu H, Xu J, et al. Leaves of Lippia triphylla improve hepatic lipid metabolism via activating AMPK to regulate lipid synthesis and degradation. J Nat Med. 2019;73(4):707-16. PubMed PMID: 31104252. Epub 05/18. eng.
11. Keane KN, Cruzat VF, Carlessi R, de Bittencourt PI, Jr., Newsholme P. Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance and β-Cell Dysfunction. Oxidative medicine and cellular longevity. 2015;2015:181643. PubMed PMID: 26257839. Pubmed Central PMCID: PMC4516838. Epub 2015/08/11. eng.
12. Ding S, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, et al. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PloS one. 2010 Aug 16;5(8):e12191. PubMed PMID: 20808947. Pubmed Central PMCID: PMC2922379. Epub 2010/09/03. eng.
13. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr. 2006 Feb;83(2):461S-5S. PubMed PMID: 16470013. Epub 2006/02/14. eng.
14. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. Molecular medicine (Cambridge, Mass). 2008 Mar-Apr;14(3-4):222-31. PubMed PMID: 18235842. Pubmed Central PMCID: PMC2215762. Epub 2008/02/01. eng.
15. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews Immunology. 2011 Feb;11(2):85-97. PubMed PMID: 21252989. Pubmed Central PMCID: PMC3518031. Epub 2011/01/22. eng.
16. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Molecular medicine (Cambridge, Mass). 2008 Nov-Dec;14(11-12):741-51. PubMed PMID: 19009016. Pubmed Central PMCID: PMC2582855. Epub 2008/11/15. eng.
17. Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing research reviews. 2012 Apr;11(2):230-41. PubMed PMID: 22186033. Epub 2011/12/22. eng.
18. Siebler J, Galle PR, Weber MM. The gut-liver-axis: endotoxemia, inflammation, insulin resistance and NASH. Journal of hepatology. 2008 Jun;48(6):1032-4. PubMed PMID: 18468548. Epub 2008/05/13. eng.
19. 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 Dec 21;444(7122):1027-31. PubMed PMID: 17183312. Epub 2006/12/22. eng.
20. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proceedings of the National Academy of Sciences of the United States of America. 2013 May 28;110(22):9066-71. PubMed PMID: 23671105. Pubmed Central PMCID: PMC3670398. Epub 2013/05/15. eng.
21. Toral M, Gómez-Guzmán M, Jiménez R, Romero M, Sánchez M, Utrilla MP, et al. The probiotic Lactobacillus coryniformis CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in obese mice. Clinical science (London, England : 1979). 2014 Jul;127(1):33-45. PubMed PMID: 24410749. Epub 2014/01/15. eng.
22. Kylin E. Studien ueber das Hypertonie-Hyperglyca "mie-Hyperurika" iesyndrom. Zentralbatt fuer Innere Medizin. 1923;44:105-27.

23. Vague J. Sexual differentiation, a factor affecting the forms of obesity. Presse Med. 1947;30:339-40.
24. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988;37(12):1595-607. PubMed PMID: 3056758. eng.
25. Kaplan NM. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Archives of internal medicine. 1989 Jul;149(7):1514-20. PubMed PMID: 2662932. Epub 1989/07/01. eng.
26. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes. 1992;41(6):715-22. PubMed PMID: 1587398. eng.
27. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998 Jul;15(7):539-53. PubMed PMID: 9686693. Epub 1998/08/01. eng.
28. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med. 1999;16(5):442-3. PubMed PMID: 10342346. eng.
29. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001 May 16;285(19):2486-97. PubMed PMID: 11368702. Epub 2001/05/23. eng.
30. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005 Sep 24-30;366(9491):1059-62. PubMed PMID: 16182882. Epub 2005/09/27. eng.
31. Bahar A, Kashi Z, Kheradmand M, Hedayatizadeh-Omran A, Moradinazar M, Ramezani F, et al. Prevalence of metabolic syndrome using international diabetes federation, National Cholesterol Education Panel-Adult Treatment Panel III and Iranian criteria: results of Tabari cohort study. Journal of Diabetes & Metabolic Disorders. 2020:1-7.
32. Nikbakht H-A, Rezaianzadeh A, Seif M, Ghaem H. Prevalence of metabolic syndrome and its components among a population-based study in south of Iran, PERSIAN Kharameh cohort study. Clinical Epidemiology and Global Health. 2020.
33. Faijer-Westerink HJ, Kengne A-P, Meeks KA, Agyemang C. Prevalence of metabolic syndrome in sub-Saharan Africa: a systematic review and meta-analysis. Nutrition, Metabolism and Cardiovascular Diseases. 2019.
34. Huang X, Hu Y, Du L, Lin X, Wu W, Fan L, et al. Metabolic syndrome in native populations living at high altitude: a cross-sectional survey in Derong, China. BMJ Open. 2020;10(1):e032840-e. PubMed PMID: 31911517. eng.
35. Sigit FS, Tahapary DL, Trompet S, Sartono E, Willems van Dijk K, Rosendaal FR, et al. The prevalence of metabolic syndrome and its association with body fat distribution in middle-aged individuals from Indonesia and the Netherlands: a cross-sectional analysis of two population-based studies. Diabetology & metabolic syndrome. 2020;12:2-. PubMed PMID: 31921359. eng.
36. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28. PubMed PMID: 15836891. eng.
37. Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. Diabetes research and clinical practice. 2003;61(1):29-37. PubMed PMID: 12849921. eng.
38. Umano GR, Caprio S. Ectopic Fat and Insulin Resistance in Youth. Insulin Resistance: Springer; 2020. p. 155-67.
39. Frithioff-Bøjsøe C, Lund MAV, Lausten-Thomsen U, Hedley PL, Pedersen O, Christiansen M, et al. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. Pediatr Diabetes. 2020;21(2):194-202. PubMed PMID: 31845423. Epub 12/26. eng.
40. Tagi VM, Giannini C, Chiarelli F. Insulin resistance in children. Front Endocrinol (Lausanne). 2019;10.
41. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of Obesity Among Adults and Youth: United States, 2015-2016. NCHS Data Brief. 2017 (288):1-8. PubMed PMID: 29155689. eng.
42. Kelsey MM, Zaepfel A, Bjornstad P, Nadeau KJ. Age-related consequences of childhood obesity. Gerontology. 2014;60(3):222-8. PubMed PMID: 24434909. Epub 2014/01/18. eng.
43. Qin X, Tang G, Zhu G, Tsoi M-F, Xu T, Zhang L, et al. Prevalence of metabolic syndrome in ethnic groups in China: a cross-sectional study. The Lancet. 2019;394:S39.
44. Shaikh RA, Siahpush M, Singh GK, Tibbits M. Socioeconomic Status, Smoking, Alcohol use, Physical Activity, and Dietary Behavior as Determinants of Obesity and Body Mass Index in the United States: Findings from the National Health Interview Survey. Int J MCH AIDS. 2015;4(1):22-34. PubMed PMID: 27622000. eng.
45. Gosadi IM. Assessment of the environmental and genetic factors influencing prevalence of metabolic syndrome in Saudi Arabia. Saudi Med J. 2016;37(1):12-20. PubMed PMID: 26739969. eng.

46. Kósa Z, Moravcsik-Kornyicki Á, Diószegi J, Roberts B, Szabó Z, Sándor J, et al. Prevalence of metabolic syndrome among Roma: a comparative health examination survey in Hungary. European journal of public health. 2015 Apr;25(2):299-304. PubMed PMID: 25231955. Epub 2014/09/19. eng.
47. Detection NCEPEPo, Adults ToHBCi. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III): National Cholesterol Education Program, National Heart, Lung, and Blood …; 2002.
48. Ladeiras-Lopes R, Teixeira P, Azevedo A, Leite-Moreira A, Bettencourt N, Fontes-Carvalho R. Metabolic syndrome severity score is associated with diastolic dysfunction and low-grade inflammation in a community-based cohort. European journal of preventive cardiology. 2019 Dec 17:2047487319895400. PubMed PMID: 31847564. Epub 2019/12/19. eng.
49. Leisegang K, Henkel R, Agarwal A. Obesity and metabolic syndrome associated with systemic inflammation and the impact on the male reproductive system. American journal of reproductive immunology (New York, NY : 1989). 2019 Nov;82(5):e13178. PubMed PMID: 31373727. Epub 2019/08/03. eng.
50. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovasc Diabetol. 2018;17(1):122.
51. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Experimental & molecular medicine. 2016;48(3):e219-e. PubMed PMID: 26964835. eng.
52. Akhtar DH, Iqbal U, Vazquez-Montesino LM, Dennis BB, Ahmed A. Pathogenesis of Insulin Resistance and Atherogenic Dyslipidemia in Nonalcoholic Fatty Liver Disease. Journal of clinical and translational hepatology. 2019 Dec 28;7(4):362-70. PubMed PMID: 31915606. Pubmed Central PMCID: PMC6943204. Epub 2020/01/10. eng.
53. Guo S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. J Endocrinol. 2014;220(2):T1-T23. PubMed PMID: 24281010. eng.
54. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. Physiol Rev. 2018;98(4):2133-223. PubMed PMID: 30067154. eng.
55. D'Oria R, Laviola L, Giorgino F, Unfer V, Bettocchi S, Scioscia M. PKB/Akt and MAPK/ERK phosphorylation is highly induced by inositols: Novel potential insights in endothelial dysfunction in preeclampsia. Pregnancy Hypertens. 2017;10:107-12. PubMed PMID: 29153661. Epub 07/10. eng.
56. Doliba NM. Pancreatic Islet Adaptation and Failure in Obesity and Diabetes. In: Ahima RS, editor. Metabolic Syndrome: A Comprehensive Textbook. Cham: Springer International Publishing; 2018. p. 1-21.
57. Rodríguez-Rodríguez E, Perea JM, López-Sobaler AM, Ortega RM. Obesity, insulin resistance and increase in adipokines levels: importance of the diet and physical activity. Nutr Hosp. 2009 Jul-Aug;24(4):415-21. PubMed PMID: 19721920. spa.
58. Lytrivi M, Castell AL, Poitout V, Cnop M. Recent Insights Into Mechanisms of β-Cell Lipo- and Glucolipotoxicity in Type 2 Diabetes. Journal of molecular biology. 2020 Mar 6;432(5):1514-34. PubMed PMID: 31628942. Pubmed Central PMCID: PMC7073302. Epub 2019/10/20. eng.
59. Owei I, Umekwe N, Provo C, Wan J, Dagogo-Jack S. Insulin-sensitive and insulin-resistant obese and non-obese phenotypes: role in prediction of incident pre-diabetes in a longitudinal biracial cohort. BMJ Open Diabetes Res Care. 2017;5(1):e000415-e. PubMed PMID: 28878939. eng.
60. Perry RJ, Shulman GI. Mechanistic Links between Obesity, Insulin, and Cancer. Trends Cancer. 2020;6(2):75-8. PubMed PMID: 32061306. Epub 01/14. eng.
61. Costa RM, Neves KB, Tostes RC, Lobato NS. Perivascular Adipose Tissue as a Relevant Fat Depot for Cardiovascular Risk in Obesity. Front Physiol. 2018;9:253-. PubMed PMID: 29618983. eng.
62. Mohammadi M, Gozashti MH, Aghadavood M, Mehdizadeh MR, Hayatbakhsh MM. Clinical Significance of Serum IL-6 and TNF-α Levels in Patients with Metabolic Syndrome. Rep Biochem Mol Biol. 2017;6(1):74-9. PubMed PMID: 29090232. eng.
63. Tangvarasittichai S, Pongthaisong S, Tangvarasittichai O. Tumor Necrosis Factor-A, Interleukin-6, C-Reactive Protein Levels and Insulin Resistance Associated with Type 2 Diabetes in Abdominal Obesity Women. Indian J Clin Biochem. 2016;31(1):68-74. PubMed PMID: 26855490. Epub 07/26. eng.
64. Das UN. Renin-angiotensin-aldosterone system in insulin resistance and metabolic syndrome. J Transl Int Med. 2016;4(2):66-72. PubMed PMID: 28191524. Epub 07/07. eng.
65. Achari AE, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci. 2017 Jun 21;18(6). PubMed PMID: 28635626. Pubmed Central PMCID: PMC5486142. Epub 2017/06/22. eng.
66. Ghosh A, Gao L, Thakur A, Siu PM, Lai CWK. Role of free fatty acids in endothelial

dysfunction. J Biomed Sci. 2017;24(1):50-. PubMed PMID: 28750629. eng.
67. Manjunath C, Rawal JR, Irani PM, Madhu K. Atherogenic dyslipidemia. Indian J Endocrinol Metab. 2013;17(6):969.
68. Semenkovich CF. Insulin resistance and atherosclerosis. The Journal of clinical investigation. 2006 Jul;116(7):1813-22. PubMed PMID: 16823479. Pubmed Central PMCID: PMC1483180. Epub 2006/07/11. eng.
69. Schoenfeld BJ, Grgic J, Ogborn D, Krieger JW. Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training: A Systematic Review and Meta-analysis. Journal of strength and conditioning research. 2017 Dec;31(12):3508-23. PubMed PMID: 28834797. Epub 2017/08/24. eng.
70. Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. Rev Endocr Metab Disord. 2013;14(1):5-12. PubMed PMID: 23306778. eng.
71. Gutiérrez A, Contreras C, Sánchez A, Prieto D. Role of Phosphatidylinositol 3-Kinase (PI3K), Mitogen-Activated Protein Kinase (MAPK), and Protein Kinase C (PKC) in Calcium Signaling Pathways Linked to the α(1)-Adrenoceptor in Resistance Arteries. Front Physiol. 2019;10:55-. PubMed PMID: 30787881. eng.
72. Prieto D, Contreras C, Sánchez A. Endothelial dysfunction, obesity and insulin resistance. Curr Vasc Pharmacol. 2014;12(3):412-26. PubMed PMID: 24846231. eng.
73. Draznin B. Molecular mechanisms of insulin resistance. Insulin Resistance: Springer; 2020. p. 55-66.
74. Zhao W, Feng H, Guo S, Han Y, Chen X. Danshenol A inhibits TNF-α-induced expression of intercellular adhesion molecule-1 (ICAM-1) mediated by NOX4 in endothelial cells. Sci Rep. 2017;7(1):12953-. PubMed PMID: 29021525. eng.
75. Ghantous CM, Azrak Z, Hanache S, Abou-Kheir W, Zeidan A. Differential Role of Leptin and Adiponectin in Cardiovascular System. Int J Endocrinol. 2015;2015:534320-. PubMed PMID: 26064110. Epub 05/03. eng.
76. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13(4):851-63. PubMed PMID: 28721154. Epub 03/31. eng.
77. Caër C, Rouault C, Le Roy T, Poitou C, Aron-Wisnewsky J, Torcivia A, et al. Immune cell-derived cytokines contribute to obesity-related inflammation, fibrogenesis and metabolic deregulation in human adipose tissue. Sci Rep. 2017;7(1):3000-. PubMed PMID: 28592801. eng.
78. Kang YE, Kim JM, Joung KH, Lee JH, You BR, Choi MJ, et al. The Roles of Adipokines, Proinflammatory Cytokines, and Adipose Tissue Macrophages in Obesity-Associated Insulin Resistance in Modest Obesity and Early Metabolic Dysfunction. PloS one. 2016;11(4):e0154003-e. PubMed PMID: 27101398. eng.
79. Ježek P, Jabůrek M, Plecitá-Hlavatá L. Contribution of Oxidative Stress and Impaired Biogenesis of Pancreatic β-Cells to Type 2 Diabetes. Antioxid Redox Signal. 2019;31(10):722-51. PubMed PMID: 30450940. Epub 01/23. eng.
80. Sun Q, Li J, Gao F. New insights into insulin: The anti-inflammatory effect and its clinical relevance. World J Diabetes. 2014;5(2):89-96. PubMed PMID: 24765237. eng.
81. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. J Intern Med. 2015;278(5):483-93. PubMed PMID: 26260307. Epub 08/11. eng.
82. Divella R, Mazzocca A, Daniele A, Sabbà C, Paradiso A. Obesity, Nonalcoholic Fatty Liver Disease and Adipocytokines Network in Promotion of Cancer. Int J Biol Sci. 2019;15(3):610-6. PubMed PMID: 30745847. eng.
83. Dharmalingam M, Yamasandhi PG. Nonalcoholic fatty liver disease and type 2 diabetes mellitus. Indian J Endocrinol Metab. 2018;22(3):421.
84. D'Adamo E, Castorani V, Nobili V. The Liver in Children With Metabolic Syndrome. Front Endocrinol (Lausanne). 2019;10:514-. PubMed PMID: 31428049. eng.
85. Wargny M, Smati S, Pichelin M, Bigot-Corbel E, Authier C, Dierry V, et al. Fatty liver index is a strong predictor of changes in glycemic status in people with prediabetes: The IT-DIAB study. PloS one. 2019;14(8):e0221524-e. PubMed PMID: 31465427. eng.
86. Jiang B, Gu T, Zhou K, Zheng Y, Guo Y, Lu Y. Fatty Liver as a Potential Surrogate for Waist Circumference in the Diagnosis of Metabolic Syndrome: A Population-Based Study among Chinese Adults. Int J Endocrinol. 2018;2018:7903982-. PubMed PMID: 29849623. eng.
87. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. The Journal of clinical endocrinology and metabolism. 2007 Sep;92(9):3490-7. PubMed PMID: 17595248. Epub 2007/06/28. eng.
88. Almendros I, García-Río F. Sleep apnoea, insulin resistance and diabetes: the first step is in the fat. The European respiratory journal. 2017 Apr;49(4). PubMed PMID: 28424366. Epub 2017/04/21. eng.
89. Kheirandish-Gozal L, Gozal D. Obstructive Sleep Apnea and Inflammation: Proof of Concept Based on Two Illustrative Cytokines. Int J Mol Sci. 2019 Jan 22;20(3). PubMed PMID: 30678164. Pubmed Central PMCID: PMC6387387. Epub 2019/01/27. eng.

90. Bingol Z, Karaayvaz EB, Telci A, Bilge AK, Okumus G, Kiyan E. Leptin and adiponectin levels in obstructive sleep apnea phenotypes. Biomark Med. 2019;13(10):865-74. PubMed PMID: 31210052. Epub 06/18. eng.
91. Lu M, Fang F, Wang Z, Wei P, Hu C, Wei Y. Association between serum/plasma levels of adiponectin and obstructive sleep apnea hypopnea syndrome: a meta-analysis. Lipids Health Dis. 2019 Jan 26;18(1):30. PubMed PMID: 30684961. Pubmed Central PMCID: PMC6347767. Epub 2019/01/28. eng.
92. Wu W-T, Tsai S-S, Shih T-S, Lin M-H, Chou T-C, Ting H, et al. The Association between Obstructive Sleep Apnea and Metabolic Markers and Lipid Profiles. PloS one. 2015;10(6):e0130279-e. PubMed PMID: 26115005. eng.
93. Mesarwi O, Polak J, Jun J, Polotsky VY. Sleep disorders and the development of insulin resistance and obesity. Endocrinol Metab Clin North Am. 2013;42(3):617-34. PubMed PMID: 24011890. eng.
94. Vgontzas AN, Bixler EO, Chrousos GP. Sleep apnea is a manifestation of the metabolic syndrome. Sleep Med Rev. 2005;9(3):211-24. PubMed PMID: 15893251. eng.
95. Coughlin S, Calverley P, Wilding J. Sleep disordered breathing--a new component of syndrome x? Obes Rev. 2001;2(4):267-74. PubMed PMID: 12119997. eng.
96. Pasquali R. Metabolic Syndrome in Polycystic Ovary Syndrome. Front Horm Res. 2018;49:114-30. PubMed PMID: 29894990. Epub 04/05. eng.
97. Ng NYH, Jiang G, Cheung LP, Zhang Y, Tam CHT, Luk AOY, et al. Progression of glucose intolerance and cardiometabolic risk factors over a decade in Chinese women with polycystic ovary syndrome: A case-control study. PLoS Med. 2019;16(10):e1002953-e. PubMed PMID: 31652273. eng.
98. Zhang B, Wang J, Shen S, Liu J, Sun J, Gu T, et al. Association of Androgen Excess with Glucose Intolerance in Women with Polycystic Ovary Syndrome. Biomed Res Int. 2018;2018:6869705-. PubMed PMID: 29707577. eng.
99. Ramezani Tehrani F, Amiri M, Behboudi-Gandevani S, Bidhendi-Yarandi R, Carmina E. Cardiovascular events among reproductive and menopausal age women with polycystic ovary syndrome: a systematic review and meta-analysis. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2020 Jan;36(1):12-23. PubMed PMID: 31385729. Epub 2019/08/07. eng.
100. Pivonello R, Menafra D, Riccio E, Garifalos F, Mazzella M, de Angelis C, et al. Metabolic Disorders and Male Hypogonadotropic Hypogonadism. Front Endocrinol (Lausanne). 2019;10:345-. PubMed PMID: 31402895. eng.
101. Schulster ML, Liang SE, Najari BB. Metabolic syndrome and sexual dysfunction. Curr Opin Urol. 2017;27(5):435-40. PubMed PMID: 28650864. eng.
102. Biobaku F, Ghanim H, Batra M, Dandona P. Macronutrient-Mediated Inflammation and Oxidative Stress: Relevance to Insulin Resistance, Obesity, and Atherogenesis. The Journal of clinical endocrinology and metabolism. 2019;104(12):6118-28. PubMed PMID: 31219543. eng.
103. Martínez-García M, Moncayo S, Insenser M, Álvarez-Blasco F, Luque-Ramírez M, Escobar-Morreale HF. Metabolic Cytokines at Fasting and During Macronutrient Challenges: Influence of Obesity, Female Androgen Excess and Sex. Nutrients. 2019 Oct 24;11(11). PubMed PMID: 31652917. Pubmed Central PMCID: PMC6893420. Epub 2019/10/28. eng.
104. Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G, Reyes-Romero MA. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. Int J Endocrinol. 2013;2013:678159-. PubMed PMID: 23690772. Epub 04/17. eng.
105. Kotas ME, Medzhitov R. Homeostasis, inflammation, and disease susceptibility. Cell. 2015 Feb 26;160(5):816-27. PubMed PMID: 25723161. Pubmed Central PMCID: PMC4369762. Epub 2015/02/28. eng.
106. Romeo GR, Lee J, Shoelson SE. Metabolic syndrome, insulin resistance, and roles of inflammation--mechanisms and therapeutic targets. Arterioscler Thromb Vasc Biol. 2012;32(8):1771-6. PubMed PMID: 22815343. eng.
107. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. The Journal of clinical investigation. 2017;127(1):1-4. PubMed PMID: 28045402. Epub 01/03. eng.
108. Church C, Horowitz M, Rodeheffer M. WAT is a functional adipocyte? Adipocyte. 2012;1(1):38-45. PubMed PMID: 23700509. eng.
109. Nishimoto Y, Tamori Y. CIDE Family-Mediated Unique Lipid Droplet Morphology in White Adipose Tissue and Brown Adipose Tissue Determines the Adipocyte Energy Metabolism. J Atheroscler Thromb. 2017;24(10):989-98. PubMed PMID: 28883211. Epub 09/05. eng.
110. McIlvride S, Mushtaq A, Papacleovoulou G, Hurling C, Steel J, Jansen E, et al. A progesterone-brown fat axis is involved in regulating fetal growth. Sci Rep. 2017;7(1):10671-. PubMed PMID: 28878263. eng.
111. Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. Nat Rev Endocrinol.

2019;15(9):507-24. PubMed PMID: 31296970. Epub 07/11. eng.
112. Smitka K, Marešová D. Adipose Tissue as an Endocrine Organ: An Update on Pro-inflammatory and Anti-inflammatory Microenvironment. Prague Med Rep. 2015;116(2):87-111. PubMed PMID: 26093665. eng.
113. Mancuso P, Bouchard B. The Impact of Aging on Adipose Function and Adipokine Synthesis. Front Endocrinol (Lausanne). 2019;10:137. PubMed PMID: 30915034. Pubmed Central PMCID: PMC6421296. Epub 2019/03/28. eng.
114. Giordano A, Nisoli E. Neuroendocrinology of Energy Balance. In: Sbraccia P, Finer N, editors. Obesity: Pathogenesis, Diagnosis, and Treatment. Cham: Springer International Publishing; 2019. p. 31-50.
115. Perry RJ, Shulman GI. The Role of Leptin in Maintaining Plasma Glucose During Starvation. Postdoc J. 2018;6(3):3-19. PubMed PMID: 29682594. eng.
116. Paniagua JA. Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome. World J Diabetes. 2016;7(19):483-514. PubMed PMID: 27895819. eng.
117. Harris RBS. Direct and indirect effects of leptin on adipocyte metabolism. Biochim Biophys Acta. 2014;1842(3):414-23. PubMed PMID: 23685313. Epub 05/17. eng.
118. Paz-Filho G, Mastronardi C, Wong M-L, Licinio J. Leptin therapy, insulin sensitivity, and glucose homeostasis. Indian J Endocrinol Metab. 2012;16(Suppl 3):S549-S55. PubMed PMID: 23565489. eng.
119. Barateiro A, Mahú I, Domingos AI. Leptin Resistance and the Neuro-Adipose Connection. Front Endocrinol (Lausanne). 2017;8:45-. PubMed PMID: 28321206. eng.
120. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? Nutrients. 2019;11(11):2704. PubMed PMID: 31717265. eng.
121. Pérez-Pérez A, Vilariño-García T, Fernández-Riejos P, Martín-González J, Segura-Egea JJ, Sánchez-Margalet V. Role of leptin as a link between metabolism and the immune system. Cytokine Growth Factor Rev. 2017;35:71-84. PubMed PMID: 28285098. Epub 03/04. eng.
122. Sun X, Wei J, Tang Y, Wang B, Zhang Y, Shi L, et al. Leptin-induced migration and angiogenesis in rheumatoid arthritis is mediated by reactive oxygen species. FEBS Open Bio. 2017;7(12):1899-908. PubMed PMID: 29226077. eng.
123. La Cava A. Leptin in inflammation and autoimmunity. Cytokine. 2017 Oct;98:51-8. PubMed PMID: 27916613. Pubmed Central PMCID: PMC5453851. Epub 2016/12/06. eng.
124. Jonas MI, Kurylowicz A, Bartoszewicz Z, Lisik W, Jonas M, Domienik-Karlowicz J, et al. Adiponectin/resistin interplay in serum and in adipose tissue of obese and normal-weight individuals. Diabetology & metabolic syndrome. 2017;9:95. PubMed PMID: 29213336. Pubmed Central PMCID: PMC5709988. Epub 2017/12/08. eng.
125. Fisman EZ, Tenenbaum A. Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? Cardiovasc Diabetol. 2014;13:103-. PubMed PMID: 24957699. eng.
126. Frankenberg ADv, Reis AF, Gerchman F. Relationships between adiponectin levels, the metabolic syndrome, and type 2 diabetes: a literature review. Arch Endocrinol Metab. 2017;61(6):614-22. PubMed PMID: 29412387. eng.
127. Combs TP, Marliss EB. Adiponectin signaling in the liver. Rev Endocr Metab Disord. 2014;15(2):137-47. PubMed PMID: 24297186. eng.
128. Balsan GA, Vieira JLdC, Oliveira AMd, Portal VL. Relationship between adiponectin, obesity and insulin resistance. Rev Assoc Med Bras (1992). 2015 Jan-Feb;61(1):72-80. PubMed PMID: 25909213. Epub 01/01. eng.
129. Taghian F, Esteki Ghashghaei F, Badami R, Esteki Ghashghaei S. Comparison the Effect of One Session Submaximal Exercise on Plasma Levels of IL6 and TNF- a in Obese and Non-Obese Women. ARYA Atheroscler. 2011 Winter;6(4):153-6. PubMed PMID: 22577435. eng.
130. Shi J, Fan J, Su Q, Yang Z. Cytokines and Abnormal Glucose and Lipid Metabolism. Front Endocrinol (Lausanne). 2019;10:703-. PubMed PMID: 31736870. eng.
131. Lopez-Sandoval J, Sanchez-Enriquez S, Rivera-Leon EA, Bastidas-Ramirez BE, Garcia-Garcia MR, Gonzalez-Hita ME. CARDIOVASCULAR RISK FACTORS IN ADOLESCENTS: ROLE OF INSULIN RESISTANCE AND OBESITY. Acta endocrinologica (Bucharest, Romania : 2005). 2018 Jul-Sep;14(3):330-7. PubMed PMID: 31149280. Pubmed Central PMCID: PMC6525782. Epub 2019/06/01. eng.
132. Fernández-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev. 2003;24(3):278-301. PubMed PMID: 12788800. eng.
133. Reiss AB, Siegart NM, De Leon J. Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? Clinical Lipidology. 2017;12(1):14-23.
134. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and

insulin resistance: cells, cytokines, and chemokines. ISRN inflammation. 2013 Dec 22;2013:139239. PubMed PMID: 24455420. Pubmed Central PMCID: PMC3881510. Epub 2014/01/24. eng.
135. Zhu W, Owen N. Sedentary behavior and health: Concepts, assessments, and interventions: Human Kinetics; 2017.
136. Speaker KJ, Fleshner M. Interleukin-1 beta: a potential link between stress and the development of visceral obesity. BMC physiology. 2012 Jun 27;12:8. PubMed PMID: 22738239. Pubmed Central PMCID: PMC3404929. Epub 2012/06/29. eng.
137. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes. 2003 Mar;52(3):812-7. PubMed PMID: 12606524. Epub 2003/02/28. eng.
138. Bing C. Is interleukin-1β a culprit in macrophage-adipocyte crosstalk in obesity? Adipocyte. 2015 Apr-Jun;4(2):149-52. PubMed PMID: 26167419. Pubmed Central PMCID: PMC4496963. Epub 2015/07/15. eng.
139. Surmi BK, Hasty AH. Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. Future lipidology. 2008;3(5):545-56. PubMed PMID: 18978945. Pubmed Central PMCID: PMC2575346. Epub 2008/11/04. eng.
140. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM, et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. Endocr Rev. 2017;38(4):267-96. PubMed PMID: 28898979. eng.
141. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol. 2015;208(5):501-12. PubMed PMID: 25733711. eng.
142. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. Int J Mol Sci. 2019 May 13;20(9). PubMed PMID: 31085992. Pubmed Central PMCID: PMC6539070. Epub 2019/05/16. eng.
143. Ye J. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. Int J Obes (Lond). 2009;33(1):54-66. PubMed PMID: 19050672. Epub 12/09. eng.
144. Avram MM, Avram AS, James WD. Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. J Am Acad Dermatol. 2007;56(3):472-92. PubMed PMID: 17317490. eng.
145. Ma X, Wang D, Zhao W, Xu L. Deciphering the Roles of PPARγ in Adipocytes via Dynamic Change of Transcription Complex. Front Endocrinol (Lausanne). 2018;9:473. PubMed PMID: 30186237. Pubmed Central PMCID: PMC6110914. Epub 2018/09/07. eng.
146. Bandera Merchan B, Tinahones FJ, Macías-González M. Commonalities in the Association between PPARG and Vitamin D Related with Obesity and Carcinogenesis. PPAR Res. 2016;2016:2308249-. PubMed PMID: 27579030. Epub 08/08. eng.
147. Ko KD, Kim KK, Lee KR. Does Weight Gain Associated with Thiazolidinedione Use Negatively Affect Cardiometabolic Health? Journal of obesity & metabolic syndrome. 2017 Jun;26(2):102-6. PubMed PMID: 31089503. Pubmed Central PMCID: PMC6484909. Epub 2017/06/01. eng.
148. Kim SM, Lun M, Wang M, Senyo SE, Guillermier C, Patwari P, et al. Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. Cell Metab. 2014;20(6):1049-58. PubMed PMID: 25456741. Epub 11/20. eng.
149. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature. 2008;453(7196):783-7. PubMed PMID: 18454136. Epub 05/04. eng.
150. MacLean PS, Higgins JA, Giles ED, Sherk VD, Jackman MR. The role for adipose tissue in weight regain after weight loss. Obes Rev. 2015;16 Suppl 1(Suppl 1):45-54. PubMed PMID: 25614203. eng.
151. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259(5091):87-91. PubMed PMID: 7678183. eng.
152. Johnson AMF, Olefsky JM. The origins and drivers of insulin resistance. Cell. 2013;152(4):673-84. PubMed PMID: 23415219. eng.
153. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation. 2003;112(12):1796-808. PubMed PMID: 14679176. eng.
154. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010;72:219-46. PubMed PMID: 20148674. eng.
155. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation. 2007 Jan;117(1):175-84. PubMed PMID: 17200717. Pubmed Central PMCID: PMC1716210. Epub 2007/01/04. eng.
156. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by

spatiotemporal differences in macrophage subtypes. Diabetes. 2008 Dec;57(12):3239-46. PubMed PMID: 18829989. Pubmed Central PMCID: PMC2584129. Epub 2008/10/03. eng.
157. Boutens L, Hooiveld GJ, Dhingra S, Cramer RA, Netea MG, Stienstra R. Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia. 2018;61(4):942-53. PubMed PMID: 29333574. Epub 01/14. eng.
158. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, 2nd, DeFuria J, Jick Z, et al. Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes. 2007;56(12):2910-8. PubMed PMID: 17848624. Epub 09/11. eng.
159. Rogero MM, Calder PC. Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients. 2018;10(4):432. PubMed PMID: 29601492. eng.
160. Carvalheira JBC, Qiu Y, Chawla A. Blood spotlight on leukocytes and obesity. Blood. 2013;122(19):3263-7. PubMed PMID: 24065242. Epub 09/24. eng.
161. Yao Y, Xu X-H, Jin L. Macrophage Polarization in Physiological and Pathological Pregnancy. Frontiers in immunology. 2019;10:792-. PubMed PMID: 31037072. eng.
162. Tian Y, Yang C, Yao Q, Qian L, Liu J, Xie X, et al. Procyanidin B2 Activates PPARγ to Induce M2 Polarization in Mouse Macrophages. Frontiers in immunology. 2019;10:1895-. PubMed PMID: 31440258. eng.
163. Han JM, Levings MK. Immune regulation in obesity-associated adipose inflammation. Journal of immunology (Baltimore, Md : 1950). 2013 Jul 15;191(2):527-32. PubMed PMID: 23825387. Epub 2013/07/05. eng.
164. Nikhra V. The Entangled Relationship between NAFLD, Insulin Resistance and Obesity (ERNIRO). EC Endocrinology and Metabolic Research. 2019;4:349-59.
165. Pardo V, González-Rodríguez Á, Guijas C, Balsinde J, Valverde ÁM. Opposite cross-talk by oleate and palmitate on insulin signaling in hepatocytes through macrophage activation. The Journal of biological chemistry. 2015;290(18):11663-77. PubMed PMID: 25792746. Epub 03/19. eng.
166. Tencerova M, Aouadi M, Vangala P, Nicoloro SM, Yawe JC, Cohen JL, et al. Activated Kupffer cells inhibit insulin sensitivity in obese mice. FASEB J. 2015;29(7):2959-69. PubMed PMID: 25805830. Epub 03/24. eng.
167. Kern L, Mittenbühler MJ, Vesting AJ, Ostermann AL, Wunderlich CM, Wunderlich FT. Obesity-Induced TNFα and IL-6 Signaling: The Missing Link between Obesity and Inflammation-Driven Liver and Colorectal Cancers. Cancers. 2018 Dec 27;11(1). PubMed PMID: 30591653. Pubmed Central PMCID: PMC6356226. Epub 2018/12/29. eng.
168. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. Journal of hepatology. 2016;64(6):1403-15. PubMed PMID: 26867490. Epub 02/08. eng.
169. De Taeye BM, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, et al. Macrophage TNF-alpha contributes to insulin resistance and hepatic steatosis in diet-induced obesity. Am J Physiol Endocrinol Metab. 2007;293(3):E713-E25. PubMed PMID: 17578885. Epub 06/19. eng.
170. Kim MH, Ahn HK, Lee EJ, Kim SJ, Kim YR, Park JW, et al. Hepatic inflammatory cytokine production can be regulated by modulating sphingomyelinase and ceramide synthase 6. International journal of molecular medicine. 2017 Feb;39(2):453-62. PubMed PMID: 28035360. Epub 2016/12/31. eng.
171. Sokolowska E, Blachnio-Zabielska A. The Role of Ceramides in Insulin Resistance. Front Endocrinol (Lausanne). 2019;10:577-. PubMed PMID: 31496996. eng.
172. Sharifnia T, Antoun J, Verriere TG, Suarez G, Wattacheril J, Wilson KT, et al. Hepatic TLR4 signaling in obese NAFLD. American journal of physiology Gastrointestinal and liver physiology. 2015 Aug 15;309(4):G270-8. PubMed PMID: 26113297. Pubmed Central PMCID: PMC4537925. Epub 2015/06/27. eng.
173. Guillot A, Tacke F. Liver Macrophages: Old Dogmas and New Insights. Hepatol Commun. 2019;3(6):730-43. PubMed PMID: 31168508. eng.
174. Egawa T, Tsuda S, Goto A, Ohno Y, Yokoyama S, Goto K, et al. Potential involvement of dietary advanced glycation end products in impairment of skeletal muscle growth and muscle contractile function in mice. Br J Nutr. 2017;117(1):21-9. PubMed PMID: 28093090. Epub 01/17. eng.
175. Bhatt M, Rudrapatna S, Banfield L, Bierbrier R, Wang P-W, Wang K-W, et al. Evaluating the evidence for macrophage presence in skeletal muscle and its relation to insulin resistance in obese mice and humans: a systematic review protocol. BMC Res Notes. 2017;10(1):374-. PubMed PMID: 28789665. eng.
176. Liu J, Liu Z. Muscle Insulin Resistance and the Inflamed Microvasculature: Fire from Within. Int J Mol Sci. 2019;20(3):562. PubMed PMID: 30699907. eng.
177. Castañer O, Schröder H. Response to: Comment on "The Gut Microbiome Profile in Obesity: A Systematic Review". Int J Endocrinol. 2018;2018:9109451-. PubMed PMID: 30671094. eng.
178. Frost F, Storck LJ, Kacprowski T, Gärtner S, Rühlemann M, Bang C, et al. A structured weight loss program increases gut microbiota

phylogenetic diversity and reduces levels of Collinsella in obese type 2 diabetics: A pilot study. PloS one. 2019;14(7):e0219489-e. PubMed PMID: 31318902. eng.
179. Sivamaruthi BS, Kesika P, Suganthy N, Chaiyasut C. A Review on Role of Microbiome in Obesity and Antiobesity Properties of Probiotic Supplements. Biomed Res Int. 2019;2019:3291367-. PubMed PMID: 31211135. eng.
180. Sun L, Ma L, Ma Y, Zhang F, Zhao C, Nie Y. Insights into the role of gut microbiota in obesity: pathogenesis, mechanisms, and therapeutic perspectives. Protein Cell. 2018;9(5):397-403. PubMed PMID: 29725936. eng.
181. Silva-Junior VLd, Lopes FdAM, Albano RM, Souza MdGCd, Barbosa CMdL, Maranhão PA, et al. Obesity and gut microbiota-what do we know so far? MedicalExpress. 2017;4(4).
182. Al-Assal K, Martinez AC, Torrinhas RS, Cardinelli C, Waitzberg D. Gut microbiota and obesity. Clinical Nutrition Experimental. 2018;20:60-4.
183. Davis CD. The Gut Microbiome and Its Role in Obesity. Nutr Today. 2016 Jul-Aug;51(4):167-74. PubMed PMID: 27795585. eng.
184. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas M-E. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med. 2016;8(1):42-. PubMed PMID: 27098727. eng.
185. Wilson K, Situ C. Systematic review on effects of diet on gut microbiota in relation to metabolic syndromes. J Clin Nutr Metab. 2017;1(2):1-12.
186. López M. EJE PRIZE 2017: Hypothalamic AMPK: a golden target against obesity? Eur J Endocrinol. 2017;176(5):R235-R46. PubMed PMID: 28232370. Epub 02/23. eng.
187. Lazar V, Ditu LM, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, et al. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. Frontiers in immunology. 2018;9:1830. PubMed PMID: 30158926. Pubmed Central PMCID: PMC6104162. Epub 2018/08/31. eng.
188. Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. Expert Rev Gastroenterol Hepatol. 2017;11(9):821-34. PubMed PMID: 28650209. Epub 06/26. eng.
189. Blackwood BP, Yuan CY, Wood DR, Nicolas JD, Grothaus JS, Hunter CJ. Probiotic Lactobacillus Species Strengthen Intestinal Barrier Function and Tight Junction Integrity in Experimental Necrotizing Enterocolitis. J Probiotics Health. 2017;5(1):159. PubMed PMID: 28638850. Epub 01/02. eng.
190. Medzhitov R. Approaching the asymptote: 20 years later. Immunity. 2009 Jun 19;30(6):766-75. PubMed PMID: 19538928. Epub 2009/06/23. eng.
191. Mukherjee S, Karmakar S, Babu SPS. TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. Braz J Infect Dis. 2016 Mar-Apr;20(2):193-204. PubMed PMID: 26775799. Epub 01/14. eng.
192. Thiriet M. Vasculopathies: Behavioral, Chemical, Environmental, and Genetic Factors: Springer International Publishing; 2019.
193. Fornari E, Maffeis C. Treatment of Metabolic Syndrome in Children. Front Endocrinol (Lausanne). 2019;10:702-. PubMed PMID: 31681173. eng.
194. Benaiges D, Parri A, Subirana I, Pedro-Botet J, Villatoro M, Ramon JM, et al. Most of qualitative dietary changes observed one year post-bariatric surgery can be achieved with a preoperative dietary intervention. Endocrinol Diabetes Nutr. 2020;67(1):20-7. PubMed PMID: 31288988. Epub 07/07. eng
spa.
195. Patel AR, Nicholson RA, Marangoni AG. Applications of Fat mimetics for the replacement of saturated and hydrogenated fat in food products. Current Opinion in Food Science. 2020.
196. Calliope SR, Samman NC. Sodium Content in Commonly Consumed Foods and Its Contribution to the Daily Intake. Nutrients. 2019;12(1):E34. PubMed PMID: 31877703. eng.
197. Greer RC, Marklund M, Anderson CAM, Cobb LK, Dalcin AT, Henry M, et al. Potassium-Enriched Salt Substitutes as a Means to Lower Blood Pressure: Benefits and Risks. Hypertension. 2020;75(2):266-74. PubMed PMID: 31838902. Epub 12/16. eng.
198. Delgado-Floody P, Álvarez C, Lusa Cadore E, Flores-Opazo M, Caamaño-Navarrete F, Izquierdo M. Preventing metabolic syndrome in morbid obesity with resistance training: Reporting interindividual variability. Nutr Metab Cardiovasc Dis. 2019;29(12):1368-81. PubMed PMID: 31383503. Epub 07/12. eng.
199. Myers J, Kokkinos P, Nyelin E. Physical Activity, Cardiorespiratory Fitness, and the Metabolic Syndrome. Nutrients. 2019;11(7):1652. PubMed PMID: 31331009. eng.
200. Wang Y, Xu D. Effects of aerobic exercise on lipids and lipoproteins. Lipids Health Dis. 2017;16(1):132-. PubMed PMID: 28679436. eng.
201. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, et al. Pharmacological management of obesity: an endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2015 Feb;100(2):342-62. PubMed PMID: 25590212. Epub 2015/01/16. eng.
202. Narayanaswami V, Dwoskin LP. Obesity: Current and potential pharmacotherapeutics and

targets. Pharmacol Ther. 2017;170:116-47. PubMed PMID: 27773782. Epub 10/20. eng.
203. Albert CL. Morbid Obesity as a Therapeutic Target for Heart Failure. Current treatment options in cardiovascular medicine. 2019 Sep 5;21(10):52. PubMed PMID: 31486922. Epub 2019/09/06. eng.
204. Apovian CM, Istfan NW. Obesity: Guidelines, Best Practices, New Research. Endocrinol Metab Clin North Am. 2016;45(3):xvii-xviii. PubMed PMID: 27519142. eng.
205. Kanagaraj M. Weight loss medications for patients: A review. Bariatric Times. 2018;15(4):6-10.
206. Allison DB, Gadde KM, Garvey WT, Peterson CA, Schwiers ML, Najarian T, et al. Controlled-release phentermine/topiramate in severely obese adults: a randomized controlled trial (EQUIP). Obesity (Silver Spring). 2012 Feb;20(2):330-42. PubMed PMID: 22051941. Pubmed Central PMCID: PMC3270297. Epub 2011/11/05. eng.
207. Gadde KM, Allison DB, Ryan DH, Peterson CA, Troupin B, Schwiers ML, et al. Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377(9774):1341-52. PubMed PMID: 21481449. Epub 04/08. eng.
208. Bray GA, Heisel WE, Afshin A, Jensen MD, Dietz WH, Long M, et al. The Science of Obesity Management: An Endocrine Society Scientific Statement. Endocr Rev. 2018;39(2):79-132. PubMed PMID: 29518206. eng.
209. Johnson DB, Quick J. Topiramate And Phentermine. StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
210. McCafferty BJ, Hill JO, Gunn AJ. Obesity: scope, lifestyle interventions, and medical management. Techniques in Vascular and Interventional Radiology. 2020:100653.
211. Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 2004;27(1):155-61. PubMed PMID: 14693982. eng.
212. Velazquez A, Apovian CM. Updates on obesity pharmacotherapy. Annals of the New York Academy of Sciences. 2018 Jan;1411(1):106-19. PubMed PMID: 29377198. Epub 2018/01/30. eng.
213. Solomon LR, Nixon AC, Ogden L, Nair B. Orlistat-induced oxalate nephropathy: an under-recognised cause of chronic kidney disease. BMJ Case Rep. 2017;2017:bcr2016218623. PubMed PMID: 29133578. eng.
214. Coutinho AK, Glancey GR. Orlistat, an under-recognised cause of progressive renal impairment. Nephrol Dial Transplant. 2013;28 Suppl 4:iv172-iv4. PubMed PMID: 24049105. Epub 09/18. eng.
215. Apovian C, Palmer K, Fain R, Perdomo C, Rubino D. Effects of lorcaserin on fat and lean mass loss in obese and overweight patients without and with type 2 diabetes mellitus: the BLOSSOM and BLOOM-DM studies. Diabetes Obes Metab. 2016;18(9):945-8. PubMed PMID: 27173586. Epub 06/22. eng.
216. Aronne L, Shanahan W, Fain R, Glicklich A, Soliman W, Li Y, et al. Safety and efficacy of lorcaserin: a combined analysis of the BLOOM and BLOSSOM trials. Postgrad Med. 2014;126(6):7-18. PubMed PMID: 25414931. eng.
217. Patel DK, Stanford FC. Safety and tolerability of new-generation anti-obesity medications: a narrative review. Postgrad Med. 2018;130(2):173-82. PubMed PMID: 29388462. Epub 02/08. eng.
218. Fujioka K, Braverman-Panza J. Answers to Clinical Questions in the Primary Care Management of People with Obesity: Pharmacologic Management. The Journal of family practice. 2016 Jul;65(7 Suppl):S16-23. PubMed PMID: 27565106. Epub 2016/08/28. eng.
219. Pi-Sunyer X, Apovian CM, McElroy SL, Dunayevich E, Acevedo LM, Greenway FL. Psychiatric adverse events and effects on mood with prolonged-release naltrexone/bupropion combination therapy: a pooled analysis. Int J Obes (Lond). 2019;43(10):2085-94. PubMed PMID: 30664661. Epub 01/21. eng.
220. Bello NT. Update on drug safety evaluation of naltrexone/bupropion for the treatment of obesity. Expert Opin Drug Saf. 2019;18(7):549-52. PubMed PMID: 31092063. Epub 05/17. eng.
221. Upadhyay J, Polyzos SA, Perakakis N, Thakkar B, Paschou SA, Katsiki N, et al. Pharmacotherapy of type 2 diabetes: An update. Metabolism. 2018;78:13-42. PubMed PMID: 28920861. Epub 09/08. eng.
222. Davies MJ, Bergenstal R, Bode B, Kushner RF, Lewin A, Skjøth TV, et al. Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes: The SCALE Diabetes Randomized Clinical Trial. JAMA. 2015;314(7):687-99. PubMed PMID: 26284720. eng.
223. Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, et al. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. N Engl J Med. 2015;373(1):11-22. PubMed PMID: 26132939. eng.
224. Wadden TA, Hollander P, Klein S, Niswender K, Woo V, Hale PM, et al. Weight

maintenance and additional weight loss with liraglutide after low-calorie-diet-induced weight loss: the SCALE Maintenance randomized study. Int J Obes (Lond). 2013;37(11):1443-51. PubMed PMID: 23812094. Epub 07/01. eng.
225. Dowarah J, Singh VP. Anti-diabetic drugs recent approaches and advancements. Bioorganic & medicinal chemistry. 2020 Mar 1;28(5):115263. PubMed PMID: 32008883. Epub 2020/02/06. eng.
226. Jamous RM, Abu-Zaitoun SY, Akkawi RJ, Ali-Shtayeh MS. Antiobesity and Antioxidant Potentials of Selected Palestinian Medicinal Plants. Evid Based Complement Alternat Med. 2018;2018:8426752-. PubMed PMID: 30026782. eng.
227. Alexandru Mihai Grumezescu AMH. Therapeutic, Probiotic, and Unconventional Foods. 2018. p. 484.
228. Aryaeian N, Sedehi SK, Arablou T. Polyphenols and their effects on diabetes management: A review. Med J Islam Repub Iran. 2017;31:134-. PubMed PMID: 29951434. eng.
229. Khalilpourfarshbafi M, Gholami K, Murugan DD, Abdul Sattar MZ, Abdullah NA. Differential effects of dietary flavonoids on adipogenesis. Eur J Nutr. 2019;58(1):5-25. PubMed PMID: 29541908. Epub 03/14. eng.
230. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. Nutrients. 2018;10(11):1618. PubMed PMID: 30400131. eng.
231. Mendoza-Sánchez M, Pérez-Ramírez IF, Wall-Medrano A, Martinez-Gonzalez AI, Gallegos-Corona MA, Reynoso-Camacho R. Chemically induced common bean (Phaseolus vulgaris L.) sprouts ameliorate dyslipidemia by lipid intestinal absorption inhibition. Journal of functional foods. 2019;52:54-62.
232. Huang J, Feng S, Liu A, Dai Z, Wang H, Reuhl K, et al. Green Tea Polyphenol EGCG Alleviates Metabolic Abnormality and Fatty Liver by Decreasing Bile Acid and Lipid Absorption in Mice. Mol Nutr Food Res. 2018;62(4):10.1002/mnfr.201700696. PubMed PMID: 29278293. Epub 01/29. eng.
233. Tenore GC, Carotenuto A, Caruso D, Buonomo G, D'Avino M, Brancaccio D, et al. A nutraceutical formulation based on Annurca apple polyphenolic extract is effective on intestinal cholesterol absorption: A randomised, placebo-controlled, crossover study. PharmaNutrition. 2018;6(3):85-94.
234. Porras D, Nistal E, Martínez-Flórez S, Pisonero-Vaquero S, Olcoz JL, Jover R, et al. Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. Free Radic Biol Med. 2017;102:188-202. PubMed PMID: 27890642. Epub 11/25. eng.
235. Choi I, Park Y, Choi H, Lee EH. Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. Biofactors. 2006;26(4):273-81. PubMed PMID: 17119273. eng.
236. Hsu C-L, Wu C-H, Huang S-L, Yen G-C. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. J Agric Food Chem. 2009;57(2):425-31. PubMed PMID: 19119847. eng.
237. Alkhalidy H, Moore W, Zhang Y, McMillan R, Wang A, Ali M, et al. Small Molecule Kaempferol Promotes Insulin Sensitivity and Preserved Pancreatic β -Cell Mass in Middle-Aged Obese Diabetic Mice. J Diabetes Res. 2015;2015:532984-. PubMed PMID: 26064984. Epub 05/07. eng.
238. Zang Y, Zhang L, Igarashi K, Yu C. The anti-obesity and anti-diabetic effects of kaempferol glycosides from unripe soybean leaves in high-fat-diet mice. Food & function. 2015;6(3):834-41. PubMed PMID: 25599885. eng.
239. Imai K, Nakachi K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. BMJ. 1995;310(6981):693-6. PubMed PMID: 7711535. eng.
240. Vitale M, Vaccaro O, Masulli M, Bonora E, Del Prato S, Giorda CB, et al. Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: The TOSCA.IT study. Clinical nutrition (Edinburgh, Scotland). 2017 Dec;36(6):1686-92. PubMed PMID: 27890487. Epub 2016/11/29. eng.
241. Pounis G, Bonaccio M, Di Castelnuovo A, Costanzo S, de Curtis A, Persichillo M, et al. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. Thromb Haemost. 2016;115(2):344-52. PubMed PMID: 26355794. Epub 09/10. eng.
242. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al. Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci. 2010;11(4):1365-402. PubMed PMID: 20480025. eng.
243. Watson RR, Preedy VR, Zibadi S. Polyphenols: Mechanisms of Action in Human Health and Disease: Elsevier Science; 2018.
244. Sayem ASM, Arya A, Karimian H, Krishnasamy N, Ashok Hasamnis A, Hossain CF. Action of Phytochemicals on Insulin Signaling Pathways Accelerating Glucose Transporter (GLUT4) Protein Translocation. Molecules. 2018;23(2):258. PubMed PMID: 29382104. eng.
245. Dhanya R, Arya AD, Nisha P, Jayamurthy P. Quercetin, a Lead Compound against Type 2 Diabetes Ameliorates Glucose Uptake via AMPK Pathway in Skeletal Muscle Cell Line. Front Pharmacol. 2017;8:336-. PubMed PMID: 28642704. eng.

246. Eid HM, Martineau LC, Saleem A, Muhammad A, Vallerand D, Benhaddou-Andaloussi A, et al. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Mol Nutr Food Res. 2010;54(7):991-1003.
247. Zhang Y, Liu D. Flavonol kaempferol improves chronic hyperglycemia-impaired pancreatic beta-cell viability and insulin secretory function. Eur J Pharmacol. 2011;670(1):325-32. PubMed PMID: 21914439. Epub 09/02. eng.
248. Kappel VD, Cazarolli LH, Pereira DF, Postal BG, Zamoner A, Reginatto FH, et al. Involvement of GLUT-4 in the stimulatory effect of rutin on glucose uptake in rat soleus muscle. J Pharm Pharmacol. 2013;65(8):1179-86. PubMed PMID: 23837585. Epub 05/16. eng.
249. Kamalakkannan N, Prince PSM. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin Pharmacol Toxicol. 2006;98(1):97-103. PubMed PMID: 16433898. eng.
250. Rodríguez-Rodríguez C, Torres N, Gutiérrez-Uribe JA, Noriega LG, Torre-Villalvazo I, Leal-Díaz AM, et al. The effect of isorhamnetin glycosides extracted from Opuntia ficus-indica in a mouse model of diet induced obesity. Food & function. 2015;6(3):805-15. PubMed PMID: 25588195. eng.
251. Liu IM, Tzeng T-F, Liou S-S, Lan T-W. Myricetin, a naturally occurring flavonol, ameliorates insulin resistance induced by a high-fructose diet in rats. Life Sci. 2007;81(21-22):1479-88. PubMed PMID: 17976658. Epub 10/02. eng.
252. Colitti M, Grasso S. Nutraceuticals and regulation of adipocyte life: premises or promises. Biofactors. 2014 Jul-Aug;40(4):398-418. PubMed PMID: 24692086. Epub 04/02. eng.
253. Peng C-H, Cheng J-J, Yu M-H, Chung D-J, Huang C-N, Wang C-J. Solanum nigrum polyphenols reduce body weight and body fat by affecting adipocyte and lipid metabolism. Food & function. 2020;11(1):483-92. PubMed PMID: 31833514. eng.
254. Hussain S, Rehman AU, Luckett DJ, Blanchard CL, Obied HK, Strappe P. Phenolic Compounds with Antioxidant Properties from Canola Meal Extracts Inhibit Adipogenesis. Int J Mol Sci. 2019;21(1):1. PubMed PMID: 31861265. eng.
255. Wu LY, Chen CW, Chen LK, Chou HY, Chang CL, Juan CC. Curcumin Attenuates Adipogenesis by Inducing Preadipocyte Apoptosis and Inhibiting Adipocyte Differentiation. Nutrients. 2019 Sep 28;11(10). PubMed PMID: 31569380. Pubmed Central PMCID: PMC6836120. Epub 2019/10/02. eng.
256. Wein S, Behm N, Petersen RK, Kristiansen K, Wolffram S. Quercetin enhances adiponectin secretion by a PPAR-gamma independent mechanism. Eur J Pharm Sci. 2010;41(1):16-22. PubMed PMID: 20580672. Epub 05/16. eng.
257. Song Y, Park HJ, Kang SN, Jang S-H, Lee S-J, Ko Y-G, et al. Blueberry peel extracts inhibit adipogenesis in 3T3-L1 cells and reduce high-fat diet-induced obesity. PloS one. 2013;8(7):e69925-e. PubMed PMID: 23936120. eng.
258. Ahn J, Lee H, Kim S, Park J, Ha T. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem Biophys Res Commun. 2008;373(4):545-9. PubMed PMID: 18586010. Epub 06/27. eng.
259. Kim HJ, You MK, Lee YH, Kim HJ, Adhikari D, Kim HA. Red pepper seed water extract inhibits preadipocyte differentiation and induces mature adipocyte apoptosis in 3T3-L1 cells. Nutrition research and practice. 2018 Dec;12(6):494-502. PubMed PMID: 30515277. Pubmed Central PMCID: PMC6277307. Epub 2018/12/06. eng.
260. Most J, Warnke I, Boekschoten MV, Jocken JWE, de Groot P, Friedel A, et al. The effects of polyphenol supplementation on adipose tissue morphology and gene expression in overweight and obese humans. Adipocyte. 2018;7(3):190-6. PubMed PMID: 29786471. Epub 05/22. eng.
261. Rivera L, Morón R, Sánchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity (Silver Spring). 2008;16(9):2081-7. PubMed PMID: 18551111. eng.
262. Kobori M, Masumoto S, Akimoto Y, Oike H. Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. Mol Nutr Food Res. 2011;55(4):530-40. PubMed PMID: 21462320. Epub 12/15. eng.
263. Overman A, Chuang CC, McIntosh M. Quercetin attenuates inflammation in human macrophages and adipocytes exposed to macrophage-conditioned media. Int J Obes (Lond). 2011;35(9):1165-72. PubMed PMID: 21224828. Epub 01/11. eng.
264. Luo C, Yang H, Tang C, Yao G, Kong L, He H, et al. Kaempferol alleviates insulin resistance via hepatic IKK/NF-κB signal in type 2 diabetic rats. Int Immunopharmacol. 2015;28(1):744-50. PubMed PMID: 26263168. Epub 08/09. eng.
265. Tanaka M, Sugama A, Sumi K, Shimizu K, Kishimoto Y, Kondo K, et al. Gallic acid regulates adipocyte hypertrophy and suppresses inflammatory gene expression induced by the paracrine interaction between adipocytes and macrophages in vitro and in vivo. Nutr Res.

2020;73:58-66. PubMed PMID: 31841748. Epub 10/24. eng.
266. Jiang Y, Du H, Liu X, Fu X, Li X, Cao Q. Artemisinin alleviates atherosclerotic lesion by reducing macrophage inflammation via regulation of AMPK/NF-κB/NLRP3 inflammasomes pathway. J Drug Target. 2020;28(1):70-9. PubMed PMID: 31094238. Epub 07/16. eng.
267. Zhao L, Cen F, Tian F, Li M-J, Zhang Q, Shen H-Y, et al. Combination treatment with quercetin and resveratrol attenuates high fat diet-induced obesity and associated inflammation in rats via the AMPKα1/SIRT1 signaling pathway. Exp Ther Med. 2017;14(6):5942-8. PubMed PMID: 29285143. Epub 10/18. eng.
268. Zhao L, Zou T, Gomez NA, Wang B, Zhu M-J, Du M. Raspberry alleviates obesity-induced inflammation and insulin resistance in skeletal muscle through activation of AMP-activated protein kinase (AMPK) α1. Nutr Diabetes. 2018;8(1):39-. PubMed PMID: 29961765. eng.
269. Kolehmainen M, Mykkänen O, Kirjavainen PV, Leppänen T, Moilanen E, Adriaens M, et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. Mol Nutr Food Res. 2012 Oct;56(10):1501-10. PubMed PMID: 22961907. Epub 2012/09/11. eng.
270. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292(12):1440-6. PubMed PMID: 15383514. eng.
271. Sánchez M, Romero M, Gómez-Guzmán M, Tamargo J, Pérez-Vizcaino F, Duarte J. Cardiovascular Effects of Flavonoids. Current medicinal chemistry. 2019;26(39):6991-7034. PubMed PMID: 30569843. Epub 2018/12/21. eng.
272. Al-Awwadi NA, Araiz C, Bornet A, Delbosc S, Cristol J-P, Linck N, et al. Extracts enriched in different polyphenolic families normalize increased cardiac NADPH oxidase expression while having differential effects on insulin resistance, hypertension, and cardiac hypertrophy in high-fructose-fed rats. J Agric Food Chem. 2005;53(1):151-7. PubMed PMID: 15631522. eng.
273. Calabriso N, Massaro M, Scoditti E, D'Amore S, Gnoni A, Pellegrino M, et al. Extra virgin olive oil rich in polyphenols modulates VEGF-induced angiogenic responses by preventing NADPH oxidase activity and expression. J Nutr Biochem. 2016;28:19-29. PubMed PMID: 26878779. Epub 10/17. eng.
274. Gómez-Guzmán M, Jiménez R, Sánchez M, Romero M, O'Valle F, Lopez-Sepulveda R, et al. Chronic (-)-epicatechin improves vascular oxidative and inflammatory status but not hypertension in chronic nitric oxide-deficient rats. Br J Nutr. 2011 Nov;106(9):1337-48. PubMed PMID: 21910946. Epub 2011/09/14. eng.
275. Galindo P, González-Manzano S, Zarzuelo MJ, Gómez-Guzmán M, Quintela AM, González-Paramás A, et al. Different cardiovascular protective effects of quercetin administered orally or intraperitoneally in spontaneously hypertensive rats. Food & function. 2012 Jun;3(6):643-50. PubMed PMID: 22441211. Epub 2012/03/24. eng.
276. Ikarashi N, Toda T, Hatakeyama Y, Kusunoki Y, Kon R, Mizukami N, et al. Anti-Hypertensive Effects of Acacia Polyphenol in Spontaneously Hypertensive Rats. Int J Mol Sci. 2018;19(3):700. PubMed PMID: 29494506. eng.
277. Medina-Remón A, Zamora-Ros R, Rotchés-Ribalta M, Andres-Lacueva C, Martínez-González MA, Covas MI, et al. Total polyphenol excretion and blood pressure in subjects at high cardiovascular risk. Nutr Metab Cardiovasc Dis. 2011 May;21(5):323-31. PubMed PMID: 20167460. Epub 2010/02/20. eng.
278. Onakpoya I, Spencer E, Heneghan C, Thompson M. The effect of green tea on blood pressure and lipid profile: a systematic review and meta-analysis of randomized clinical trials. Nutr Metab Cardiovasc Dis. 2014;24(8):823-36. PubMed PMID: 24675010. Epub 01/31. eng.
279. Cassidy A, O'Reilly É J, Kay C, Sampson L, Franz M, Forman JP, et al. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr. 2011 Feb;93(2):338-47. PubMed PMID: 21106916. Pubmed Central PMCID: PMC3021426. Epub 2010/11/26. eng.
280. Lavefve L, Howard LR, Carbonero F. Berry polyphenols metabolism and impact on human gut microbiota and health. Food & function. 2020 Jan 29;11(1):45-65. PubMed PMID: 31808762. Epub 2019/12/07. eng.
281. Wang P, Li D, Ke W, Liang D, Hu X, Chen F. Resveratrol-induced gut microbiota reduces obesity in high-fat diet-fed mice. Int J Obes (Lond). 2020;44(1):213-25. PubMed PMID: 30718820. Epub 02/04. eng.
282. Bitzer ZT, Elias RJ, Vijay-Kumar M, Lambert JD. (-)-Epigallocatechin-3-gallate decreases colonic inflammation and permeability in a mouse model of colitis, but reduces macronutrient digestion and exacerbates weight loss. Mol Nutr Food Res. 2016;60(10):2267-74. PubMed PMID: 27218415. Epub 06/20. eng.
283. Cremonini E, Wang Z, Bettaieb A, Adamo AM, Daveri E, Mills DA, et al. (-)-Epicatechin protects the intestinal barrier from high fat diet-induced permeabilization: Implications for steatosis and insulin resistance. Redox Biol. 2018;14:588-99. PubMed PMID: 29154190. Epub 11/07. eng.
284. Gu L, Li N, Gong J, Li Q, Zhu W, Li J. Berberine ameliorates intestinal epithelial tight-junction damage and down-regulates myosin light chain kinase pathways in a mouse model of

endotoxinemia. J Infect Dis. 2011;203(11):1602-12. PubMed PMID: 21592990. eng.
285. Bernardi S, Del Bo C, Marino M, Gargari G, Cherubini A, Andrés-Lacueva C, et al. Polyphenols and Intestinal Permeability: Rationale and Future Perspectives. J Agric Food Chem. 2020;68(7):1816-29. PubMed PMID: 31265272. Epub 07/02. eng.
286. Dueñas M, Muñoz-González I, Cueva C, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C, et al. A survey of modulation of gut microbiota by dietary polyphenols. Biomed Res Int. 2015;2015:850902-. PubMed PMID: 25793210. Epub 02/22. eng.
287. Moreno-Indias I, Sánchez-Alcoholado L, Pérez-Martínez P, Andrés-Lacueva C, Cardona F, Tinahones F, et al. Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients. Food & function. 2016;7(4):1775-87. PubMed PMID: 26599039. eng.
288. Fernandez-Raudales D, Hoeflinger JL, Bringe NA, Cox SB, Dowd SE, Miller MJ, et al. Consumption of different soymilk formulations differentially affects the gut microbiomes of overweight and obese men. Gut Microbes. 2012 Nov-Dec;3(6):490-500. PubMed PMID: 22895080. Epub 08/16. eng.
289. Ismail A, Ikram EHK, Nazri HSM. Roselle (Hibiscus sabdariffa L.) seeds-nutritional composition, protein quality and health benefits. Food. 2008;2(1):1-16.
290. Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U, et al. Effects of roselle (Hibiscus sabdariffa Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in Salmonella typhimurium and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in F344 rats. Food Chem Toxicol. 1999;37(6):591-601. PubMed PMID: 10478827. eng.
291. Wright CI, Van-Buren L, Kroner CI, Koning MMG. Herbal medicines as diuretics: a review of the scientific evidence. J Ethnopharmacol. 2007;114(1):1-31. PubMed PMID: 17804183. Epub 07/31. eng.
292. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L. Biomed Pharmacother. 2018;102:575-86. PubMed PMID: 29597091. Epub 04/05. eng.
293. Maganha EG, da Costa Halmenschlager R, Rosa RM, Henriques JAP, de Paula Ramos ALL, Saffi J. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chem. 2010;118(1):1-10.
294. Moyano G, Sáyago-Ayerdi SG, Largo C, Caz V, Santamaria M, Tabernero M. Potential use of dietary fibre from Hibiscus sabdariffa and Agave tequilana in obesity management. Journal of Functional Foods. 2016;21:1-9.
295. Formagio ASN, Ramos DD, Vieira MC, Ramalho SR, Silva MM, Zárate NAH, et al. Phenolic compounds of Hibiscus sabdariffa and influence of organic residues on its antioxidant and antitumoral properties. Braz J Biol. 2015 Jan-Mar;75(1):69-76. PubMed PMID: 25945622. eng.
296. Zakaria FR, Prangdimurti E, Damanik R. Anti-inflammatory of purple roselle extract in diabetic rats induced by streptozotocin. Procedia Food Science. 2015;3:182-9.
297. Peter EL, Rumisha SF, Mashoto KO, Malebo HM. Ethno-medicinal knowledge and plants traditionally used to treat anemia in Tanzania: a cross sectional survey. J Ethnopharmacol. 2014;154(3):767-73. PubMed PMID: 24835027. Epub 05/14. eng.
298. Tsai T-C, Huang H-P, Chang Y-C, Wang C-J. An anthocyanin-rich extract from Hibiscus sabdariffa linnaeus inhibits N-nitrosomethylurea-induced leukemia in rats. J Agric Food Chem. 2014;62(7):1572-80. PubMed PMID: 24471438. Epub 02/06. eng.
299. Aziz Z, Wong SY, Chong NJ. Effects of Hibiscus sabdariffa L. on serum lipids: a systematic review and meta-analysis. J Ethnopharmacol. 2013;150(2):442-50. PubMed PMID: 24120746. Epub 10/10. eng.
300. Gbolade A. Ethnobotanical study of plants used in treating hypertension in Edo State of Nigeria. J Ethnopharmacol. 2012;144(1):1-10. PubMed PMID: 22975417. Epub 09/10. eng.
301. Prasongwatana V, Woottisin S, Sriboonlue P, Kukongviriyapan V. Uricosuric effect of Roselle (Hibiscus sabdariffa) in normal and renal-stone former subjects. J Ethnopharmacol. 2008;117(3):491-5. PubMed PMID: 18423919. Epub 03/14. eng.
302. Ribeiro RdA, de Barros F, de Melo MM, Muniz C, Chieia S, Wanderley M das G, et al. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of São Paulo, Brasil. J Ethnopharmacol. 1988;24(1):19-29. PubMed PMID: 3199837. eng.
303. Olivares-Vicente M, Barrajon-Catalan E, Herranz-Lopez M, Segura-Carretero A, Joven J, Encinar JA, et al. Plant-Derived Polyphenols in Human Health: Biological Activity, Metabolites and Putative Molecular Targets. Curr Drug Metab. 2018;19(4):351-69. PubMed PMID: 29468962. eng.
304. Beltrán-Debón R, Rodríguez-Gallego E, Fernández-Arroyo S, Senan-Campos O, Massucci FA, Hernández-Aguilera A, et al. The acute impact of polyphenols from Hibiscus sabdariffa in metabolic homeostasis: an approach combining metabolomics and gene-expression analyses. Food & function. 2015;6(9):2957-66. PubMed PMID: 26234931. eng.

305. Villalpando-Arteaga EV, Mendieta-Condado E, Esquivel-Solís H, Canales-Aguirre AA, Gálvez-Gastélum FJ, Mateos-Díaz JC, et al. Hibiscus sabdariffa L. aqueous extract attenuates hepatic steatosis through down-regulation of PPAR-γ and SREBP-1c in diet-induced obese mice. Food & function. 2013;4(4):618-26. PubMed PMID: 23389749. Epub 02/07. eng.
306. Alarcon-Aguilar FJ, Zamilpa A, Perez-Garcia MD, Almanza-Perez JC, Romero-Nuñez E, Campos-Sepulveda EA, et al. Effect of Hibiscus sabdariffa on obesity in MSG mice. J Ethnopharmacol. 2007;114(1):66-71. PubMed PMID: 17765418. Epub 07/27. eng.
307. Hansawasdi C, Kawabata J, Kasai T. Hibiscus acid as an inhibitor of starch digestion in the Caco-2 cell model system. Bioscience, biotechnology, and biochemistry. 2001;65(9):2087-9. PubMed PMID: 11676026. eng.
308. Carvajal-Zarrabal O, Hayward-Jones PM, Orta-Flores Z, Nolasco-Hipólito C, Barradas-Dermitz DM, Aguilar-Uscanga MG, et al. Effect of Hibiscus sabdariffa L. dried calyx ethanol extract on fat absorption-excretion, and body weight implication in rats. J Biomed Biotechnol. 2009;2009:394592-. PubMed PMID: 19756159. Epub 09/10. eng.
309. Chang H-C, Peng C-H, Yeh D-M, Kao E-S, Wang C-J. Hibiscus sabdariffa extract inhibits obesity and fat accumulation, and improves liver steatosis in humans. Food & function. 2014;5(4):734-9. PubMed PMID: 24549255. Epub 02/19. eng.
310. Pettinelli P, Videla LA. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. The Journal of clinical endocrinology and metabolism. 2011;96(5):1424-30. PubMed PMID: 21325464. Epub 02/16. eng.
311. Herranz-López M, Fernández-Arroyo S, Pérez-Sanchez A, Barrajón-Catalán E, Beltrán-Debón R, Menéndez JA, et al. Synergism of plant-derived polyphenols in adipogenesis: perspectives and implications. Phytomedicine. 2012;19(3-4):253-61. PubMed PMID: 22280831. Epub 01/24. eng.
312. Kim M-S, Kim J-K, Kim H-J, Moon S-R, Shin B-C, Park K-W, et al. Hibiscus extract inhibits the lipid droplet accumulation and adipogenic transcription factors expression of 3T3-L1 preadipocytes. J Altern Complement Med. 2003;9(4):499-504. PubMed PMID: 14499025. eng.
313. Ezzat SM, Salama MM, Seif El-Din SH, Saleh S, El-Lakkany NM, Hammam OA, et al. Metabolic profile and hepatoprotective activity of the anthocyanin-rich extract of Hibiscus sabdariffa calyces. Pharm Biol. 2016;54(12):3172-81. PubMed PMID: 27564372. Epub 08/26. eng.
314. Parodi TV, Cunha MA, Becker AG, Zeppenfeld CC, Martins DI, Koakoski G, et al. Anesthetic activity of the essential oil of Aloysia triphylla and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, Rhamdia quelen. Fish Physiol Biochem. 2014;40(2):323-34. PubMed PMID: 23974669. Epub 08/22. eng.
315. Parodi TV, Vargas APdC, Krewer C, Flores ÉMdM, Baldisserotto B, Heinzmann BM, et al. Chemical composition and antibacterial activity of Aloysia triphylla (L'Hérit) Britton extracts obtained by pressurized CO2 extraction. Brazilian Archives of Biology and Technology. 2013;56(2):283-92.
316. Santos-Gomes PC, Fernandes-Ferreira M, Vicente AM. Composition of the essential oils from flowers and leaves of vervain [Aloysia triphylla (L'Herit.) Britton] grown in Portugal. Journal of Essential Oil Research. 2005;17(1):73-8.
317. Pascual ME, Slowing K, Carretero E, Sánchez Mata D, Villar A. Lippia: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol. 2001;76(3):201-14. PubMed PMID: 11448540. eng.
318. Argueta L.A. RCM. Atlas de las plantas de la Medicina Tradicional Mexicana: Instituto Nacional Indigenista, México; 1994.
319. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol. 2005;97(2):305-11. PubMed PMID: 15707770. Epub 01/05. eng.
320. Di Leo Lira P, van Baren CM, López S, Molina A, Heit C, Viturro C, et al. Northwestern Argentina: a center of genetic diversity of lemon verbena (Aloysia citriodora PALÁU, Verbenaceae). Chemistry & biodiversity. 2013 Feb;10(2):251-61. PubMed PMID: 23418172. Epub 2013/02/19. eng.
321. Sánchez-Marzo N, Lozano-Sánchez J, Cádiz-Gurrea MdlL, Herranz-López M, Micol V, Segura-Carretero A. Relationships Between Chemical Structure and Antioxidant Activity of Isolated Phytocompounds from Lemon Verbena. Antioxidants (Basel). 2019;8(8):324. PubMed PMID: 31434276. eng.
322. Bilia AR, Giomi M, Innocenti M, Gallori S, Vincieri FF. HPLC-DAD-ESI-MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. J Pharm Biomed Anal. 2008;46(3):463-70. PubMed PMID: 18155378. Epub 11/17. eng.
323. Quirantes-Piné R, Herranz-López M, Funes L, Borrás-Linares I, Micol V, Segura-Carretero A, et al. Phenylpropanoids and their metabolites are the major compounds responsible for blood-cell protection against oxidative stress after administration of Lippia citriodora in rats.

Phytomedicine. 2013;20(12):1112-8. PubMed PMID: 23827667. Epub 07/01. eng.
324. Portmann E, Nigro MML, Reides CG, Llesuy S, Ricco RA, Wagner ML, et al. Aqueous extracts of Lippia turbinata and Aloysia citriodora (Verbenaceae): assessment of antioxidant capacity and DNA damage. Int J Toxicol. 2012;31(2):192-202. PubMed PMID: 22427199. Epub 03/16. eng.
325. Boix-Castejón M, Herranz-López M, Pérez Gago A, Olivares-Vicente M, Caturla N, Roche E, et al. Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial. Food & function. 2018;9(6):3173-84. PubMed PMID: 29862395. eng.
326. Ciulu M, Cádiz-Gurrea MdlL, Segura-Carretero A. Extraction and Analysis of Phenolic Compounds in Rice: A Review. Molecules. 2018;23(11):2890. PubMed PMID: 30404149. eng.
327. Ejtahed H-S, Tito RY, Siadat S-D, Hasani-Ranjbar S, Hoseini-Tavassol Z, Rymenans L, et al. Metformin induces weight loss associated with gut microbiota alteration in non-diabetic obese women: a randomized double-blind clinical trial. Eur J Endocrinol. 2018:EJE-18-0826.R1. PubMed PMID: 30540558. eng.
328. Herranz-López M, Barrajón-Catalán E, Segura-Carretero A, Menéndez JA, Joven J, Micol V. Lemon verbena (Lippia citriodora) polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms. Phytomedicine. 2015;22(6):605-14.
329. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World J Gastroenterol. 2014;20(43):16079-94. PubMed PMID: 25473159. eng.
330. Gurrola-Díaz CM, García-López PM, Sánchez-Enríquez S, Troyo-Sanromán R, Andrade-González I, Gómez-Leyva JF. Effects of Hibiscus sabdariffa extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). Phytomedicine. 2010;17(7):500-5. PubMed PMID: 19962289. Epub 12/03. eng.
331. Peng C-H, Chyau C-C, Chan K-C, Chan T-H, Wang C-J, Huang C-N. Hibiscus sabdariffa polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. J Agric Food Chem. 2011;59(18):9901-9. PubMed PMID: 21870884. Epub 09/06. eng.
332. Liu M-J, Li J-X, Guo H-Z, Lee K-M, Qin L, Chan K-M. The effects of verbascoside on plasma lipid peroxidation level and erythrocyte membrane fluidity during immobilization in rabbits: a time course study. Life Sci. 2003;73(7):883-92. PubMed PMID: 12798414. eng.
333. Wong IY, He ZD, Huang Y, Chen ZY. Antioxidative activities of phenylethanoid glycosides from Ligustrum purpurascens. J Agric Food Chem. 2001;49(6):3113-9. PubMed PMID: 11410017. eng.
334. Avila JG, de Liverant JG, Martínez A, Martínez G, Muñoz JL, Arciniegas A, et al. Mode of action of Buddleja cordata verbascoside against Staphylococcus aureus. J Ethnopharmacol. 1999;66(1):75-8. PubMed PMID: 10432210. eng.
335. Ohno T, Inoue M, Ogihara Y, Saracoglu I. Antimetastatic activity of acteoside, a phenylethanoid glycoside. Biol Pharm Bull. 2002;25(5):666-8. PubMed PMID: 12033512. eng.
336. Cassidy A, Minihane A-M. The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr. 2017;105(1):10-22. PubMed PMID: 27881391. Epub 11/23. eng.
337. Sireswar S, Dey G. Matrix-wise evaluation of in vivo and in vitro efficiencies of L. rhamnosus GG-fortified beverages. Food research international (Ottawa, Ont). 2019;119:908-19. PubMed PMID: 30884731. Epub 10/28. eng.
338. Leyva-Jiménez FJ, Lozano-Sánchez J, Borrás-Linares I, Arráez-Román D, Segura-Carretero A. Comparative study of conventional and pressurized liquid extraction for recovering bioactive compounds from Lippia citriodora leaves. Food research international (Ottawa, Ont). 2018;109:213-22. PubMed PMID: 29803444. Epub 04/17. eng.
339. Rodríguez-Nogales A, Algieri F, Garrido-Mesa J, Vezza T, Utrilla MP, Chueca N, et al. Differential intestinal anti-inflammatory effects of Lactobacillus fermentum and Lactobacillus salivarius in DSS mouse colitis: impact on microRNAs expression and microbiota composition. Mol Nutr Food Res. 2017;61(11):10.1002/mnfr.201700144. PubMed PMID: 28752563. Epub 08/29. eng.
340. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, et al. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics. 2008;9:386-. PubMed PMID: 18803844. eng.
341. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7. PubMed PMID: 17586664. Epub 06/22. eng.
342. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 2014;30(21):3123-4. PubMed PMID: 25061070. Epub 07/23. eng.
343. Zarzuelo MJ, Jiménez R, Galindo P, Sánchez M, Nieto A, Romero M, et al. Antihypertensive effects of peroxisome

proliferator-activated receptor-β activation in spontaneously hypertensive rats. Hypertension. 2011 Oct;58(4):733-43. PubMed PMID: 21825230. Epub 2011/08/10. eng.
344. Huang TW, Chang CL, Kao ES, Lin JH. Effect of Hibiscus sabdariffa extract on high fat diet-induced obesity and liver damage in hamsters. Food & nutrition research. 2015;59:29018. PubMed PMID: 26475512. Pubmed Central PMCID: PMC4608971. Epub 2015/10/18. eng.
345. Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK. Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. Endocrinology. 2006 Nov;147(11):5340-51. PubMed PMID: 16873530. Epub 2006/07/29. eng.
346. Herranz-López M, Olivares-Vicente M, Encinar JA, Barrajón-Catalán E, Segura-Carretero A, Joven J, et al. Multi-Targeted Molecular Effects of Hibiscus sabdariffa Polyphenols: An Opportunity for a Global Approach to Obesity. Nutrients. 2017 Aug 20;9(8). PubMed PMID: 28825642. Pubmed Central PMCID: PMC5579700. Epub 2017/08/22. eng.
347. Fernández-Arroyo S, Herranz-López M, Beltrán-Debón R, Borrás-Linares I, Barrajón-Catalán E, Joven J, et al. Bioavailability study of a polyphenol-enriched extract from Hibiscus sabdariffa in rats and associated antioxidant status. Mol Nutr Food Res. 2012 Oct;56(10):1590-5. PubMed PMID: 22893520. Epub 2012/08/16. eng.
348. Xue B, Kahn BB. AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. The Journal of physiology. 2006 Jul 1;574(Pt 1):73-83. PubMed PMID: 16709629. Pubmed Central PMCID: PMC1817809. Epub 2006/05/20. eng.
349. Bijland S, Mancini SJ, Salt IP. Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation. Clinical science (London, England : 1979). 2013 Apr;124(8):491-507. PubMed PMID: 23298225. Epub 2013/01/10. eng.
350. Carling D, Thornton C, Woods A, Sanders MJ. AMP-activated protein kinase: new regulation, new roles? The Biochemical journal. 2012 Jul 1;445(1):11-27. PubMed PMID: 22702974. Epub 2012/06/19. eng.
351. Yang Z, Kahn BB, Shi H, Xue BZ. Macrophage alpha1 AMP-activated protein kinase (alpha1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. The Journal of biological chemistry. 2010 Jun 18;285(25):19051-9. PubMed PMID: 20421294. Pubmed Central PMCID: PMC2885183. Epub 2010/04/28. eng.
352. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-85. PubMed PMID: 28776086. Pubmed Central PMCID: PMC5552828. Epub 2017/08/05. eng.
353. Acedo SC, Gambero S, Cunha FG, Lorand-Metze I, Gambero A. Participation of leptin in the determination of the macrophage phenotype: an additional role in adipocyte and macrophage crosstalk. In vitro cellular & developmental biology Animal. 2013 Jun;49(6):473-8. PubMed PMID: 23708919. Epub 2013/05/28. eng.
354. Gavito AL, Bautista D, Suarez J, Badran S, Arco R, Pavón FJ, et al. Chronic IL-6 Administration Desensitizes IL-6 Response in Liver, Causes Hyperleptinemia and Aggravates Steatosis in Diet-Induced-Obese Mice. PloS one. 2016;11(6):e0157956. PubMed PMID: 27333268. Pubmed Central PMCID: PMC4917096. Epub 2016/06/23. eng.
355. Al-Qahtani SM, Bryzgalova G, Valladolid-Acebes I, Korach-André M, Dahlman-Wright K, Efendić S, et al. 17β-Estradiol suppresses visceral adipogenesis and activates brown adipose tissue-specific gene expression. Hormone molecular biology and clinical investigation. 2017 Jan 1;29(1):13-26. PubMed PMID: 27831918. Epub 2016/11/11. eng.
356. Shepherd PR, Kahn BB. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. N Engl J Med. 1999 Jul 22;341(4):248-57. PubMed PMID: 10413738. Epub 1999/07/22. eng.
357. Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Molecular aspects of medicine. 2013 Feb;34(1):39-58. PubMed PMID: 23159341. Epub 2012/11/20. eng.
358. Bergstrom KS, Guttman JA, Rumi M, Ma C, Bouzari S, Khan MA, et al. Modulation of intestinal goblet cell function during infection by an attaching and effacing bacterial pathogen. Infection and immunity. 2008 Feb;76(2):796-811. PubMed PMID: 17984203. Pubmed Central PMCID: PMC2223480. Epub 2007/11/07. eng.
359. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cellular and molecular life sciences : CMLS. 2013 Feb;70(4):631-59. PubMed PMID: 22782113. Epub 2012/07/12. eng.
360. Vallianou NG, Stratigou T, Tsagarakis S. Microbiome and diabetes: Where are we now? Diabetes research and clinical practice. 2018 Dec;146:111-8. PubMed PMID: 30342053. Epub 2018/10/21. eng.
361. Blandino G, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. Diabetes & metabolism. 2016 Nov;42(5):303-15. PubMed PMID: 27179626. Epub 2016/05/18. eng.
362. Lu X, Liu J, Zhang N, Fu Y, Zhang Z, Li Y, et al. Ripened Pu-erh Tea Extract Protects Mice from Obesity by Modulating Gut Microbiota

Composition. J Agric Food Chem. 2019 Jun 26;67(25):6978-94. PubMed PMID: 31070363. Epub 2019/05/10. eng.
363. Rastmanesh R. High polyphenol, low probiotic diet for weight loss because of intestinal microbiota interaction. Chemico-biological interactions. 2011 Jan 15;189(1-2):1-8. PubMed PMID: 20955691. Epub 2010/10/20. eng.
364. Choque Delgado GT, Tamashiro W. Role of prebiotics in regulation of microbiota and prevention of obesity. Food research international (Ottawa, Ont). 2018 Nov;113:183-8. PubMed PMID: 30195512. Epub 2018/09/10. eng.
365. Kim B, Feldman EL. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Experimental & molecular medicine. 2015 Mar 13;47(3):e149. PubMed PMID: 25766618. Pubmed Central PMCID: PMC4351418. Epub 2015/03/15. eng.
366. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: Review of the underlying molecular mechanisms. Journal of cellular physiology. 2019 Jun;234(6):8152-61. PubMed PMID: 30317615. Epub 2018/10/15. eng.
367. Boi SK, Buchta CM, Pearson NA, Francis MB, Meyerholz DK, Grobe JL, et al. Obesity alters immune and metabolic profiles: New insight from obese-resistant mice on high-fat diet. Obesity (Silver Spring). 2016 Oct;24(10):2140-9. PubMed PMID: 27515998. Pubmed Central PMCID: PMC5039085. Epub 2016/08/16. eng.
368. Yan ZK, Yang ZJ, Chen F. [Effect of electroacupuncture stimulation of "Housanli" (ST 36) and "Zhongwan" (CV 12) on serum leptin and hepatocellular JAK 2-STAT 3 signaling in obese rats]. Zhen ci yan jiu = Acupuncture research. 2015 Feb;40(1):1-5. PubMed PMID: 25845212. Epub 2015/04/08. chi.
369. Lyons CL, Roche HM. Nutritional Modulation of AMPK-Impact upon Metabolic-Inflammation. Int J Mol Sci. 2018 Oct 9;19(10). PubMed PMID: 30304866. Pubmed Central PMCID: PMC6213547. Epub 2018/10/12. eng.
370. Genser L, Aguanno D, Soula HA, Dong L, Trystram L, Assmann K, et al. Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes. The Journal of pathology. 2018 Oct;246(2):217-30. PubMed PMID: 29984492. Epub 2018/07/10. eng.
371. Cani PD. Crosstalk between the gut microbiota and the endocannabinoid system: impact on the gut barrier function and the adipose tissue. Clin Microbiol Infect. 2012;18 Suppl 4:50-3. PubMed PMID: 22647050. eng.
372. Vezza T, Rodríguez-Nogales A, Algieri F, Garrido-Mesa J, Romero M, Sánchez M, et al. The metabolic and vascular protective effects of olive (Olea europaea L.) leaf extract in diet-induced obesity in mice are related to the amelioration of gut microbiota dysbiosis and to its immunomodulatory properties. Pharmacological research. 2019 Dec;150:104487. PubMed PMID: 31610229. Epub 2019/10/15. eng.
373. Anhê FF, Varin TV, Le Barz M, Desjardins Y, Levy E, Roy D, et al. Gut Microbiota Dysbiosis in Obesity-Linked Metabolic Diseases and Prebiotic Potential of Polyphenol-Rich Extracts. Current obesity reports. 2015 Dec;4(4):389-400. PubMed PMID: 26343880. Epub 2015/09/08. eng.
374. Rao R. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. Hepatology (Baltimore, Md). 2009 Aug;50(2):638-44. PubMed PMID: 19575462. Pubmed Central PMCID: PMC6209509. Epub 2009/07/04. eng.
375. Porras D, Nistal E, Martínez-Flórez S, González-Gallego J, García-Mediavilla MV, Sánchez-Campos S. Intestinal Microbiota Modulation in Obesity-Related Non-alcoholic Fatty Liver Disease. Front Physiol. 2018;9:1813. PubMed PMID: 30618824. Pubmed Central PMCID: PMC6305464. Epub 2019/01/09. eng.
376. Bouter KE, van Raalte DH, Groen AK, Nieuwdorp M. Role of the Gut Microbiome in the Pathogenesis of Obesity and Obesity-Related Metabolic Dysfunction. Gastroenterology. 2017 May;152(7):1671-8. PubMed PMID: 28192102. Epub 2017/02/14. eng.
377. Derrien M, Vaughan EE, Plugge CM, de Vos WM. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. International journal of systematic and evolutionary microbiology. 2004 Sep;54(Pt 5):1469-76. PubMed PMID: 15388697. Epub 2004/09/25. eng.
378. Díaz-de-Cerio E, Rodríguez-Nogales A, Algieri F, Romero M, Verardo V, Segura-Carretero A, et al. The hypoglycemic effects of guava leaf (Psidium guajava L.) extract are associated with improving endothelial dysfunction in mice with diet-induced obesity. Food research international (Ottawa, Ont). 2017 Jun;96:64-71. PubMed PMID: 28528109. Epub 2017/05/22. eng.
379. Kobayasi R, Akamine EH, Davel AP, Rodrigues MA, Carvalho CR, Rossoni LV. Oxidative stress and inflammatory mediators contribute to endothelial dysfunction in high-fat diet-induced obesity in mice. Journal of hypertension. 2010 Oct;28(10):2111-9. PubMed PMID: 20616756. Epub 2010/07/10. eng.

ABBREVIATIONS

Ach	Acetylcholine
AMPK	Amp activated protein kinase
AUC	Area under the curve
BAT	Brown adipose tissue
BMI	Body mass index
C/EBP	CCAAT-enhancer-binding protein
CETP	Cholesterol ester transport protein
CVD	Cardiovascular diseases
EGCG	(-)-epigallocatechin-3-gallate
eNOS	Endothelium Nitric oxide synthase
ET-1	Endothelin-1
F/B	*Firmicutes*/*Bacteroidetes* ratio
FAS	Fatty acid synthase
FDA	Food and drug administration
FFA	Free fatty acid
GA	Gallic acid
GLP-1	Glucagon like-.peptide 1
Glut	Glucose transporter type
HDL	High-density lipoprotein
HFD	High-fat diet
HOMA-IR	Homeostatic model assessment for insulin resistance
HSE	*Hibiscus sabdariffa* extract
IKKB	Inhibitor of nuclear factor κB Kinase subunit beta
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IR	Insulin receptor
IRS-1	Insulin receptor substrate-1
JNK	c-jun N-terminal kinase
LCE	*Lippia citriodora* extract
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
M1	Classically activated macrophages

M2	Alternatively activated macrophages
MAPK	Mitogen activated protein kinase
MCP	Monocyte chemoattractant protein
MetS	Metabolic syndrome
MS	HPLC-ESI-QTOF-MS
MUC	Mucin
NFκB	Nuclear factor κB
NO	Nitric oxide
PcoA	Principal component analysis
PI3K	Phosphoinositide 3-kinase
PPAR	Peroxisome proliferator activated receptors
ROS	Reactive oxygen species
SCFA	Short-chain fatty acid
SREBP	Sterol regulatory element-binding protein
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol
TFF	Trefoil factor
TG	Triglyceride
TLR	Toll-like receptor
TNF	Tumour necrosis factor
VLDL	Very low-density lipoprotein
WAT	Withe adipose tissue
ZO	Zonula occludens

ANNEX

PUBLICATIONS

Diez-Echave, P., Vezza, T., Rodríguez-Nogales, A., Ruiz-Malagón, A. J., Hidalgo-García, L., Garrido-Mesa, J., Molina-Tijeras, J. A., Romero, M., Robles-Vera, I., Pimentel-Moral, S., Borras-Linares, I., Arráez-Román, D., Segura-Carretero, A., Micol, V., García, F., Duarte, J., Rodríguez-Cabezas, M. E., & Gálvez, J. (2020). The prebiotic properties of Hibiscus sabdariffa extract contribute to the beneficial effects in diet-induced obesity in mice. *Food research international (Ottawa, Ont.)*, *127*, 108722. https://doi.org/10.1016/j.foodres.2019.108722.

Pacheco, M. T., , Vezza, T., , **Diez-Echave, P**., , Utrilla, P., , Villamiel, M., , & Moreno, F. J., (2018). Anti-inflammatory bowel effect of industrial orange by-products in DSS-treated mice. *Food & function*, *9*(9), 4888–4896. https://doi.org/10.1039/c8fo01060a.

CONTRIBUCIONES A CONGRESOS

Vezza T; Molina-Tijeras JA; Ruiz-Malagón AJ; Hidalgo-García L; **Díez-Echave P**; Rodríguez-Sojo MJ; Rodríguez-Nogales A; Romero M; Robles Vera I; Martín-García B; Gómez-Caravaca AM; Arráez-Román D; Segura-Carretero A; Duarte J; Rodríguez-Cabezas ME; Gálvez J. Efecto antiinflamatorio de un extracto de hoja de olivo en un modelo de trasplante de microbiota fecal (FMT) en ratones obesos. Tipo de participación: Póster. Congreso: XI Workshop Sociedad Española de Microbiota, Probióticos y Prebióticos (SEMiPyP 2020) Lugar de celebración: Granada (España). Fecha: 12-14/02/2020.

Rodríguez-Sojo MJ; **Díez-Echave P**; Garrido-Mesa J; Vezza T; Rodríguez-Nogales A; Algieri F; Rodríguez-Nogales A; Rodríguez E; Langa S; Arques JL; Rodríguez-Cabezas ME; Gálvez J. Efecto inmunomodulador del *Lactobacillus paracasei* INIAp272 en colitis inducida por DSS en ratones. Tipo de participación: Comunicación oral. Congreso: XI Workshop Sociedad Española de Microbiota, Probióticos y Prebióticos (SEMiPyP 2020). Lugar de celebración: Granada (España). Fecha: 12-14/02/2020.

Molina-Tijeras JA; Vezza T; **Díez-Echave P**; Hidalgo-García L; Ruiz-Malagón AJ; Rodríguez-Sojo MJ; Bañuelos O; Olivares M; Rodríguez-Cabezas ME; Gálvez J. Impacto del probiótico *Lactobacillus fermentum* CECT5716 sobre el proceso inflamatorio asociado a la obesidad experimental en ratones. Tipo de participación: Comunicación oral. Congreso: XI Workshop Sociedad Española de Microbiota, Probióticos y Prebióticos (SEMiPyP 2020). Lugar de celebración: Granada (España). Fecha: 12-14/02/2020.

Ruiz-Malagón AJ; Vezza T; Hidalgo-García L; **Díez-Echave P**; Molina-Tijeras JA; Rodríguez-Sojo MJ; Rodríguez-Cabezas ME; Marchal JA; Gálvez J. La microbiota de ratones obesos incrementa el proceso tumoral e inflamatorio en cáncer colorrectal. Tipo de participación: Póster. Congreso: XI Workshop Sociedad Española de Microbiota, Probióticos y Prebióticos (SEMiPyP 2020). Lugar de celebración: Granada (España). Fecha: 12-14/02/2020.

Hidalgo-García L; Molina-Tijeras JA; Vezza T; **Diez-Echave P**; Ruiz-Malagón AJ; Rodríguez-Sojo MJ; Olivares M; Huertas F; Anderson P; Anderson P; Rodríguez-Cabezas ME. Analysis of mesenchymal stromal cells immunomodulation by different TLRs agonists and the probiotic *Lactobacillus fermentum* CECT5716. Tipo de participación: Comunicación oral. Congreso: XI Reunión de Jóvenes Farmacólogos de Andalucía. Lugar de celebración: Málaga (España). Fecha: 31/05/2019.

Rodríguez-Sojo MJ; Vezza T; **Diez-Echave P**; Hidalgo-García L; Ruiz-Malagón AJ; Molina-Tijeras JA; Rodríguez-Cabezas ME; Gálvez J. Beneficial effects of agomelatine on liver inflammation in obese mice. Tipo de participación: Comunicación oral. Congreso: XI Reunión de Jóvenes Farmacólogos de Andalucía. Lugar de celebración: Málaga (España). Fecha: 31/05/2019.

Ruiz-Malagón AJ; Vezza T; **Diez-Echave P**; Hidalgo-García L; Molina-Tijeras JA; Rodríguez-Sojo MJ; Baños A; Rodríguez-Cabezas ME; Gálvez J. The immunomodulatory properties of PTSO contribute to its beneficial effects in diet-induced obesity in mice. Tipo de participación: Comunicación oral. Congreso: XI Reunión de Jóvenes Farmacólogos de Andalucía. Lugar de celebración: Málaga (España). Fecha: 31/05/2019.

Vezza T; Rodriguez-Nogales A; Algieri F; Garrido-Mesa J; Molina-Tijeras JA; Hidalgo-García L; Rodriguez-Sojo MJ; Ruiz-Malagón A; **Diez-Echave P**; Romero M; Sánchez M; Toral M; García F; Utrilla MP; Duarte J; Rodríguez-Cabezas ME; Gálvez J. The metabolic protective effects of Olive (Olea europaea L.) leaf extract in diet-induced obesity in mice is related to the restoration of gut microbiota dysbiosis and to its immunomodulatory properties. Tipo de participación: Comunicación oral. Congreso: XI Reunión de Jóvenes Farmacólogos de Andalucía. Lugar de celebración: Málaga (España). Fecha: 31/05/2019.

Molina-Tijeras JA; Vezza T; **Diez-Echave P**; Hidalgo-García L; Ruiz-Malagón AJ; Rodríguez-Sojo MJ; Bañuelos O; Olivares M; Rodríguez-Cabezas ME; Gálvez J. The probiotic *Lactobacillus fermentum* CECT5716 shows anti-obesity properties in diet-induced obesity mice. Tipo de participación: Comunicación oral. Congreso: XI Reunión de Jóvenes Farmacólogos de Andalucía. Lugar de celebración: Málaga (España). Fecha: 31/05/2019.

Molina-Tijeras JA; Hidalgo-García L; **Diez-Echave P**; Ruiz-Malagón A; Vezza T; Muñoz-Almagro N; Sabater C; Rodríguez-Cabezas ME; Gálvez J; Utrilla MP. Anti-inflammatory properties of oligosaccharides derived from *Cynara scolymusin* in DSS-induced colitis in mice. Tipo de participación: Póster. Congreso: I Congreso de Investigadores del PTS Lugar de celebración: Granada (España). Fecha: 13-15 /02/2019.

Rodríguez-Sojo MJ; Vezza T; **Diez-Echave P**; Hidalgo-García L; Garrido-Mesa J; Gálvez J. Beneficial effects of aglomelatine on obesity associated liver inflammation in mice. Tipo de participación: Póster. Congreso: I Congreso de Investigadores del PTS. Lugar de celebración: Granada (España). Fecha: 13-15 /02/201.

Diez-Echave P; Vezza T; Hidalgo-García L; Garrido-Mesa J; Pimentel-Moral S; Segura-

Carretero A; Rodríguez-Cabezas ME; Gálvez J. Effect of a *Hibiscus sabdariffa* extract in liver inflammation and intestinal epithelial permeability in obese mice. Tipo de participación: Póster. Congreso: I Congreso de Investigadores del PTS Lugar de celebración: Granada (España). Fecha: 13-15 /02/2019.

Ruiz-Malagón AJ; Molina-Tijeras JA; **Diez-Echave P**; Hidalgo-García L; Vezza T; Rodríguez-Cabezas ME; Lozano-Pérez A; Cenis JL; Gálvez J. Effect of a *Morus alba* leaf extract in an experimental model of obesity: impact on liver steatosis and inflammation. Tipo de participación: Póster. Congreso: I Congreso de Investigadores del PTS. Lugar de celebración: Granada (España). Fecha: 13-15 /02/2019.

Hidalgo-García L; Rodríguez- Cabezas ME; Molina-Tijeras JA; Vezza T; **Diez-Echave P**; Huertas F; Becerra-Massare P; Gálvez J; Anderson P. TNF-α and IFN-γ induce anantiinflammatory phenotype in intestinal mesenchymal stromal cells. Tipo de participación: Póster. Congreso: I Congreso de Investigadores del PTS. Lugar de celebración: Granada (España). Fecha: 13-15/02/2019.

Molina-Tijeras J.A.; Hidalgo-García L.; **Diez-Echave P**.; Ruiz-Malagón A.; Vezza T.; Muñoz-Almagro N.; Sabater C.; Rodríguez-Cabezas ME.; Gálvez J.; Utrilla M.P. Antiinflammatory properties of oligosaccharides derived from *Cynara scolymus*in DSS-induced colitis in mice. Tipo de participación: Póster. Congreso: X Reunión de Jóvenes Farmacólogos. Lugar de celebración: Granada (España). Fecha:3/07/2018.

Diez-Echave P.; Vezza T.; Hidalgo-García L.; Garrido-Mesa J.; Pimentel-Moral S.; Segura-Carretero A.; Rodríguez-Cabezas ME.; Gálvez J. Effect of a *Hibiscus sabdariffa* extract in liver inflammation and intestinal epithelial permeability in obese mice. Tipo de participación: Póster. Congreso: X Reunión de Jóvenes Farmacólogos. Lugar de celebración: Granada (España). Fecha:3/07/2018.

Ruiz-Malagon A.; Molina-Tijeras J.A.; **Diez-Echave P**.; Hidalgo-García L.; Vezza T.; Rodríguez-Cabezas ME.; Cenis J.L.; Gálvez J. Effect of a *Morus alba* leaf extract in experimental model of obesity: impact on liver steatosis and inflammation. Tipo de participación: Póster. Congreso: X Reunión de Jóvenes Farmacólogos. Lugar de celebración: Granada (España). Fecha:3/07/2018.

J.A. Molina-Tijeras, L. Hidalgo-García, **P. Diez-Echave**, A. Ruiz-Malagón, T. Vezza, N. Muñoz-Almagro, C. Sabater, M.E. Rodríguez-Cabezas, J. Gálvez, M.P. Utrilla. Anti-inflammatory properties of oligosaccharides derived from *Cynara scolymus* in DSS-induced colitis in mice. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

M.D. Lorente, **P. Diez-Echave**, T. Vezza, A. Ruiz-Malagón, J.A. Molina-Tijeras, L. Hidalgo-García, M.E. Rodríguez-Cabezas, A. Lozano-Pérez, J.L. Cenis,. J. Gálvez Effects of quercetin loaded silk fibroin nanoparticles in DSS experimental colitis in mice. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-

08/09/2018

M.E. Rodríguez-Cabezas, T. Vezza, **P. Diez-Echave**, L. Hidalgo-García, J. Garrido-Mesa, M.M. Martínez-Lao, J. Gálvez Beneficial effects of agomelatine on obesity associated liver inflammation in mice. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

P. Diez-Echave, T. Vezza, L. Hidalgo-García, J. Garrido-Mesa, S. Pimentel-Moral, A. Segura-Carretero, M.E. Rodríguez-Cabezas, J. Gálvez. Effect of a *Hibiscus sabdariffa* extract in liver inflammation and intestinal epithelial permeability in obese mice. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018

L. Hidalgo-García, J.A. Molina-Tijeras, **P. Diez-Echave**, A. Ruiz-Malagón, A.W. Lopes, S.M. Zucolotto, G. Coelho, M.E. Rodríguez-Cabezas, J. Gálvez. Intestinal anti-inflammatory effects of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* extracts exert intestinal anti-inflammatory effects in experimental colitis. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

A. Ruiz-Malagón, J.A. Molina-Tijeras, **P. Diez-Echave**, L. Hidalgo-García, T. Vezza, M.E. Rodríguez-Cabezas, A. Lozano-Pérez, J.L. Cenis, J. Gálvez. Effect of a *Morus alba* leaf extract in an experimental model of obesity: Impact on liver steatosis and inflammation. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

C. Gálvez-Lorente, J. García-García, **P. Diez-Echave**, T. Vezza, L. Hidalgo-García, J. Garrido-Mesa, F.J. Leyva-Jimenez, A. Segura-Carretero, M.E. Rodríguez-Cabezas, Gálvez J. Effects of *Lippia citriodora* in high-fat diet-fed mice: NAFLD and altered intestinal permeability Tipo de participación: Póster. Gálvez Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

L. Hidalgo-García, M.E. Rodríguez-Cabezas, J.A. Molina-Tijeras, T. Vezza, **P. Diez-Echave**, A.J. Ruiz-Malagón, F. Huertas, P. Becerra-Massare, J. Gálvez, P. Anderson. TNF-α and IFN-γ induce an anti-inflammatory phenotype in intestinal mesenchymal stromal cells. Tipo de participación: Póster. Congreso: Falk Symposium 219 2018. Lugar celebración: Kyoto (Japón) Fecha: 07-08/09/2018.

Garrido-Mesa N., Garrido-Mesa J., Vezza T., Hidalgo-García L., Garrido-Barros M., Rodríguez-Nogales A., **Diez-Echave P**., Algieri F., Utrilla-Navarro M.P., Rodríguez-Cabezas M.E., Gálvez J. Immunomodulatory effect of minocycline in intestinal inflammation. Tipo de participación: Póster. Congreso: Falk Symposium 209 2017. Lugar celebración: Berlín (Alemania) Fecha: 06-07/10/2017.

P. Díez-Echave, J. Garrido-Mesa, T. Vezza, A. Rodríguez-Nogales, F. Algieri, M.P. Utrilla, E. Rodríguez, S. Langa, J.L. Arques, M.E. Rodríguez-Cabezas, J. Gálvez. The immunomodulatory properties of the probiotic *Lactobacillus reuteri* INIA P572 contribute to

its intestinal antiinflammatory effects in DSS-colitis in mice. Tipo de participación: Póster. Congreso: Falk Symposium 209 2017. Lugar celebración: Berlín (Alemania) Fecha: 06-07/10/2017

A. Ruiz-Malagón, **P. Díez-Echave**, J. Garrido-Mesa, T. Vezza,. A. Rodríguez-Nogales, F. Algieri, M.P. Utrilla, E. Rodríguez, S. Langa, J.L. Arques, M.E. Rodríguez-Cabezas, J. Galvez. Immunomodulatory effects of the probiotic *Lactobacillus paracasei* INIA P272 in DSS-colitis: Impact on immune cell populations. Tipo de participación: Póster. Congreso: Falk Symposium 209 2017. Lugar celebración: Berlín (Alemania) Fecha: 06-07/10/2017.

Hidalgo-García, P. Anderson, V. de Araujo Farias, A. Rodríguez-Nogales, F. Algieri, T. Vezza, J. Garrido-Mesa, M.P. Utrilla, F. Huertas, **P. Diez-Echave**, P. Becerra-Massare, M.E. Rodriguez-Cabezas, J. Galvez. Characterization of intestinal stromal stem cells for its future use in the treatment of inflammatory bowel disease. Tipo de participación: Póster. Congreso: Falk Symposium 209 2017. Lugar celebración: Berlin (Alemania) Fecha: 06-07/10/2017.

Diez-Echave P., Vezza T, Algieri F., Rodríguez-Nogales A., Garrido-Mesa J., Toral M., Romero M. , Sánchez M. , Rodríguez-Pérez C., Gómez-Caravaca A.M., Segura- Carretero A., Rodríguez-Cabezas ME., Gálvez J. Titulo: Effects of olive leaf extract in high-fat diet-fed mice. Tipo de participación: Póster. Congreso: 37 CONGRESO SEF 2017. Lugar celebración: Barcelona (España) Fecha: 18-21/06/2017.

Diez-Echave P., Vezza T, Algieri F., Rodríguez-Nogales A., Garrido-Mesa J., Toral M., Romero M., Sánchez M., Rodríguez-Pérez C., Gómez-Caravaca A.M., Segura- Carretero A., Rodríguez-Cabezas ME., Gálvez J. Effects of olive leaf extract in high-fat diet-fed mice: impact on vascular dysfunction. Tipo de participación: Comunicación oral. Congreso: IX Reunión de Jóvenes Farmacólogos. Lugar celebración: Sevilla (España). Fecha: 07/06/2017.

De Montijo, S., **Diez-Echave, P**., Moreno, E., Ruiz-Bravo, A., Jiménez-Valera, M. Modulación de la producción de factor necrosante de tumores (Tnf-α) en cultivos de células RAW por *Lactobacillus plantarum* C4. Tipo de participación: Comunicación oral. Congreso: VII Workshop Probióticos, Prebióticos y Salud: Evidencia Científica. Tipo de participación: Comunicación oral. Lugar celebración: Sevilla (España). Fecha: 28-29/01/2016.

OTROS MÉRITOS

Formación en protección y experimentación animal para la capacitación de las funciones a) cuidado de los animales de experimentación, b) eutanasia y c) realización de experimentos Entidad de titulación: Instituto de Investigación y Tipo de entidad: Organismo Público de Formación Agraria y Pesquera Investigación. Fecha de titulación: 10/07/2017.

Introducción al diseño de nucleótidos para PCR Entidad de titulación: Universidad de Granada . Ciudad entidad titulación: Granada, Andalucía, España Fecha de inicio-fin: 24/04/2018 - 26/04/2018.

Multicolor Panel Building by Flow Cytometry, practical workshop. Entidad de titulación:

Centro de Investigación biomédica (CIBM). Ciudad entidad titulación: Granada, Andalucía, España. Fecha de inicio-fin: 15/03/2018 - 15/03/2018.

Introducción a la estadística en ciencias de la salud. Entidad de titulación: Universidad de Granada. Ciudad entidad titulación: Granada, Andalucía, España. Fecha de inicio-fin: 06/02/2018 - 08/02/2018.

Métodos alternativos en toxicología usando el Pez Cebra. Entidad de titulación: Neuron BIO. Ciudad entidad titulación: Granada, Andalucía, España. Fecha: 19/10/2017 - 20/10/2017.

Scientific research, publishing and ethics. Entidad de titulación: Universidad de Granada. Ciudad entidad titulación: Granada, Andalucía, España. Fecha: 25/09/2017 - 28/09/2017.

Discover wide-ranging approaches and opportunities in Multicolor flow cytometry. Título de la subespecialidad: BD Horizon TM Global Tour Flow Cytometry Seminar. Entidad de titulación: Instituto de Parasitología y Biomedicina López Neyra..Ciudad entidad titulación: Granada, Andalucía, España. Fecha 19/11/2016 - 19/11/2016.

Experimental Genome Science Entidad de titulación: Univesity of Pennsylvania Fecha de finalización: 20/01/2014.

Epidemics: The dynamics of infectious Diseases. Entidad de titulación: The Pennsylvania State University. Fecha de finalización: 10/01/2014.

ESTANCIAS DE INVESTIGACIÓN EN OTROS CENTROS

Movilidad internacional de estudiantes de programas de doctorado Universidad de Granada. Centro: Helmholtz-Zentrum, Munich, Alemania. Supervisor: Siegfried Ussar. Fecha: 15/06/2019 - 15/09/2019 (3 meses).

Departamento de farmacología

Universidad de Granada