Programa de Doctorado en Bioingeniería

Ingredientes basados en polifenoles vegetales con aplicaciones en patologías asociadas a la obesidad

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INFORMAN:

Que D./Dña. *"Marina Boix Castejón"* ha realizado bajo nuestra supervisión el trabajo titulado "**Ingredientes basados en polifenoles vegetales con aplicaciones en patologías asociadas a la obesidad**" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

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Director de la Tesis Doctoral	Codirectora de la Tesis Doctoral
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INFORMA:

Que Dña. *Marina Boix Castejón* ha realizado bajo la supervisión de nuestro Programa de Doctorado el trabajo titulado **"Ingredientes basados en polifenoles vegetales con aplicaciones en patologías asociadas a la obesidad"** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmo para los efectos oportunos, en Elche a ..... de ..... de 202....

Dra. *Piedad de las Nieves de Aza Moya*

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"Si pudiésemos dar a cada individuo la cantidad adecuada de nutrición y ejercicio, ni muy poco ni demasiado, habríamos encontrado el camino más seguro hacia la salud."

Hipócrates

Resumen

RESUMEN

La obesidad clasificada según la Organización Mundial de la Salud (OMS) como una pandemia mundial, está asociada a varios trastornos metabólicos, tales como aumento de la masa de tejido adiposo, resistencia a la insulina, dislipidemia e hipertensión, entre otros. La obesidad presenta como abordaje principal una restricción calórica y un aumento de la actividad física, pero la implementación de estas estrategias conlleva el abandono a largo plazo y una recaída en la recuperación del peso perdido. Ello es debido principalmente, a la lenta movilización de las grasas almacenadas en el tejido adiposo, lo que a largo plazo desmotiva al paciente, recayendo en la ganancia de peso.

La evidencia emergente sugiere el uso de ciertos compuestos derivados de plantas como potencial herramienta terapéutica. Estos compuestos de naturaleza polifenólica actuarían como antioxidantes en base a evidencias previas, siendo el desequilibrio oxidativo un mecanismo subyacente en el desarrollo de la obesidad poco caracterizado. La proteína quinasa activada por AMP parece ejercer un papel regulador en tales efectos a través de su capacidad para modular la homeostasis energética, el gasto energético diario total y el manejo de lípidos. Hasta la fecha, la mayoría de los efectos de los compuestos polifenólicos en el abordaje de la obesidad se han corroborado únicamente en modelos animales hiperlipidémicos demostrando que el consumo continuado de polifenoles prevenía la enfermedad del hígado graso y mejoraba el metabolismo lipídico.

El objetivo fundamental de la presente tesis ha sido evaluar mediante diversos estudios de intervención en voluntarios, si el extracto derivado de la combinación de polifenoles de *Hibiscus sabdariffa* (HS) y *Lippia citriodora* (LC) pueden tener aplicaciones importantes en patologías relacionadas con el estrés metabólico (estrés oxidativo y glucolipotoxicidad), demostrando su mejora en las alteraciones asociadas con la obesidad. El abordaje novedoso de la presente tesis se basa en la realización de estudios poblacionales representativos que intentan trasladar los resultados *in vitro* y en modelos animales al ámbito clínico con pacientes humanos. En este contexto, se ha estudiado la posible modulación de biomarcadores relacionados con el apetito, así como el control de la hipertensión (muy frecuente en personas con sobrepeso y obesidad) mediante una monitorización continua de la tensión arterial en pre-hipertensos e hipertensos de grado I.

Los resultados de la presente tesis confirmaron la eficacia de la combinación polifenólica de HS y LC, demostrado ser coadyuvantes en el abordaje de la obesidad. Los extractos de HS y LC mejoraron los parámetros antropométricos, perfil lipídico, así como un mantenimiento de la pérdida de peso a largo plazo mediante la modulación de los biomarcadores del apetito. Además, se observó una reducción significativa de la presión arterial sistólica y diastólica diaria promedio, así como en la presión arterial diastólica y sistólica diurna, diastólica nocturna y en el porcentaje reductor o "dipper" en hipertensos de grado I. Se puede sugerir que la combinación polifenólica de HS y LC ha mostrado ser un activo potencial en el desarrollo de nuevas estrategias en el tratamiento de los trastornos alimentarios por sobre ingesta y los trastornos metabólicos asociados, proporcionando un nuevo marco de investigación.

ABSTRACT

Obesity classified by the World Health Organization (WHO) as a global pandemic, is associated with several metabolic disorders, such as increased adipose tissue mass, insulin resistance, dyslipidemia and hypertension, among others. In general, obesity treatment presents a combination of approaches based on caloric restriction and an increase in physical activity, but the implementation of these strategies leads to long-term abandonment and a relapse in the recovery of lost weight. This is mainly due to the slow mobilization of fats stored in adipose tissue, which in the long term demotivates the patient, coming back into weight gain.

Emerging evidence suggests *the use of* potential plant-derived compounds with therapeutic properties. These compounds of polyphenolic nature would *function* as antioxidants based on previous evidence, being oxidative stress an underlying mechanism in the development of obesity poorly characterized. AMP-activated protein kinase appears to exert a regulatory role in such effects through its ability to modulate energy homeostasis, total daily energy expenditure, and lipid management. To date, most of the effects of polyphenolic compounds in the approach to obesity have been corroborated only in hyperlipidemic animal models demonstrating that continued consumption of polyphenols prevented fatty liver disease and improved lipid metabolism.

The main objective of this thesis has been to evaluate through various intervention studies in volunteers, if the extract derived from the combination of polyphenols of Hibiscus sabdariffa (HS) and Lippia citriodora (LC) can have key applications in pathologies related to metabolic stress (oxidative stress and glucolipotoxicity), demonstrating improvement in the alterations associated with obesity. The novel approach of this thesis is based on the realization of representative population studies that try to transfer the results in vitro and in animal models to the clinical setting with human patients. In this context, the possible modulation of biomarkers related to appetite has been studied, as well as the control of hypertension (*quite* common in overweight and obese people) through continuous monitoring of blood pressure in pre-hypertensive and grade I hypertensive patients.

The results of this thesis confirmed the effectiveness of the combination polyphenols from HS and LC, working as coadjuvants in the approach to obesity. HS and LC extracts improved anthropometric parameters, lipid profile, as well as a maintenance of long-term

weight loss by modulating appetite biomarkers. In addition, a significant reduction in mean daily systolic/diastolic blood pressure was observed, as well as in diastolic/systolic daytime, diastolic nocturnal and dipper blood pressure in grade I hypertensive patients. It can be suggested that the polyphenolic combination of HS and LC present a potential in the development of new strategies for the treatment of overeating disorders and associated metabolic disorders, providing a new framework for research.

Abreviaturas

ABREVIATURAS

AAMI, Association for the Advancement of Medical Instrumentation

AMPK, Proteína quinasa activada por AMP

BHS, British Hypertension Society

CCK, Colecistoquinina

CRH, Hormona liberadora de corticotropina

ECV, Enfermedades cardiovasculares

ENT, Enfermedades no transmisibles

ESH, European Society of Hypertension

GLP-1, Péptido análogo del glucagón tipo 1

HS, *Hibiscus sabdariffa*

HTA, Hipertensión arterial

IMC, Índice de Masa Corporal

KS, Kolmogorov-Smirnov

LC, *Lippia citriodora*

MAPA, Monitoreo ambulatorio de presión arterial

MCH, Hormona concentradora de melanina

NPY, Neuropéptido Y

OMS, Organización Mundial de la Salud

OXM, Oxintomodulina

PA, Presión Arterial

PAC, Presión Aórtica Central

PAM, Presión Arterial Media

PD, Presión diastólica

PP, Presión de Pulso

PS, Presión sistólica

PYY, Péptido Tirosina-Tirosina

SNC, Sistema Nervioso Central

GI, Tracto gastrointestinal

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ÍNDICE

Introducción General

1. OBESIDAD Y SOBREPESO

1.1 Obesidad y Sobrepeso. La epidemia del siglo XXI

La prevalencia de la obesidad está aumentando en todo el mundo. Según la Organización Mundial de la Salud (OMS) la obesidad casi se ha triplicado desde 1975. El 39% de los adultos mayores de 18 años tenían sobrepeso en 2016 y el 13% eran obesos [1].

Según las estimaciones en Europa, en el año 2022 el sobrepeso y la obesidad afectaba casi al 60% de los adultos. Con un continuo incremento de la prevalecía, la obesidad representa una de las mayores amenazas para la salud de nuestra sociedad. Según las últimas estimaciones, únicamente en la Región Europea se sugiere que el sobrepeso y la obesidad causan más de 1,2 millones de muertes cada año, siendo la cuarta causa más importante después de hipertensión, diabetes y consumo de tabaco [2].

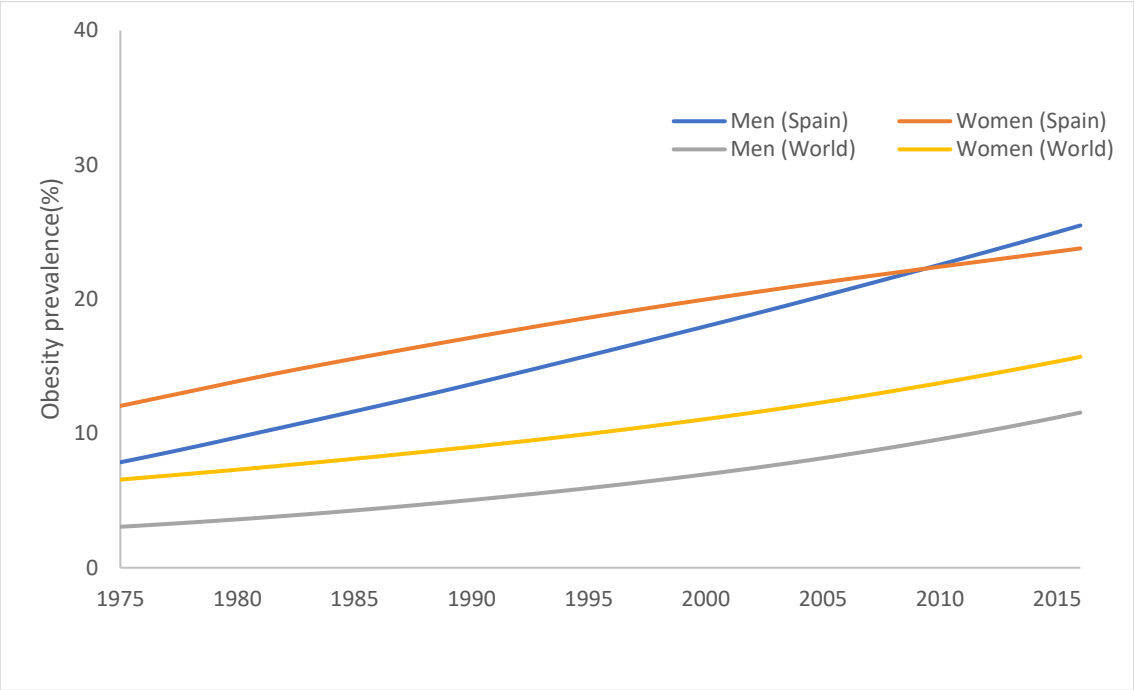

Figura 1: Comparativa de la tendencia de la prevalencia de obesidad desde el año 1975 hasta el año 2015 entre España y a nivel mundial. En el año 2015, el porcentaje de hombres obesos representó el 25.5% en España y el 11.6% a nivel mundial, mientras que en mujeres obesas el porcentaje representó el 23.8% en España y el 15.7% a nivel mundial. Adaptación de los datos publicados por la NCD-RisC a partir de una población de participantes adultos desde 1975 hasta 2015 [3].

La obesidad se asocia con una gran disminución de la esperanza de vida y se ha convertido en un importante problema de salud mundial. La NCD *Risk Factor Collaboration* (NCD-RisC), una red de investigadores y profesionales de la salud de todo el mundo coordinada por el Centro Colaborador de la OMS en Vigilancia de las enfermedades no transmisibles (ENT) y Epidemiología en el Imperial College de Londres, manifiesta que la prevalencia de la obesidad ha aumentado de forma notoria desde 1975 a 2016 (Fig. 1) [4].

Según la OMS, el Índice de Masa Corporal (IMC) es el indicador más usado para identificar el sobrepeso y/o la obesidad en adultos. Se calcula dividiendo el peso de una persona en kilogramos por el cuadrado de su talla en metros (kg/m^2). Según esta definición, la OMS clasifica a los adultos con sobrepeso cuando el IMC es igual o superior a 25 kg/m^2 y a los adultos con obesidad cuando el IMC es igual o superior a 30 kg/m^2 (Fig. 2). El IMC es el método más generalizado para estimar el estado nutricional de la población, pero debe considerarse como valor aproximado ya que es una variable que puede diferir de gran manera según el adulto. Así, puede tener serias limitaciones ya que es un indicador que no distingue entre género, edad o porcentaje de grasa corporal [5].

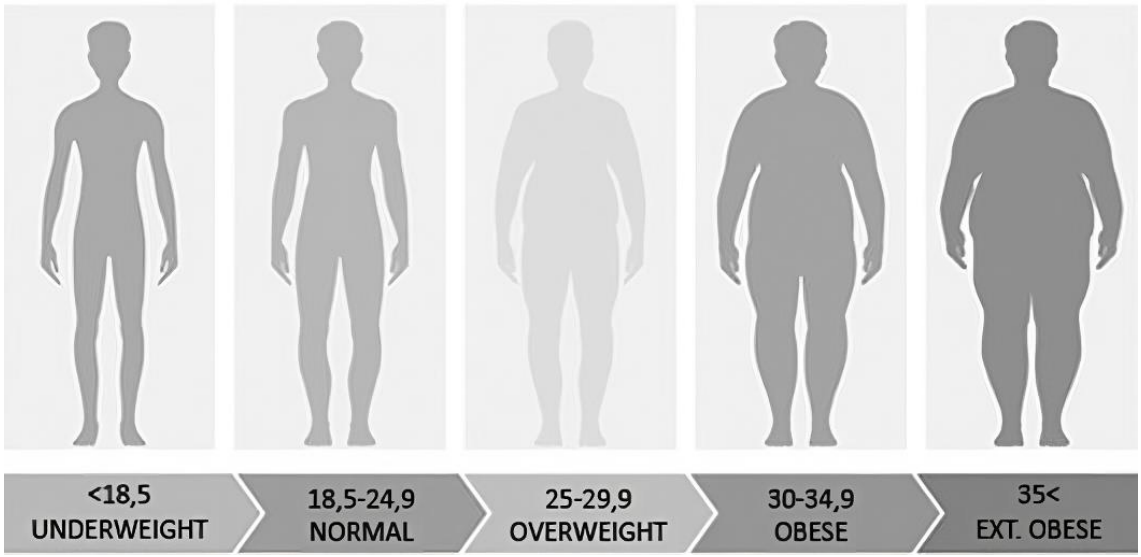

Figura 2: Clasificación según la OMS del estado nutricional de acuerdo con el IMC. Datos obtenidos a partir del informe "Obesity: preventing and managing the global epidemic" publicado en el año 2000 por la OMS [6].

El sobrepeso y la obesidad son el resultado de una acumulación anormal o excesiva de grasa que puede ser perjudicial para la salud debida a la alteración del equilibrio energético entre la ingesta y el gasto energético. Está interrelacionada con una cascada de trastornos

que incluyen hipertensión, resistencia a la insulina, enfermedad renal crónica, aterosclerosis, patologías cardiacas y vasculares. De aquí surge el término síndrome metabólico, cuya prevalencia promedio mundial es del 31% y se caracteriza por ser una condición patológica con un aumento de la masa de tejido adiposo, resistencia a la insulina, hipertensión e hiperlipidemia y se asocia con un incremento del doble de riesgo de enfermedad coronaria [7, 8].

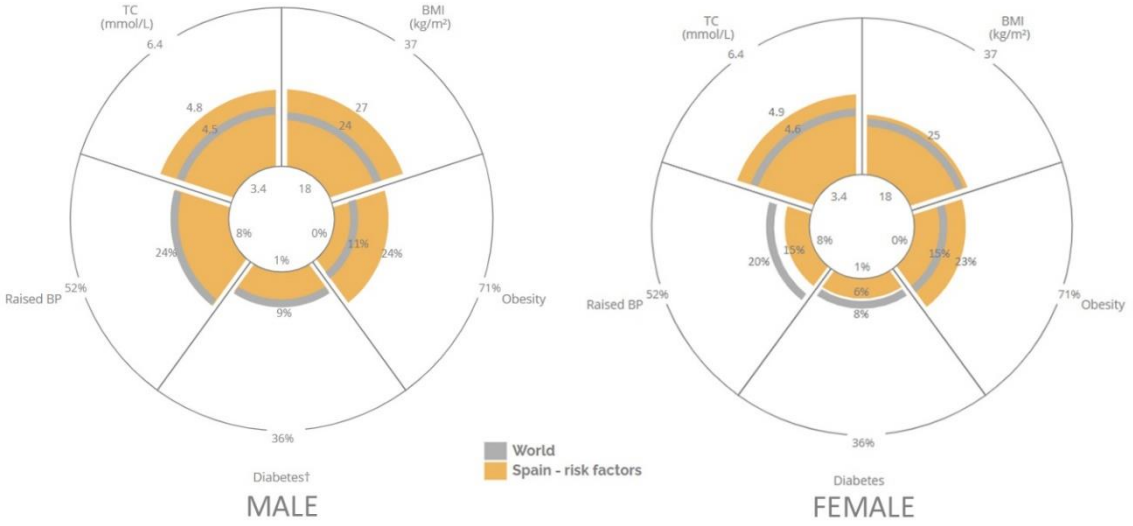

Figura 3: Datos comparativos de diferentes factores de riesgo en adultos: IMC (del inglés BMI), obesidad, diabetes, hipertensión (del inglés, Blood Pressure, BP) y colesterol total (del inglés, Total Cholesterol, TC) en el año 2014 en España y a nivel mundial. Adaptación de los datos publicados por la NCD-RisC a partir de una población de participantes adultos [3].

Tal y como se observa en la figura 3, en el año 2014 el valor medio del IMC en mujeres adultas fue de 25 kg/m^2 tanto en España como a nivel mundial y en hombres adultos de 27 kg/m^2 en España y de 24 kg/m^2 a nivel mundial. Así mismo, según la NCD-RisC, el porcentaje de mujeres adultas con obesidad representó el 23% en España y el 15% a nivel mundial, mientras que en hombres adultos fue el 24% en España y el 11% a nivel mundial.

Entre los factores de riesgo que se destacan en la gráfica de la figura 3, la diabetes representó el 8% en mujeres adultas y el 9% en hombres adultos a nivel mundial. Por otro lado, el porcentaje de mujeres adultas con hipertensión representó el 20% a nivel mundial y en hombres adultos representó el 24% a nivel mundial. Estas cifras ponen de manifiesto la estrecha relación de estas patologías con la obesidad, cuya prevalencia, según la OMS, ha alcanzado proporciones epidémicas en Europa.

1.2 Hipertensión

Las enfermedades cardiovasculares (ECV) son la principal causa de muerte en los países industrializados y están estrechamente relacionadas con la hipertensión arterial (HTA). La HTA es uno de los principales factores de riesgo cardiovascular. Las enfermedades cardiovasculares, la enfermedad coronaria, el accidente cerebrovascular, la insuficiencia cardíaca y la arteriopatía obliterante periférica son la principal causa de muerte en los países industrializados. La correlación entre HTA y riesgo de ECV es muy positiva, no obstante, la coexistencia con otras condiciones patológicas aumenta el riesgo en gran medida [9, 10]. La HTA es un síndrome cardiovascular progresivo que se define por la presencia de una elevación crónica de la presión arterial sistémica por encima de un cierto valor umbral, que surge de una etiología compleja e interrelacionada. Todo ello supone un grave problema de salud pública y un significativo gasto sanitario.

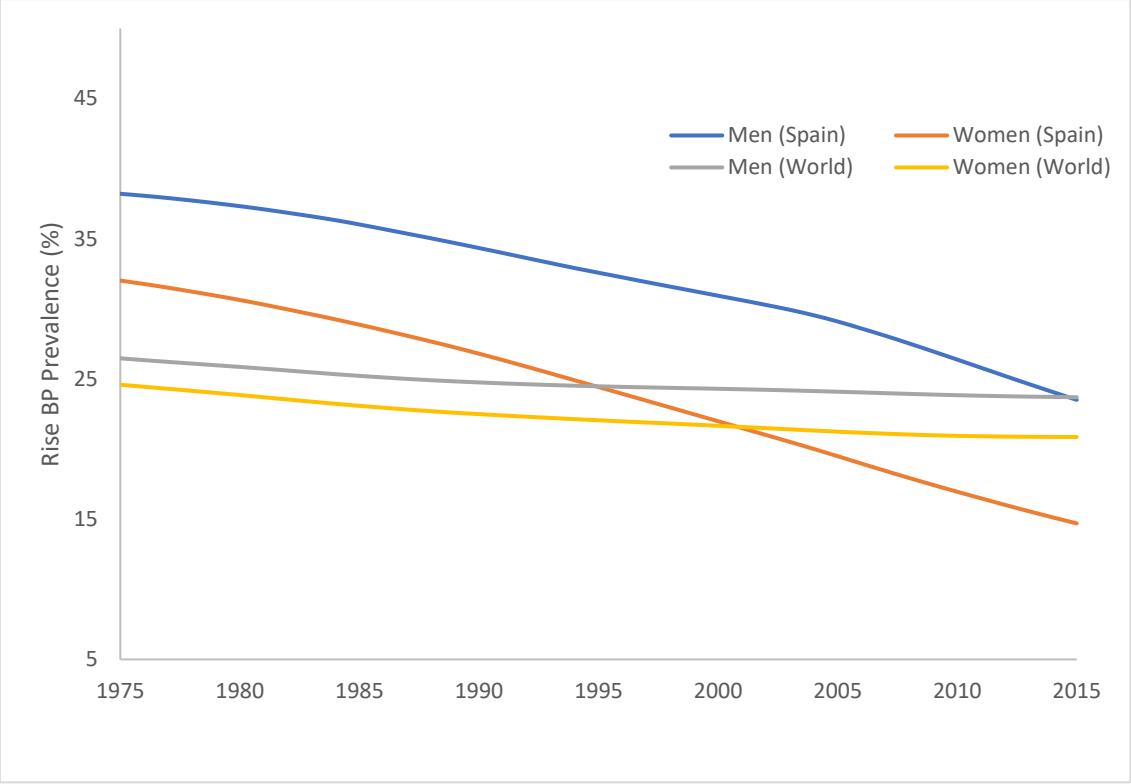

Figura 4: Comparativa de la tendencia de la prevalencia de la hipertensión desde el año 1975 hasta el año 2015 entre España y a nivel mundial. Adaptación de los datos publicados por la NCD-RisC a partir de una población de participantes adultos desde 1975 hasta 2015.

Según los datos proporcionados por la NCD-RisC sobre los factores de riesgo para las ENT, en el año 2015 el porcentaje de hombres hipertensos representó el 23.5 % en España y el 24.1% a nivel mundial, mientras que en mujeres hipertensas el porcentaje representó el 14.7 % en España y el 20.1 % a nivel mundial, mostrando una tendencia a la disminución de la prevalencia de la hipertensión desde el año 1975 hasta el año 2015 en España comparado a nivel mundial (Fig. 4).

Según la Sociedad Europea de Cardiología y el Colegio Americano de Cardiología, los valores normales de presión arterial son <120 mmHg para la presión sistólica (PS) y <80 mmHg para la presión diastólica (PD) [11]. Si el rango de un individuo está en 120-139 mmHg para PS y 80-89 mmHg para PD, se le diagnostica prehipertensión, siendo la etapa más leve. Esta etapa se ve agravada por el hecho de que la mayoría de pacientes con prehipertensión padecen sobrepeso u obesidad, asociados generalmente a alteraciones metabólicas, oxidativas e inflamatorias. El grado 1 de hipertensión se caracteriza por valores ≥ 140 mmHg para la presión arterial sistólica y ≥ 90 mmHg para la presión arterial diastólica. El grado 2 se caracteriza por la presencia de marcadores difusos de enfermedad y/o signos tempranos de ECV, correspondiendo a ≥ 160 mmHg para la presión arterial sistólica y ≥ 100 para la presión arterial diastólica [12]. Y finalmente, en el grado 3, cuyos individuos presentan evidencia clínica de daño manifiesto de órganos diana, la presión arterial sistólica es ≥ 180 mmHg y ≥ 110 para la presión arterial diastólica [13].

La creciente evidencia sugiere que la epidemia de sobrepeso y obesidad subyace a muchas enfermedades metabólicas comunes. Está bien establecido que el aumento excesivo de peso asociado con un aumento de la distribución de la adiposidad visceral se acompaña de diversas alteraciones que contribuyen a un estado hipertensivo y que representa del 65% al 75% del riesgo de hipertensión primaria humana [14].

1.2.1 Parámetros para evaluar la Hipertensión Arterial

Para establecer el diagnóstico de HTA existen diversos parámetros que evalúan el grado de control del paciente hipertenso, pero todos ellos parten de una primera evaluación en consulta a través de la medida de la Presión Arterial (PA) mediante manómetro. Hasta la fecha es la técnica principal para valorar al paciente, aunque presenta numerosas limitaciones si no se dispone de un dispositivo médico ambulatorio que evalúe fuera de consulta. En consulta puede producirse el efecto de la "bata blanca" y proporcionar un

valor sesgado. Por otro lado, las determinaciones fuera de consulta (oficina de Farmacia) podrían evitar este problema, pero no suelen ser habituales.

Por todo ello, en los últimos ensayos clínicos se han empezado a evaluar mediante técnicas nuevas y complementarias de medición de la PA con el objetivo de mejorar el grado de exactitud y precisión para clasificar correctamente al paciente hipertenso.

Existen dispositivos validados según los estándares clínicos internacionales que pueden proporcionar valores adicionales a la PA y así obtener una monitorización más completa. Entre ellos, una solución de Monitorización Ambulatoria de la Presión Arterial (MAPA) de 24 horas, es un pequeño dispositivo de muñeca (Bpro). Este dispositivo captura los datos de las ondas de pulso arterial de los usuarios y los convierte en lecturas de MAPA de 24 horas mediante un algoritmo propio. BPro recoge los datos de la onda del pulso arterial en intervalos de 15 minutos. Cuando finaliza el periodo de 24 horas, los datos se transmiten a un software que genera un informe detallado de diversos índices de presión arterial:

- La Presión de Pulso (PP) evalúa la rigidez de la arteria aorta. Cuanto mayor es la presión de pulso, mayor es la rigidez y el daño de los vasos sanguíneos. Se obtiene de la siguiente ecuación PP= PS-PD.
- La Presión Arterial Media (PAM) evalúa la presión de perfusión de los órganos corporales. Su estudio indica si existe o no suficiente riego sanguíneo y/o riesgo de isquemia. La PAM puede aproximarse empleando la siguiente fórmula:
- PAM = TD + 1/3 (PS-PD).
- La Presión Aórtica Central (PAC) o PA existente en la raíz aórtica. Es el principal factor de carga hemodinámica que afecta al musculo miocárdico. La aparición de nuevos métodos permite su determinación indirecta de forma no invasiva. La falta de reducción de la PAC determinaría el elevado riesgo cardiovascular residual de pacientes con HTA y alertando del riesgo de angiopatía.

Recientes estudios que evalúan el valor pronóstico de los anteriores parámetros de forma domiciliaria en relación con la morbilidad cardiovascular han proporcionado datos interesantes. Aunque sigue siendo necesario nuevos estudios prospectivos para evaluar y confirmar cifras diagnósticas y así evaluar debidamente el tratamiento antihipertensivo acorde a cada individuo.

2. TRATAMIENTOS FRENTE A LA OBESIDAD

2.1 Enfoques terapéuticos para el tratamiento de la obesidad y trastornos metabólicos derivados

Un estilo de vida saludable, dieta equilibrada y actividad físicas adecuada es la mejor estrategia para prevenir y tratar la obesidad. Las estrategias de reducción de la ingesta energética y/o estimular el gasto energético representan uno de los enfoques más estudiados. A pesar de que la pérdida de peso es eficaz para el control de las patologías derivadas de la obesidad, son numerosos los pacientes que no pueden mantener a lo largo del tiempo una pérdida adecuada mediante modificaciones en el estilo de vida y una adquisición de hábitos saludables con una mayor actividad física y una ingesta reducida de energía. Existen otros enfoques diferentes como la farmacoterapia y la cirugía bariátrica [15].

Aunque el objetivo terapéutico más importante para los pacientes hipertensos con obesidad debería ser tratar las causas subyacentes de la obesidad, son pocos los tratamientos farmacológicos disponibles que otorguen una pérdida de peso a largo plazo de manera segura y eficaz. Generalmente cuando a un individuo se le diagnostica prehipertensión se recomienda adoptar ciertos hábitos de estilo de vida para ayudar a reducir su presión arterial antes de pasar a los medicamentos hipotensores. Las mejoras de la presión arterial no son inmediatas y a menudo los pacientes abandonan los cambios de estilo de vida, facilitando la progresión de la enfermedad.

Los fármacos anti-obesogénicos muestran muy buen funcionamiento entre las personas con obesidad inducida por la dieta, pero presentan numerosos efectos secundarios graves, como insomnio, ansiedad, hepatotoxicidad entre otros [16, 17]. Se estima que únicamente el 20% de las personas con sobrepeso que experimentan una pérdida de peso significativa son capaces de mantener el peso perdido a largo plazo [18]. La evidencia sugiere que existe una mayor adherencia a la pérdida de peso cuando los individuos logran mantenerse en su peso durante 2 años o más, consiguiendo implementar estrategias de dieta y ejercicio y reduciendo así el riesgo de una recuperación posterior del sobrepeso perdido.

De esta situación viene la creciente demanda en la búsqueda de tratamientos y nuevas farmacoterapias alternativas que examinen si la pérdida de peso sostenida en sujetos puede revertir estos cambios y que no solo proporcionen una mejoría en la reducción del peso

corporal, sino que también estén dirigidas a tratar los trastornos derivados de la obesidad como la hipertensión, la dislipemia, la hiperglucemia o la resistencia a la insulina.

Por tanto, el tratamiento farmacológico de la obesidad está lejos de obtener resultados prometedores en una realidad cercana y es necesaria la búsqueda de nuevas moléculas efectoras que modulen las vías metabólicas [19-21], nuevas pautas específicas y terapias anti-obesogénicas más efectivas sin causar efectos adversos en el paciente.

En esta línea, las medicinas tradicionales a base de plantas desempeñan un papel destacado en la estrategia para la lucha contra la obesidad. Según la OMS, más del 80% de la población mundial emplea fármacos de origen vegetal y una gran cantidad de medicamentos sintéticos de la actualidad tienen como origen las plantas medicinales [22].

Las propiedades farmacológicas de diversos extractos vegetales son motivo de estudio en numerosos campos, y empleados con distintos fines terapéuticos [23]. Los extractos de plantas y compuestos puros son empleados para abordar patologías relacionadas con el síndrome metabólico y su mecanismo de acción suele ser de origen multifactorial.

Las investigaciones recientes apoyan que las plantas que tradicionalmente han sido empleadas para tratar diversas enfermedades, pueden ser una potente fuente de moléculas bioactivas capaces de intervenir en multitud de mecanismos metabólicos. Esto confirma que los fitoquímicos son excelentes candidatos para abordar patologías multifactoriales como la obesidad.

En las últimas décadas la comunidad científica ha recopilado suficientes evidencias para postular que numerosas plantas medicinales pueden considerarse una buena alternativa en el tratamiento del control de peso por su actividad contra la obesidad, tanto en estudios *in vitro* e *in vivo*, como también en ensayos clínicos. Los suplementos contra la obesidad derivados de plantas se han considerado una mejor alternativa ya que no presentan efectos secundarios notorios [24].

2.2 Polifenoles como nutracéuticos: Función anti-obesidad de los polifenoles de plantas

La investigación de compuestos vegetales bioactivos de metabolitos secundarios derivados de plantas con efectos promotores de la salud es cada vez más creciente. Tienen un efecto sobre el organismo humano y las investigaciones recientes los han planteado como hipótesis para reducir el riesgo de patologías crónicas [25, 26]. Los polifenoles son moléculas que se caracterizan por la presencia de uno o más grupos fenólicos en sus estructuras y se encuentran naturalmente en plantas, frutas, verduras, cacao y té, entre otros (Fig. 5).

Estos compuestos antioxidantes se consideran nutracéuticos con eficacia preventiva y con la capacidad potencial de detener o revertir enfermedades relacionadas con el estrés oxidativo [27]. En este contexto, se ha estudiado un efecto promotor de la salud de los polifenoles para mitigar o contrarrestar las enfermedades crónicas degenerativas.

Figura 5: Ejemplo de imagen de alimentos y plantas ricos en polifenoles. Imagen extraída de Adobe Images.

En función de los grupos fenoles que contengan en su estructura, pueden agruparse los polifenoles en distintas clases: ácidos fenólicos (ácidos hidroxibenzoicos y ácidos cinámicos), flavonoides (flavonoles, flavonas, isoflavonas, flavanonas, flavanoles y antocianos), estilbenos y lignanos (Fig. 6) [23]. Además, los polifenoles pueden encontrarse asociados a otras moléculas como carbohidratos o ácidos orgánicos lo que les confiere propiedades físicas y químicas diferentes y una enorme diversidad estructural y aumentando así su espectro de acción. Esto les confiere numerosas funciones antitumorales, antioxidantes, antimicrobianas, antihipertensivas y antivirales [28].

Por otro lado, a lo largo de los años numerosos estudios demuestran el papel beneficioso de los polifenoles sobre la salud, demostrando que pueden ser una valiosa herramienta para tratar enfermedades multifactoriales asociadas a la obesidad [29, 30].

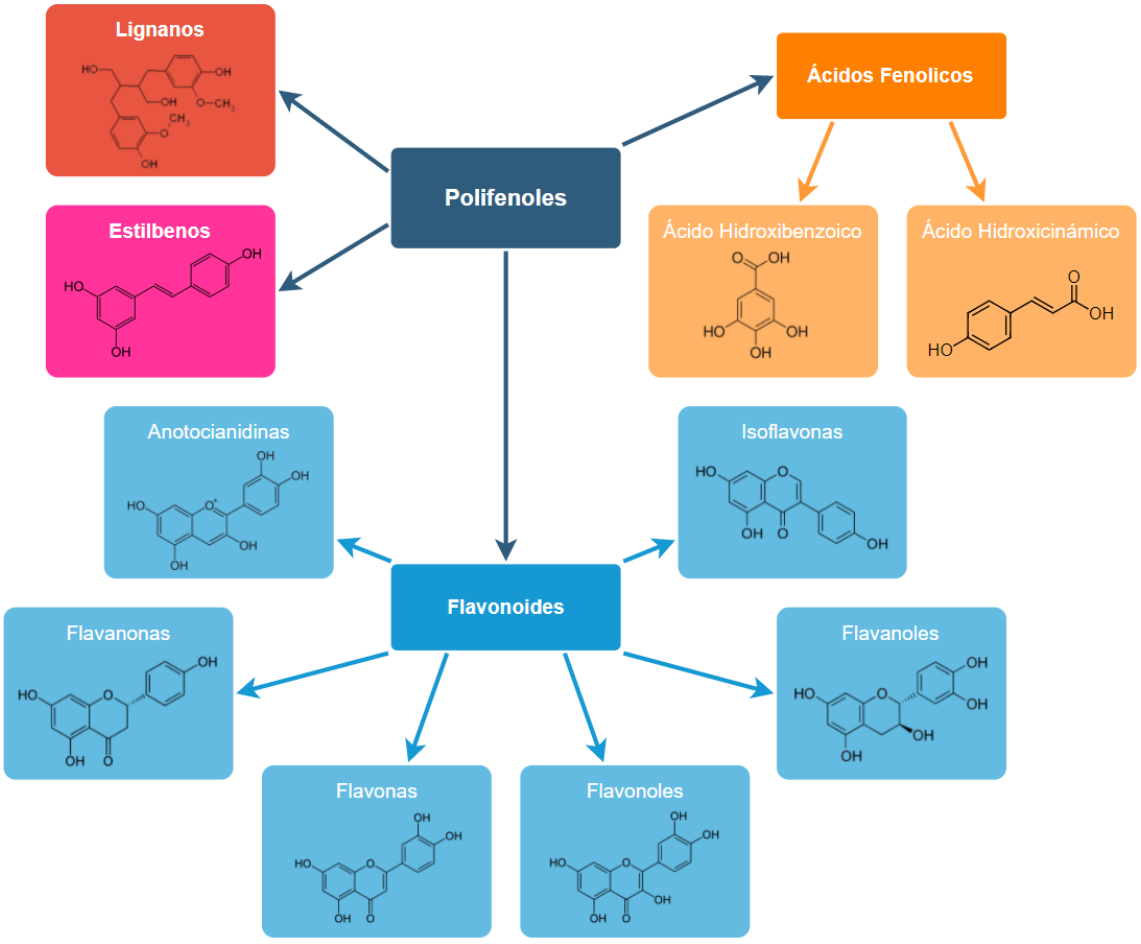

Figura 6: Clasificación de los polifenoles. Esquema representativo de las principales familias y subfamilias de polifenoles con sus estructuras químicas [23].

2.3 Extractos polifenólicos de *Hibiscus sabdariffa* y *Lippia citriodora*.

Los extractos vegetales comestibles de *Hibiscus sabdariffa* (HS) y *Lippia citriodora* (LC) han acumulado suficiente evidencia científica para convertirse en un óptimo nutracéutico en el tratamiento de patologías como la obesidad y otros trastornos metabólicos relacionados, como la hipertensión o la diabetes.

Los extractos ricos en polifenoles de HS y LC (Fig.7) han demostrado en recientes estudios *in vitro* reducir la acumulación de lípidos intracelulares, disminuir el estrés oxidativo, así

como la inflamación inducidos por la hiperglucemia en el tejido adiposo a través de la regulación de diferentes vías metabólicas [20, 31-36]. La evidencia científica emergente sugiere la capacidad de un mecanismo multidireccional, capacidad para potenciar la oxidación de ácidos grasos, activar el sensor energético de la proteína quinasa activada por AMP (AMPK), regulación en procesos relacionados con el estrés y la inflamación [20, 37].

Figura 7: *Hibiscus sabdariffa* (A), *Lippia citriodora* (B). Imágenes extraídas de Adobe Images y Pexels [38, 39].

2.3.1 Hibiscus sabdariffa

Hibiscus sabdariffa se cultiva ampliamente en numerosos países [40] y es comúnmente conocida como roselle, pertenece a la familia de las Malvaceae. Esta planta se usa a menudo en la medicina tradicional, es rica en fitoquimicos como polifenoles, especialmente antocianinas, polisacáridos y ácidos orgánicos, aprovechándose todas sus partes [41].

La infusión de hibisco se ha usado de forma muy popular por sus posibles propiedades terapéuticas en enfermedades crónicas comunes, hipertensión, hiperlipidemia, obesidad, diabetes, resfriados o infecciones del tracto urinario [40, 42].

La evidencia acumulada recoge múltiples efectos en modelos celulares y animales, así como en humanos debidos al consumo del hibisco. Entre ellos destacan potentes propiedades antioxidantes, antiinflamatorias, protección cardiovascular, antihiperlipidémicas, antihipertensivas, antiagregantes plaquetarias, diuréticas, antimicrobianas, anticancerígenas, hepatoprotectoras, antitumorales e inmunomoduladoras. Además, mejora varias afecciones relacionadas con la obesidad [43, 44] [20, 45] por lo que dicho extracto tiene un enorme potencial en los usos terapéuticos en la sociedad actual.

Los potenciales efectos en las funciones metabólicas son numerosos, mostrando efectos concurrentes en la actividad mitocondrial, el estrés oxidativo y la inflamación, así como en la homeostasis energética, considerando el impacto de los polifenoles del hibisco como un coadyuvante para la protección cardiovascular [45].

La actividad biológica de HS se atribuye al contenido polifenólico, los cálices de la flor de Jamaica son ricos en ácidos orgánicos, siendo el ácido de hibisco el compuesto más representativo, aunque también hay que considerar ácidos fenólicos, como el ácido clorogénico, y derivados de flavonoles, fenilpropanoides y antocianinas [46] (Tabla 1 y Figura 8).

Hibiscus sabdariffa	
Compuesto	**Familia**
Ácido Hibiscus	Ácidos Orgánicos/ Ácidos Dicarboxílicos
Delphinidina-3-sambubiosido **Cyanidina-3-sambubiosido**	Flavonoides/ Anthocianinas
Ácido clorogénico **Digalato de metilo** **Ácido cumaroilquínico** **Ácido 5-O- Cafeoilshikímico**	Ácidos Fenólicos
Myricetina-3-arabinogalactosa **Quercetina-3-sambubiosido** **Quercetina-3-rutinosido** **Leucosido** **Quercetina-3-glucosido** **Kaempferol-3-O-rutinosido** **Miricetina** **Quercetina**	Flavonoides/ Flavonoles
Metil epigalocatequina	Flavonoides/ Flavonoles
N-Feruloyltyramina	Otros/ Tiraminas

Tabla 1 – Compuestos del *Hibiscus sabdariffa* agrupados en familia según su estructura química. Adaptado del trabajo publicado por Olivares-Vicente et al. [46].

En ensayos clínicos, la administración oral de varios extractos acuosos de HS ejerce una potencial regulación metabólica y protección del hígado, normalizando los niveles de glucosa, reduciendo esteatosis hepática y los perfiles de lípidos, facilitando la pérdida de peso, mejorando los parámetros antropométricos, o modulando los biomarcadores del apetito en pacientes con síndrome metabólico u obesidad [35, 47]. Estudios preclínicos en ratones obesos han demostrado la capacidad de estos polifenoles para reducir significativamente el aumento de peso corporal al inhibir la acumulación de grasa y mejorar la tolerancia a la glucosa [48, 49]

2.3.2 Lippia citriodora

Lippia citriodora (syn. *Lippia triphylla*, *Aloysia triphylla*) conocida popularmente como hierbaluisa, es una planta aromática, popular por sus diversos usos, perteneciente a la familia de las Verbenaceae [46].

La infusión de las hojas de LC se ha utilizado tradicionalmente como alimento o especia, para el tratamiento de alteraciones del sueño y la ansiedad, antiespasmódico, enfermedades respiratorias, digestivas y musculares, cardiotónico y tranquilizante [50] [51].

Los extractos de LC contienen polifenoles como compuestos bioactivos, especialmente fenilpropanoides, cuyo compuesto más abundante es el verbascósido, pero también contiene iridoides y flavonoides (Tabla 2 y Figura 8) [52, 53]. Entre ellos, los fenilpropanoides son la principal clase de compuestos que han mostrado gran actividad antioxidante.

El verbascósido como principal compuesto bioactivo, ha demostrado actividad antioxidante cuando se administra a ratas, mejorando la actividad antioxidante de la glutatión peroxidasa y la glutatión reductasa y reduciendo la actividad de la mieloperoxidasa [54] [55].

La metformina, un conocido fármaco antidiabético, capaz de promover la pérdida de peso en pacientes obesos [56], parece tener un mecanismo similar al mostrado por los derivados fenólicos, modulando la actividad de AMPK en adipocitos 3T3-L1 hipertróficos inducidos con altas concentraciones de glucosa [57].

El extracto de LC ha demostrado en un modelo *in vitro* de adipocitos 3T3-L1 hipertróficos resistentes a la insulina, una reducción de la acumulación de triglicéridos y de especies reactivas de oxígeno (ROS), confirmando así su potencial antioxidante [36].

Por otro lado, estudios en humanos han mostrado beneficios antiobesidad, modulando los biomarcadores del apetito, facilitando la pérdida de peso y mejorando los parámetros antropométricos [35].

Lippia citriodora	
Compuesto	**Familia**
Shanziside	Iridoides glicósidos
Gardósido	
Tevésido	
Verbascósido	Fenilpropanoides
Cistanósido F	
β -Hidroxiverbascósido/	
β-Hidroxiisoverbascósido	
Campenósido I	
Eucovósido	
Martinósido	
Luteolina-7-diglucurónido	Flavonoides
Crisoeriol-7-diglucurónido	
Acacetina-7-diglucurónido	

Tabla 2 – Compuestos de la *Lippia citriodora* agrupados en familia según su estructura química. Adaptado del trabajo publicado por Olivares-Vicente et al. [46].

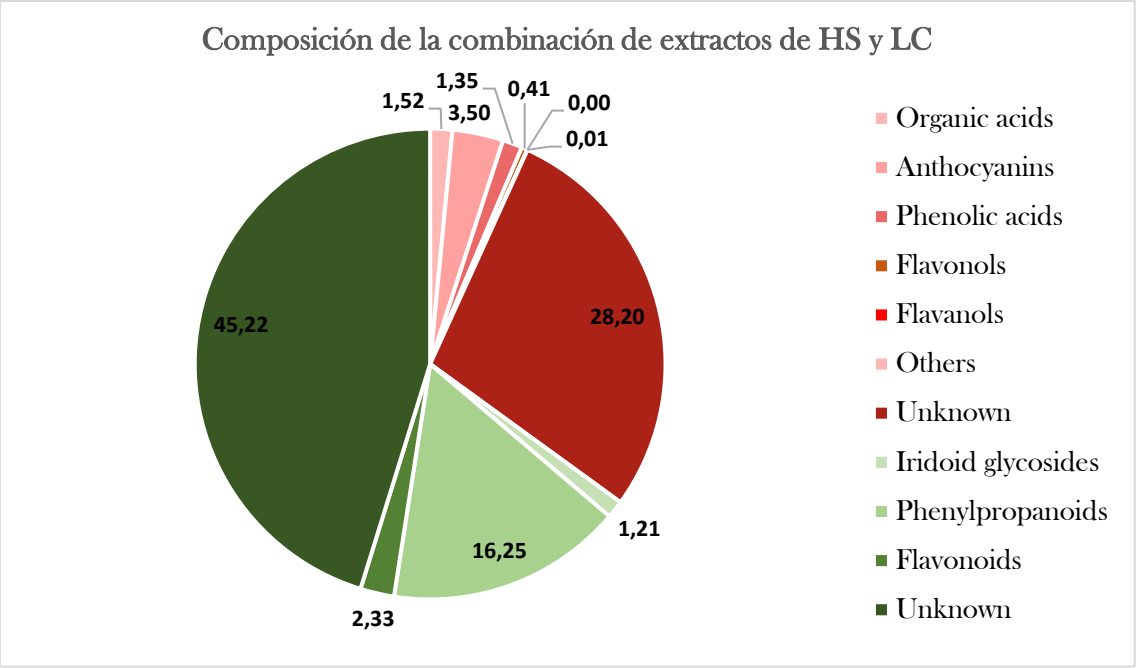

Figura 8 – Porcentaje de las diferentes familias de compuestos identificados en los extractos de HS y LC [46].

3. EL CONTROL DEL APETITO

En los últimos años, el interés de los investigadores por conocer cómo se regulan las sensaciones que regulan las conductas alimentarias ha crecido exponencialmente. La prevalencia de la obesidad y sobrepeso ha ido aumentando, considerando así una vinculación directa con el síndrome metabólico. Por todo ello, se puede confirmar que existe una estrecha relación entre el comportamiento de la ingesta de alimentos, la sensación de apetito, saciedad y hambre, con el síndrome metabólico.

Es conocido que la obesidad es una patología multifactorial determinantemente influida por factores genéticos, ambientales, culturales, económicos y conductuales entre otros. Las sensaciones de hambre, el apetito, la saciedad y el balance energético se regulan por un sistema neuroendocrino redundante que se encuentra en centros a nivel del hipotálamo.

El hipotálamo es la principal zona del sistema nervioso central (SNC) encargado de regular las interacciones entre las diferentes regiones del cerebro involucradas en la conducta alimentaria. Sin embargo, el control de la ingesta de alimentos no es un proceso que se produzca sólo en el SNC, están implicadas otras señales periféricas y regiones cerebrales extra-hipotalámicas [58, 59]. Es aquí cuando si se producen fallos en el circuito neuroendocrino y surgen patologías que alteran el sistema metabólico.

Son numerosas las señales que integran la regulación neuroendocrina de la dieta, pudiéndose realizar una clasificación en función de la duración de su acción:

- corto plazo: proceso de hambre / apetito y su regulación.
- largo plazo: proceso responsable de llenado de las reservas corporales / saciedad.

La clasificación en función de sus efectos se subdivide en:

1) efectos orexigénicos: aquellos que activan las vías anabólicas a través de un deseo intrínseco de ingerir alimentos, es decir, una mayor sensación de hambre y apetito, e inhibición del gasto energético [60].

2) efectos anorexigénicos: los que activan las vías catabólicas, activando las señales de llenado y saciedad gastrointestinal.

En las últimas décadas, han sido numerosas las investigaciones relacionadas con la obesidad y el descubrimiento de hormonas gastrointestinales, así como las vías del sistema nervioso central que influyen en la ingesta de alimentos y el gasto energético. A pesar de la evidencia acumulada, los mecanismos fisiopatológicos y los factores complejos involucrados que regulan el equilibrio energético no están esclarecidos en la actualidad [14, 35]. El manejo de la obesidad y la propiedad de supresión del apetito de ciertos compuestos naturales podrían ser potencialmente útiles en dietas con restricción energética.

3.1 El concepto de brecha energética

Los pacientes con obesidad o sobrepeso no consiguen adoptar pautas saludables ni soportar dietas con restricciones calóricas durante periodos prolongados. En el caso de adoptar y conseguir estas medidas, los pacientes tienen el consecuente déficit energético y pérdida de peso, experimentando hambre y gasto energético reducido. Este término es denominado “brecha energética” [35].

La evidencia sugiere que la expresión de neuropéptidos y péptidos intestinales tiene un papel importante en el aumento de la sensación de hambre como respuesta a la pérdida de peso. Por lo que se puede confirmar que son numerosos los factores metabólicos que contribuyen a la brecha energética [61].

Este fenómeno explicaría la disminución de hormonas como leptina y/o resistina en situaciones de restricción calórica, correlacionadas posiblemente con la disminución de adiposidad en casos de pacientes pautados bajo un régimen dietético. Es por ello por lo que se puede postular, que el consumo de nutracéuticos de origen vegetal podría contribuir modulando los biomarcadores del apetito y además reestablece el equilibrio entre el hambre y el gasto energético [35].

3.2 Hormonas implicadas en el control de la ingesta de alimentos

Existen numerosas hormonas implicadas en la regulación del apetito. La gran mayoría de estas hormonas son sintetizadas en sistemas endocrinos difusos como el tracto gastrointestinal, sistema Nervioso Central (SNC) o el tejido adiposo. La existencia de hormonas de origen gastrointestinal ha despertado un gran interés, dada la importancia de los trastornos de la ingesta de alimentos en relación con la obesidad.

En la industria farmacéutica existen numerosos fármacos evaluados por su capacidad para reducir el peso corporal, entre los que se encuentran los derivados de hormonas producidas y secretadas por el tracto gastrointestinal (GI) o el tejido adiposo [62].

Hasta la fecha y a pesar de las numerosas investigaciones, la relevancia fisiológica de cada una de ellas, aún no se ha establecido de manera precisa. Se desconocen los mecanismos de acción de estas señales endocrinas que modulan la homeostasis energética, aunque la evidencia es clara en que son fundamentales en la regulación de la ingestión de alimentos. El avance en el conocimiento de estas adipohormonas podría contribuir en la mejora terapéutica de las enfermedades crónicas y el tratamiento de la obesidad, mediante el desarrollo de nuevas moléculas. Actualmente, la relevancia fisiológica de dichas hormonas está por establecer.

El control de la ingesta alimentaria está conformado por diversas señales, como los estímulos cognitivos, señales procedentes del tracto gastrointestinal, señales originadas en el sistema nervioso central y la naturaleza bioquímica del nutriente, entre otros.

La clasificación inicial sería hormonas que incrementan la ingesta (efecto orexígeno) y hormonas que disminuyen la ingesta (efecto anorexígeno). Algunos ejemplos se muestran en la Tabla 3.

Principales hormonas que modulan la saciedad	Efecto sobre la ingesta de alimentos	Origen de las señales
Colecistoquinina (CCK)	↓	GI
Amilina	↓	GI
Péptido análogo del glucagón tipo 1 (GLP-1)	↓	GI
Péptido Tirosina-Tirosina (PYY)	↓	GI
Oxintomodulina (OXM)	↓	GI
Leptina	↓	GI
Grelina	↑	GI
Neuropéptido Y (NPY)	↑	SNC
Hormona concentradora de melanina (MCH)	↑	SNC
Hormona liberadora de corticotropina (CRH)	↓	SNC

Tabla 3 – Clasificación de hormonas moduladoras de saciedad.

3.2.1 Hormonas anorexigénicas

La mayoría de las hormonas de origen gastrointestinal que intervienen en el control de la ingestión, se conocen desde hace décadas. Se liberan como respuesta a la ingesta provocando un efecto saciante de corta duración, pero a día de hoy su relevancia fisiológica, así como su mecanismo de acción aún no ha terminado de esclarecerse. Se conoce que su producción y secreción se correlaciona con el estado nutricional del individuo.

Las principales hormonas peptídicas anorexigénicas con efecto saciante, son: CCK, PYY, GLP-1. Otros péptidos de origen gastrointestinal, con efecto saciante igualmente son: OXM y la amilina, entre otros, cuyas características, efectos y mecanismos son menos conocidos (Tabla 4).

Algunas hormonas junto con sus receptores parecen tener una clara relación con la inhibición de hormonas orexigénicas. Así CCK, una de las señales de saciedad más estudiadas junto con su receptor CCK-1, intervienen en la inhibición de la grelina, estimulando un efecto saciante mediante la estimulación de PYY.

Péptidos como PYY tienen la capacidad de inhibir la secreción de péptidos con efecto orexígeno como NPY, lo que provoca una disminución del apetito y un aumento del gasto calórico.

El freno ileal, se denomina al mecanismo por el cual parece regularse el tránsito de los nutrientes a través del tracto gastrointestinal, es decir, controla la velocidad y produce una regulación de la motilidad intestinal por las grasas. Es aquí donde hormonas como GLP-1 y PYY parecen jugar un papel fundamental al inducir este mecanismo, esencial en la regulación de la ingesta.

Actualmente han irrumpido en el mercado nuevos fármacos agonistas del receptor GLP-1 que parecen propiciar una pérdida de peso significativas de alrededor del 15%. Los agonistas del receptor GLP-1 han demostrado ser una alternativa para el tratamiento de la diabetes tipo 2, reducción de perímetros y el peso corporal del paciente [63]. Su uso ha aumentado drásticamente en los últimos años, aunque en Estados Unidos y España lleva aprobado desde hace décadas. Sin embargo, la evidencia ha demostrado grandes diferencias en la magnitud de los efectos de estos agentes y en la frecuencia de los efectos adversos, no debiendo considerarse como agentes de primer uso.

Hormonas	Acción
CCK	Inhibición moderada de la motilidad y vaciamiento gástrico, disminuyendo el tamaño de la porción alimenticia e induciendo la saciedad.
PYY	Inhibe el apetito.
OXM	Inhibe el apetito.
Amilina	Inhibe el vaciamiento gástrico y la secreción de ácidos gástricos.
GLP-1	Inhibe el vaciamiento gástrico y estimula la saciedad.
GLP-2	Modula la motilidad y tránsito intestinal.
Leptina	Principal regulador a largo plazo de la conducta alimenticia y del peso corporal. Regulación de la homeostasis energética. Los niveles circulantes de leptina tienen relación directa con los depósitos de grasa corporal y son reflejo del balance energético.
Insulina	Papel fundamental en la regulación del metabolismo de los nutrientes calóricos. Los niveles circulantes de insulina están en relación directa con la cantidad de tejido adiposo.

CRH	Regula el balance energético e influye en la respuesta ante el estrés. Anomalías en la señalización de sus receptores se relaciona con la fisiopatología de desórdenes alimenticios.

Tabla 4: Ejemplos de hormonas anorexigénicas.

3.2.2 Hormonas orexigénicas

Hormonas	Acción
NPY	Regula de la conducta nutricional favoreciendo el consumo de alimentos, la secreción de insulina, la liberación hepática de glucosa, la actividad de la lipoproteína lipasa y la termogénesis.
Grelina	Estimula el apetito a corto y largo plazo, eleva el peso corporal, disminuye la utilización de grasa.
MCH	Estimula el apetito.

Tabla 5: Ejemplos de hormonas orexigénicas.

En lo que respecta a las hormonas orexigénicas, la grelina secretada por el tracto digestivo es la primera hormona conocida de origen gastrointestinal con capacidad activadora del apetito (efecto orexigénico) (Tabla 5). Parece jugar un papel fundamental en la ingesta alimentaria, mediante acciones tanto en las vías orexigénicas como anorexigénicas del hipotálamo.

La mayoría de los resultados que se han obtenido acerca de los mecanismos que regulan hambre y saciedad se han realizado en modelos animales, y no siempre pueden ser extrapolados a los seres humanos. No obstante, los resultados muestran un evidente complejo sistema neuroendocrino.

Objetivos

Dadas las evidencias científicas con diferentes extractos polifenólicos de plantas obtenidas en el grupo de investigación en el que se enmarca el presente proyecto de Tesis Doctoral, el objetivo general que se plantea es demostrar que las fracciones polifenólicas de ciertas materias vegetales como HS y LC pueden ser coadyuvantes en el abordaje de la obesidad, así como en otras patologías metabólicas asociadas a la obesidad. Para ello, se han propuesto los siguientes objetivos específicos:

1.OBJETIVOS DEL CAPÍTULO 1

El objetivo de este capítulo es evaluar la capacidad de un suplemento dietético basado en una combinación de extractos polifenólicos de HS y LC, en un estudio de intervención en sujetos con sobrepeso u obesidad, así como evaluar los biomarcadores modulados por polifenoles, relacionados con el apetito y los efectos diferenciales en parámetros antropométricos, metabólicos y hematológicos asociados a los trastornos metabólicos por un exceso de peso corporal.

2. OBJETIVOS DEL CAPÍTULO 2

Estudios previos han indicado la posibilidad de que un producto nutracéutico botánico basado en la combinación de extractos de HS y LC tenga propiedades hipotensivas en personas con sobrepeso y obesidad. En este contexto se decidió evaluar un nutracéutico, rico en compuestos polifenólicos en un ensayo aleatorizado y controlado con placebo en individuos prehipertensos e hipertensos en grado 1, y examinar los parámetros antropométricos y sanguíneos, así como en la presión arterial.

3. OBJETIVOS DEL CAPITULO 3

El último objetivo de este proyecto es ofrecer un resumen de los datos acumulados en la bibliografía sobre bioactivos vegetales para regular el apetito y mejorar la enfermedad de la obesidad. Para ello, se evaluó la evidencia científica de estudios recientes de extractos y compuestos bioactivos específicos que se utilizan para suprimir el apetito / hambre y su capacidad para aumentar la sensación de plenitud / saciedad. Para comprender y

profundizar en el tema se han recopilado los procesos de regulación del apetito más relevantes según la evidencia hoy en día, en relación con las adipohormonas más significativas implicadas en los mecanismos de control del hambre / saciedad.

Material y Métodos

MATERIALES Y MÉTODOS

1. Materiales

1.1 Formulación del suplemento

Las cápsulas de MetabolAid® (número de solicitud de patente P201731147) fueron proporcionadas por Monteloeder SL (Elche, Alicante, España). MetabolAid® contiene 500 mg de una combinación optimizada de extractos polifenólicos de *Hibiscus sabdariffa* y *Lippia citriodora*, con un contenido final de 3,5% de antocianinas y 15% de verbascósido (% de peso seco, p / p) medido por cromatografía líquida de alto rendimiento (HPLC) junto con detección por espectrometría de masas.

2. Métodos

2.1 Estudio de intervención en sujetos con sobrepeso. Biomarcadores relacionados con el apetito.

Se llevó a cabo un ensayo controlado por placebo, doble ciego y aleatorizado de 8 semanas de duración en 54 mujeres con sobrepeso.

2.2 Sujetos de la intervención

Los participantes fueron reclutados en un Centro Dietético en la ciudad de Elche (Alicante, España) durante el año 2016. Se reclutaron mujeres con un índice de masa corporal (IMC) entre 25-34,9 kg/m^2. 70 participantes respondieron al reclutamiento inicial, de las cuales y según los criterios establecidos, fueron seleccionadas 54 participantes, comprendidas entre los 30 y los 75 años. La distribución aleatoria de las participantes establecida según el IMC comprendió: grupo experimental L1 (n = 25; IMC = 29,84) con 14 sobrepeso y 11 obesas, y grupo L2-placebo (n = 22; IMC = 29,95) con 12 sobrepeso y 10 obesas.

Los criterios de exclusión que se establecieron incluyeron la presencia de cualquier patología relacionada con la obesidad, uso de medicamentos recetados para la

hipercolesterolemia o hipertensión, consumo de suplementos / medicamentos antioxidantes, consumo frecuente de alcohol y embarazo / lactancia.

2.3 Mediciones antropométricas y análisis de sangre

Se tomaron medidas antropométricas al inicio del estudio, a los 30 y 60 días del período de intervención, e incluyeron el peso corporal, altura e índice de masa corporal (IMC) que se derivó del peso corporal y la altura utilizando la ecuación IMC = Peso corporal (kg) / altura^2 (m).

Los pliegues cutáneos del tríceps, bíceps y abdominal, y las circunferencias del brazo y el abdomen. Las circunferencias abdominales (AC) en dos sitios diferentes: en la parte anterior a medio camino entre la apófisis xifoides del esternón y el ombligo y lateralmente entre el extremo inferior de la caja torácica y las crestas ilíacas (AC1) y a nivel del ombligo (AC2).

Se midieron con un calibre de pliegues cutáneos o una cinta métrica, respectivamente.

El porcentaje de grasa corporal se derivó de los perímetros AC1 y AC2 mediante la ecuación de Weltman.

Además, se midieron la presión arterial sistólica y diastólica en reposo y la frecuencia cardíaca al inicio, 30 y 60 días de la intervención utilizando un tensiómetro Ecomed (Medisana Healthcare SL, Barcelona, España).

2.4 Péptidos y hormonas circulantes

Se obtuvieron muestras de sangre de la vena antecubital después de un ayuno nocturno en vacutainer con EDTA al inicio, 30 y 60 días del estudio.

El plasma extraído de los sujetos asignados aleatoriamente al grupo de Suplemento (L1) o Placebo (L2), se aisló inmediatamente mediante procedimientos estándar y las muestras se almacenaron a -80ºC hasta el momento de la medición.

Se utilizó el kit de inmunoensayo ProcartaPlex® de la división eBioscience de Affymetrix (inmunoensayo múltiple por conveniencia y paneles Mix & Match) para determinar los niveles de FGF-23, péptido similar al glucagón-1 (GLP-1), grelina, insulina, leptina, péptido

C, PYY y resistina. Para medir los niveles de los marcadores de apetito y saciedad antes mencionados se utilizó el analizador Luminex (Thermo Fisher Scientific).

2.5 Cuestionarios

2.5.1 Escala Visual Analógica y Cuestionarios SF-36

La Escala Visual Analógica (EVA), cuestionario validado empleado al inicio y a los 15, 30, 45 y 60 días de la intervención, se utilizó para registrar el hambre, la saciedad, la plenitud, el consumo prospectivo de alimentos, el deseo de comer algo graso, salado, dulce o sabroso y palatabilidad de las comidas.

El cuestionario de salud validado SF-36 (Optum™ SF-36v2® Health Survey) está compuesto por 36 ítems que evalúan el estado de salud tanto positivo como negativo. Los 36 ítems cubren las siguientes escalas: salud general, función física, rol físico, dolor corporal, vitalidad, función social, rol emocional y salud mental. Se utilizó al principio y al final del estudio, para evaluar el estado de salud subjetivo del individuo.

Se realizó un cuestionario de frecuencia alimentaria semicuantitativo validado y un recordatorio de dieta de 24 horas.

2.6. Análisis estadístico

El análisis estadístico de los estudios de intervención se llevó a cabo utilizando el software Graphpad Prism. Los resultados se expresaron como media $\pm$ desviación estándar (DE).

Los datos antropométricos, bioquímicos y de signos vitales (intergrupo), cuestionarios EVA y SF-36 se analizaron mediante la prueba t de Student para datos no apareados.

Por otro lado, datos antropométricos, signos vitales (estadísticos intragrupo) y los péptidos de saciedad y las hormonas se analizaron mediante la prueba t de Student pareada. Las variables de resultado se evaluaron para determinar su conformidad con la distribución normal mediante una prueba de Kolmogorov-Smirnov (KS).

Los valores obtenidos a través de los dispositivos BPro® se analizaron mediante ANOVA de una variable. Las variables de resultado se evaluaron para determinar su cumplimiento con la distribución normal mediante una prueba de Shapiro-Wilk.

3. Estudio de intervención en sujetos pre-hipertensos y en grado 1

3.1 Sujetos de la intervención

Los participantes fueron reclutados por el servicio del sistema de análisis de información sanitaria ALUMBRA. Se seleccionaron 563 pacientes prehipertensos e hipertensos en grado 1 del Centro de Salud Raval ubicado en la ciudad de Elche (España). Se excluyeron 479 pacientes por no cumplir con los criterios de inclusión. Finalmente, 84 pacientes fueron incluidos en el estudio.

3.2 Mediciones antropométricas y análisis de sangre

Se tomaron medidas antropométricas al inicio del estudio, la semana 2 y la semana 6 del período de intervención, incluido el peso corporal y la altura. El porcentaje de grasa, masa magra y agua corporal se determinó mediante un dispositivo de análisis de impedancia bioeléctrica (BIA) (Tanita BC-545N, Tanita Corporation, Japón). El índice de masa corporal (IMC) se derivó del peso corporal y la altura mediante la ecuación IMC = Peso corporal (kg) / altura^2 (m2).

Los análisis de sangre se realizaron en ayunas, al inicio y al final del estudio.

3.3 Determinaciones de presión arterial

La determinación de la presión arterial sistólica y diastólica en reposo y la frecuencia cardiaca se registraron al comienzo del estudio, la semana 1, la semana 2, la semana 4 y la semana 6 de la intervención utilizando un monitor de tensión validado MC3100 + de BP (HealthStats, Singapur) y siguiendo los estándares propuestos por la Sociedad Europea de Hipertensión (Williams et al., 2018).

El dispositivo proporcionado a cada paciente durante un día en diferentes momentos de la intervención, BPro® (HealthStats, Singapur) es un monitor de presión arterial continuo que registra una vista macroscópica precisa de los patrones de presión arterial durante 24 h. BPro® está validado por BHS (British Hypertension Society), por AAMI (Association for the Advancement of Medical Instrumentation) y por ESH (European Society of Hypertension) (MedTach, 2019). Todas estas determinaciones se realizaron al inicio del estudio, y a 1, 2, 4 y 6 semanas de consumo del producto.

Otros parámetros proporcionados por el dispositivo BPro® son:

- % Dipper: Se calculó de acuerdo con la ecuación (PA sistólica diurna media - PA sistólica nocturna media) x 100% / PA sistólica diurna media). Los valores obtenidos se asocian a un pronóstico diferente. Valores de 10% - 15% se considera el patrón más saludable y valores ≤ 0% perfiles con peor pronóstico.
- Presión sistólica aórtica central (CASP): Determina el promedio del movimiento progresivo de la onda de pulso en un punto considerando la frecuencia cardíaca del punto dividida por 4. Por lo tanto, CASP permite comparar la presión intracardíaca con respecto a la presión periférica (braquial). El rango CASP = 101-136 mmHg y CASP> 117 mmHg indica riesgo de angiopatía.
- Presión de pulso (PP): PP indica flexibilidad de las paredes de los vasos. PP se determina como la diferencia entre la presión sistólica (SP) y la presión diastólica (DP): PP = SP-DP. Una PP> 50 mmHg indica riesgo de aterosclerosis.
- Presión arterial media (MAP): Indica la presión de perfusión media en los diferentes órganos. MAP> 60 mmHg indica un buen patrón de perfusión con bajo riesgo de isquemia y MAP <60 mmHg indica un mayor riesgo de isquemia en órganos periféricos.

RESULTADOS

Capítulo 1

Food & function, 2018, 9(6), 3173–3184.

Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial

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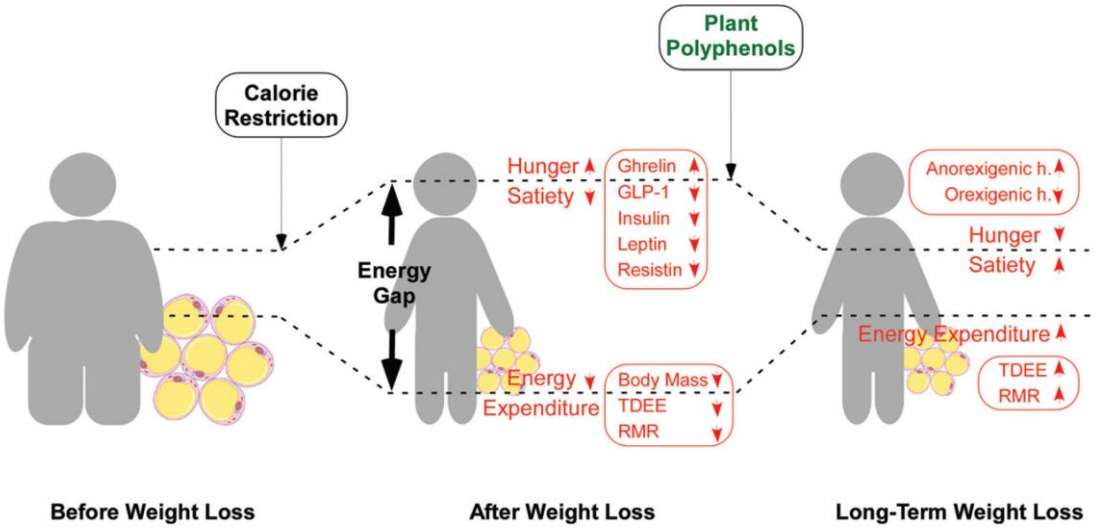

RESUMEN DE LOS RESULTADOS

Valorando los estudios previos y las evidencias científicas con diferentes extractos polifenólicos de plantas obtenidas en el grupo de investigación, a través de ensayos en modelos celulares y animales y demostrando ser coadyuvantes en el abordaje de la obesidad, se evaluó la suplementación de 500 mg de una combinación de los extractos de LC e HS junto con una dieta isocalórica en mujeres con un IMC 25–34,9 kg m^{-2} en un ensayo doble ciego, aleatorizado y controlado por placebo durante 8 semanas. Los resultados mostraron una mejora general de los parámetros antropométricos, disminución de la presión arterial y frecuencia cardíaca y una percepción más positiva en el estado de salud general, evaluado a través del cuestionario SF-36 validado, en el grupo que consumía el suplemento comparado con el control. Asimismo, se obtuvo plasma de muestras de sangre en ayunas al inicio a los 30 y 60 días del estudio. Los resultados fueron significativos en el grupo suplementado, donde se observó que los polifenoles vegetales aumentaron las hormonas anorexigénicas y disminuyeron las hormonas orexigénicas.

Al finalizar el estudio, el grupo que recibió el suplemento mostró una mayor disminución del peso corporal respecto a los grupos controles: −2.08 ± 0.30 kg en el grupo control *vs* −3.48 ± 0.40 ($p < 0.05$) en el grupo LC-HS. El pliegue tricipital presentó una notoria disminución: -1.64 ± 0.29 cm ($p < 0,001$) en el grupo suplementado *vs* −0.15 ± 0.15 en el grupo control. La circunferencia abdominal al nivel del ombligo (AC2) se redujo en 2,57 ± 0,34 cm ($p < 0.01$) en el grupo LC-HS, frente a 0,80 ± 0,55 cm en el grupo control. La circunferencia del brazo también mostró una disminución significativa: −0,22 ± 0,23 cm en el grupo placebo *vs* −0,32 ± 0,08 cm ($p < 0,001$) en el grupo experimental.

La grasa corporal y la circunferencia de la cadera disminuyó con el tiempo en ambos grupos, aunque el efecto fue más pronunciado en el grupo LC-HS. El grupo experimental perdió significativamente más grasa corporal en comparación con el grupo placebo: −0,83 ± 0,08 % ($p < 0.0001$) vs. −0,45 ± 0,07 %. La disminución de circunferencia de la cadera en el grupo experimental fue más pronunciada: −3,50 ± 0,37 cm ($p < 0.0001$) *vs* −1,30 ± 0,28 cm en el grupo placebo. Todos los parámetros antropométricos disminuyeron después del período de intervención de dos meses.

Respecto a los signos vitales, los parámetros de frecuencia cardíaca y presión arterial mostraron diferencias significativas entre el grupo que tomaba el suplemento y el grupo placebo. Los resultados mostraron una disminución significativa en la frecuencia cardíaca

de los sujetos del grupo experimental pasando de 73,3 a 68,6 pulsaciones por minuto (ppm) frente a 71 ppm al inicio y 72 ppm al finalizar el estudio en el grupo control. Se observó una disminución significativa la presión arterial sistólica y diastólica en el grupo experimental tras los 60 días de intervención. La presión arterial sistólica descendió de 117,4 mmHg a menos de 113,9 mmHg y la presión arterial diastólica pasó de 73,4 mmHg a 69,5 mmHg en el grupo experimental, frente a los resultados de presión sistólica del grupo placebo: 114,5mmHg, al inicio *vs* 115,2 mmHg al final. La presión diastólica pasaba en el grupo placebo de 73,73 mmHg al inicio, a 73,73 mmHg al final.

En las EVA analizadas se observaron diferencias estadísticamente significativas entre los dos grupos y en los diferentes momentos de evaluación a los 15, 30, 45 y 60 días. La sensación de apetito y hambre en el grupo que tomaba el suplemento disminuyó significativamente. La sensación promedio de hambre disminuyó de 5,92 (día 15) a 2,58 (día 60) en el grupo LC-HS, mientras que se observó un aumento de 6,18 (día 15) a 6,41 (día 60) en el grupo placebo. Asimismo, los cuestionarios SF-36 confirmaron una marcada mejoría en la percepción subjetiva de la calidad de vida en comparación con el grupo placebo

El análisis en plasma de los marcadores relacionados con la obesidad presentó cambios significativos en el grupo experimental en comparación con el grupo L2. GLP-1 aumentó significativamente en el grupo LC-HS mientras que disminuyó en el grupo placebo. Por el contrario, leptina disminuyó significativamente en el grupo experimental al final de la intervención en comparación con el grupo placebo. La grelina presentó un aumento significativo únicamente en el grupo placebo. Los resultados y cambios observados parecen concordar con la evolución positiva de la patología en términos de reducción de peso, brindando información sobre cambios en la adiposidad, presencia de resistencia a la insulina y saciedad.

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Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial

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Trial design: Plant-derived polyphenols have shown potential to alleviate obesity-related pathologies by a multi-targeted mechanism in animal models and human intervention studies. A dietary supplement based on a combination of *Lippia citriodora* (LC) and *Hibiscus sabdariffa* (HS) polyphenolic extracts was assayed in a double blind and placebo-controlled intervention study with 54 overweight subjects. *Methods*: Blood pressure, body weight, height, triceps, biceps and abdominal skinfold thickness, and arm and abdominal circumferences were taken at the baseline, 30 and 60 days of the intervention period. The validated Visual Analogue Scale used to record hunger and satiety-related sensations was passed at the beginning and at 15, 30, 45 and 60 days of the intervention. Subjective health status was assessed through the validated SF-36 questionnaire at the beginning and end of the study. Finally, plasma from fasting blood samples was obtained at the beginning, 30 and 60 days of the study. *Results*: The results showed an improvement of anthropometric measurements, decreased blood pressure and heart rate and a more positive perception in the overall health status. We also observed that plant polyphenols increased anorexigenic hormones (glucagon-like peptide-1) and decreased orexigenic hormones (ghrelin). *Conclusions*: Based on previous evidence we postulate that AMP-activated protein kinase may have a role in such effects through its capability to modulate energy homeostasis, total daily energy expenditure and lipid management. Although further research may be required, we propose that this polyphenolic combination may be used for weight management by increasing long-term weight loss maintenance through the modulation of appetite biomarkers. This may help to avoid the undesired weight regain typical of calorie restriction diets.

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Introduction

The worldwide prevalence of obesity has nearly tripled between 1975 and 2016, reaching more than 1.9 billion adults, of which over 650 million were obese. Obesity has been classified according to the World Health Organization (WHO) as a pandemic associated with several metabolic disorders, including increased adipose tissue mass, endothelial dysfunction, dyslipidemia, hypertension, atherosclerosis and insulin resistance.[1] Altogether, they constitute a complex pathology known as metabolic syndrome. There seem to be three interconnected reasons for obesity to onset: hyperphagy, caloric imbalance (excess food intake with low energy expenditure) and activation of cell mechanisms to favor excess energy storage as fat.[2–4]

Obesity is generally treated with a combination of approaches, including adopting a dietary intervention focused on calorie restriction, exercise and psychological support in order to achieve the real goals of weight reduction and efficient metabolic control of the pathology.[5,6] However, these strategies are difficult to implement in the overweight population in the long-term, where the patients tend to abandon treatment and regain the lost weight. As a result, obesity as well as the related noncommunicable diseases are common and often go unnoticed and untreated.

In weight stable obesity, energy intake and expenditure are balanced. As a result of an energy-restricted diet, the subsequent energy deficit and weight loss lead to hunger and reduced energy expenditure. This discordance between appetite and energy expenditure is named the "energy gap" and

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leads to a body metabolic adaptation focused to regain the lost weight. Peripheral signals of energy and nutrient deprivation are sent to the hypothalamus, which increases orexigenic hormones such as ghrelin (oriented to induce hunger), while reducing the contribution of the components of the total daily energy expenditure (TDEE), such as the resting metabolic rate (RMR) (burn less calories when inactive), the thermic effect of food (energy expenditure in response to food ingestion) and thermogenesis.[7–10] Then, dietary or lifestyle strategies focused on promoting less hunger and greater satiation should be adopted to minimize weight regain.

Obesity is a complex pathology with many factors involved, including the presence of chronic, low-level inflammatory components, altered energetic metabolism and an oxidative imbalance.[11] Such complexity is one of the reasons why many times it is necessary to include alternative approaches in the weight loss intervention, such as bariatric surgery, pharmacology, and nutritional intervention with dietary supplements or nutraceuticals.[6] All these approaches are part of the "Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–20" developed by the WHO to fight global obesity.

Emerging scientific evidence indicates that dietary supplements may become an alternative for the obesity management and other metabolic disorders. Recent *in vitro* studies suggest that polyphenolic extracts from *Lippia citriodora* (LC) and *Hibiscus sabdariffa* (HS) reduce intracellular lipid accumulation and decrease high glucose-induced oxidative stress and inflammation in adipose tissue through the regulation of different metabolic pathways.[12–17] The accumulated evidence suggests that a multi-targeted mechanism (regulation of signaling and energy-sensitive pathways, oxidative stress and inflammation-related processes, mitochondrial functionality and membrane-dependent processes) is involved rather than the simple modulation of oxidative stress.[17–20] Most of these effects have been corroborated in hyperlipidemic animal models in which the continuous consumption of these polyphenolic extracts prevented fatty liver disease and improved lipid metabolism. The blood pressure lowering effects have also been corroborated in patients with metabolic syndrome.[14]

Furthermore, a nutraceutical product, which combined LC and HS polyphenolic extracts with *in vitro* AMP-activated protein kinase (AMPK)-activating properties,[13,16] was developed to explore its potential use in alleviating obesity-related pathologies. Therefore, this intervention study was developed with the objective of assessing anthropometric and biochemical parameters in connection with satiety measurements in order to explain the observed effects. This was performed using validated questionnaires and analyzing circulating hunger- and satiety-related hormones and peptides.

Materials and methods

Supplement formulation

MetabolAid® (Patent application number P201731147) capsules were kindly provided by Monteloeder SL (Elche, Alicante, Spain). MetabolAid® contains an optimized combination of polyphenolic extracts from *Hibiscus sabdariffa* and *Lippia citriodora*, with a final content of 3.5% anthocyanins and 15% verbascoside (% dry weight, w/w) as measured by high performance liquid chromatography coupled with mass spectrometry detection.[12]

Subjects

Participants were recruited from a Dietetics Shopping Centre in Elche (Alicante, Spain) during 2016. The participant flow diagram is indicated in Fig. 1. Using the inclusion criteria (females with a body mass index, BMI, 25–34.9 kg m^{-2}), 70 prospective participants responded to the initial recruitment. Exclusion criteria included presence of any obesity-related pathology, use of prescribed medication for hypercholesterolemia or hypertension, consumption of antioxidant supplements/drugs, frequent alcohol consumption and being pregnant/lactating. Based on the above criteria, 54 eligible participants, aged 30–75 years old, passed a telephone-based health screening. The assignment of women to the different groups was carried out by the research team using sequentially numbered containers.

Random distribution as measured by BMI gave the following figures: L1-experimental group (n = 25; BMI = 29.84) with 14 overweight and 11 obese, and L2-placebo group (n = 22; BMI = 29.95) with 12 overweight and 10 obese. Since the average BMI of the participants under study was 29.9 and 26 participants were overweight, as a way of simplification, throughout the manuscript the term overweight will be used to refer weight status suffered by the average of the participants.

Prior to participating in the study, subjects were informed by the investigators about the product and the study procedures. All subjects provided a written informed consent before participating that was approved by the Ethics Committee of Miguel Hernández University (reference IB.

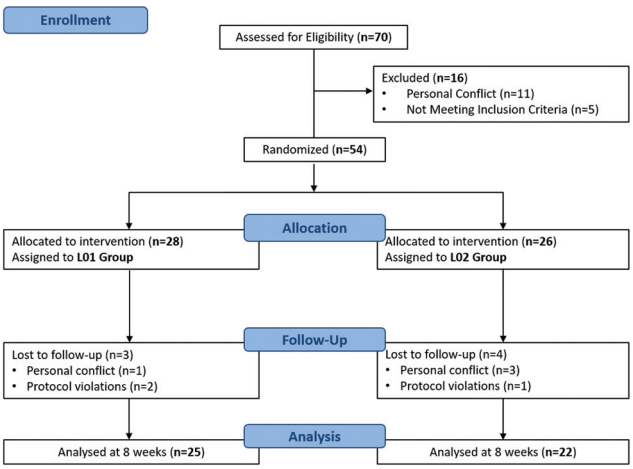

Fig. 1 Participant flow diagram. L1 corresponds to the dietary supplement group and L2 to the placebo group.

ER.01.15). In addition, the study was conducted in accordance to the Helsinki Declaration (1983 version).

Trial design

The study was an 8-week, randomized, double blind (participants and field researchers did not know the type of capsules assigned), placebo-controlled trial. After recruitment, subjects were randomly assigned into the L2-placebo (n = 26) or L1-experimental group (n = 28) in a 1 : 1 ratio using the Excel program (Fig. 1). The L1 group (mean age 51) received one capsule containing 500 mg of LC (35%)–HS (65%) per day. The L2 group (mean age 51) received one capsule daily of placebo (500 mg of crystalline microcellulose). MetabolAid® and placebo capsules were prepared so that they were the same size, odor and color. Trial participants were instructed to take one capsule 20–30 min prior to breakfast every day for two months.

Throughout both prior and post intervention periods, all participants completed a validated semiquantitative food frequency questionnaire and a 24-hour diet recall. Data showed that the trial participants did not follow a balanced, varied and complete diet. To calculate the energy requirements of each participant, the sedentary life of the subjects was taken into account. The dietary patterns were isocaloric diet equal in total energy (2200 kcal per day energy intake), energy density, dietary fiber and macronutrient with normal hydration. Participants were instructed by a qualified dietician to walk for at least 30 minutes per day.

Compliance of the subjects with the ingestion of capsules and diet was assessed at each clinic visit or by telephone interviews every week. Measurements were taken at the beginning and after 30 and 60 days of the study, unless otherwise stated. A total of 3 and 4 participants were excluded from L2 and L1 groups, respectively (Fig. 1).

Intervention

Secondary outcome measures include anthropometric determinations, blood pressure measurements and completion of validated questionnaires (VAS and SF-36). Anthropometric measurements were taken at the baseline, 30 and 60 days of the intervention period, and included body weight, height, triceps, biceps and abdominal skinfold thickness, and arm and abdominal circumferences. Abdominal circumferences (AC) were measured at two different sites: anteriorly midway between the xiphoid process of the sternum and the umbilicus and laterally between the lower end of the rib cage and iliac crests (AC1) and at the umbilicus level (AC2). Body weight and height were measured using a scale with a height-measuring rod. Body mass index (BMI) was derived from body weight and height using the equation BMI = body weight (kg)/height^2 (m). Skinfold thickness and circumferences were measured using a skinfold caliper and metric tape, respectively. Body fat percentage was derived from AC1 and AC2 perimeters using the Weltman equation.[21] In addition, resting systolic and diastolic blood pressures and heart rate were measured at the beginning, 30 and 60 days of the intervention using an Ecomed blood pressure monitor (Medisana Healthcare SL, Barcelona, Spain).

The validated Visual Analogue Scale (VAS) was used to record hunger, satiety, fullness, prospective food consumption, desire to eat something fatty, salty, sweet or savory, and palatability of meals. VAS was passed at rest at the beginning and 15, 30, 45 and 60 days of the intervention. In addition, to assess the individual's subjective health status, patients completed the validated SF-36 questionnaire at the beginning and end of the study (Optum™ SF-36v2® Health Survey). The SF-36 Health Questionnaire is composed of 36 items and assesses both positive and negative health status. The 36 items cover the following scales: general health, physical function, physical role, body pain, vitality, social function, emotional role and mental health. Higher scores of SF-36 correspond to the optimal health perception in each item.

Finally, blood samples were obtained from the antecubital vein in EDTA vacutainers after overnight fasting at the beginning, 30 and 60 days of the study. Plasma was isolated immediately by standard procedures[22] and samples were stored at −80 °C until the moment of measurement.

Peptides and circulating hormones

Primary outcome measures include satiety-related peptides. Plasma was extracted from subjects randomly assigned to the Supplement (L1) or Placebo (L2) group. The Immunoassay kit ProcartaPlex® of the eBioscience division of Affymetrix (multiple immunoassay for convenience and Mix & Match panels) was used to determine the levels of FGF-23, glucagon-like peptide-1 (GLP-1), ghrelin, insulin, leptin, C-peptide, PYY and resistin. To measure the levels of the abovementioned appetite and satiety markers the Luminex analyzer (Thermo Fisher Scientific) was used.

The changes in the different parameters and hormones related to the circulating satiety-related peptides at the beginning of the study and at 60 days were analyzed. The statistical study was carried out with the help of the software program Graphpad Prism, evaluating the evolution of the parameters studied and comparing the value at the beginning of the study with those obtained at the end of the study for the same individual.

Theoretical estimation of sample size

As we were interested as a primary outcome in detecting changes in circulating satiety-related peptides within each group after 2 months following the polyphenol extract intake, we considered an effect size (d = 1.53) with a two-sided 5% significance level and a power of 80%. This allowed obtaining a minimal sample size of around 8 participants per group.

Stopping guidelines

The only rule considered to stop the study was related with the valid number of observations needed to obtain statistically significant results. In addition, a particular participant could be excluded from the study, once included in the trial, for the following reasons: protocol violations, personal reasons and pregnancy.

Statistical analysis

Statistical analysis was carried out using the Graphpad Prism software. Results were expressed as means ± standard deviation (SD). Anthropometric and vital signs (inter-group), VAS and SF-36 data were analyzed using Student's unpaired *t*-test. By contrast, anthropometric and vital signs data (intra-group statistical) and satiety peptides and hormones were analyzed using Student's paired *t*-test. Outcome variables were assessed for conformance to the normal distribution and transformed if necessary by a KS-test. Reported *p*-values were two-sided and values of 0.05 or less (0.01, 0.001 and 0.0001) were considered statistically significant for comparisons to the baseline (beginning of the study) and between groups (L1 *vs.* L2) at the same time point of the study. The results of the SF-36 questionnaire were analyzed using the Health Outcomes Scoring Software.

Results

Anthropometric and vital parameters

Baseline characteristics of the two groups were well matched and no significant differences were detected at the beginning of the study in the Supplement (L1) *vs.* Placebo (L2) groups. Participants were recruited from February to March 2016 and the intervention occurred during April–May 2016, with a follow-up at the end of April 2016. The results indicated an overall improvement of the anthropometric data in the L1 group compared to L2 after two months, especially regarding body weight, triceps skinfold thickness, body fat and hip circumference. All parameters decreased after the two-month intervention period. The L1 group presented a higher decrease of triceps skinfold thickness compared to the control group (−0.15 ± 0.15 cm in L2 *vs.* −1.64 ± 0.29 cm in L1, $p < 0.001$) (Table 2). Hip circumference decreased over time in both groups, although the effect was more pronounced in the L1 group (−3.50 ± 0.37 cm) compared to the placebo group (−1.30 ± 0.28 cm, $p < 0.0001$). Consistent with this data, AC2 was reduced by 2.57 ± 0.34 cm in the L1 group, as opposed to only 0.80 ± 0.55 cm in the L2 group, $p < 0.01$. Arm circumference also exhibited a significant decrease compared to the placebo group (−0.22 ± 0.23 cm in L2 *vs.* −0.32 ± 0.08 cm in L1, $p < 0.001$). Body fat decreased over time in both groups but the experimental group (L1) lost significantly more body fat (−0.83 ± 0.08%) compared to the placebo group (−0.45 ± 0.07%) (Table 2).

In addition, significant differences were detected regarding heart rate and blood pressure parameters between the group taking the supplement and the placebo group. A significant decrease was observed in the heart rate of subjects in the L1 group after 60 days, evolving from 73.3 to 68.6 beats per minute (bpm) (Table 1). Also, systolic and diastolic blood pressures significantly decreased in the L1 group after 60 days. Specifically, systolic blood pressured dropped from 117.4 mmHg to below 113.9 mmHg, while diastolic pressure went from 73.4 mmHg to below 69.5 mmHg (Table 1).

Appetite assessment

The scores for hunger, satiety, fullness and prospective food consumption are shown in Fig. 2 for both groups. The data show statistically significant differences between the two groups and at the different time points (15, 30, 45 and 60 days). In the Visual Analogue Scales analysed, statistically significant differences were seen with regard to feelings of appetite, hunger and satiation in the group having the supplement compared to placebo after 1 month, these differences being greater after 45 days and 2 months of product consumption. According to the obtained scores, the consumption of the poly-

Table 1 Anthropometric measurements and vital signs at the baseline and after one and two months. Intra-group statistical analysis at endpoint compared to the baseline expressed as means ± SD. Significance was established at: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

	Placebo (L2)			Supplement (L1)		
Anthropometric parameters	Baseline	Month 1	Month 2	Baseline	Month 1	Month 2
Body weight (kg)	75.64 ± 12.92	74.20 ± 12.62****	73.56 ± 12.57****	75.26 ± 9.06	72.80± 9.45****	71.78 ± 9.06****
Body mass index (kg m^{-2})	29.78 ± 4.19	29.15 ± 4.08****	28.95 ± 4.01****	29.60 ± 3.40	28.60 ± 3.52****	28.26 ± 3.46****
Arm circumference (cm)	31.20 ± 3.80	31.23 ± 3.80	30.98 ± 4.10	30.58 ± 1.67	30.47 ± 1.83	30.26 ± 1.76****
AC1 (cm)	94.02 ± 13.03	92.84 ± 12.86***	92.05 ± 13.16****	90.96 ± 9.03	88.96 ± 9.18****	88.01 ± 8.90****
AC2 (cm)	100.7 ± 14.01	100.5 ± 14.21	99.90 ± 14.54**	96.42 ± 7.93	94.84 ± 7.87****	93.85 ± 7.95****
Hip circumference (cm)	108.8 ± 8.73	108.15 ± 8.55**	107.5 ± 8.48***	110.4 ± 7.23	108.2 ± 7.56****	106.9 ± 7.48****
Triceps skinfold thickness (mm)	43.25 ± 9.28	43.15 ± 9.34	43.10 ± 9.42	41.62 ± 8.18	40.96 ± 8–08**	39.98 ± 7.97****
Biceps skinfold thickness (mm)	41.53 ± 14.46	41.25 ± 14.35*	41.09 ± 14.35*	38.33 ± 10.63	37.61 ± 10.87*	36.87 ± 10.52****
Abdominal skinfold thickness (mm)	35.72 ± 11.15	35.39 ± 10.68	34.39 ± 10.68	41.45 ± 12.89	39.72 ± 12.95***	38.60 ± 13.08***
% Body fat	44.98 ± 2.71	44.69 ± 2.65***	44.53 ± 2.64****	44.66 ± 2.04	44.10 ± 2.10****	43.83 ± 2.06****
Vital signs						
Heart rate (bpm)	71.41 ± 8.89	71.95 ± 8.85	72.09 ± 9.24	73.32 ± 9.70	70.84 ± 7.54*	68.64 ± 7.07****
Systolic pressure (mm Hg)	114.5 ± 23.26	115.1 ± 23.55*	115.2 ± 23.13*	117.4 ± 12.13	115.1 ± 12.82**	113.9 ± 12.5****
Diastolic pressure (mm Hg)	73.73 ± 10.57	74.05 ± 11.50	73.73 ± 10.70	73.40 ± 5.00	71.28 ± 5.89****	69.48 ± 6.62****

Abbreviations used: bpm, beats per minute.

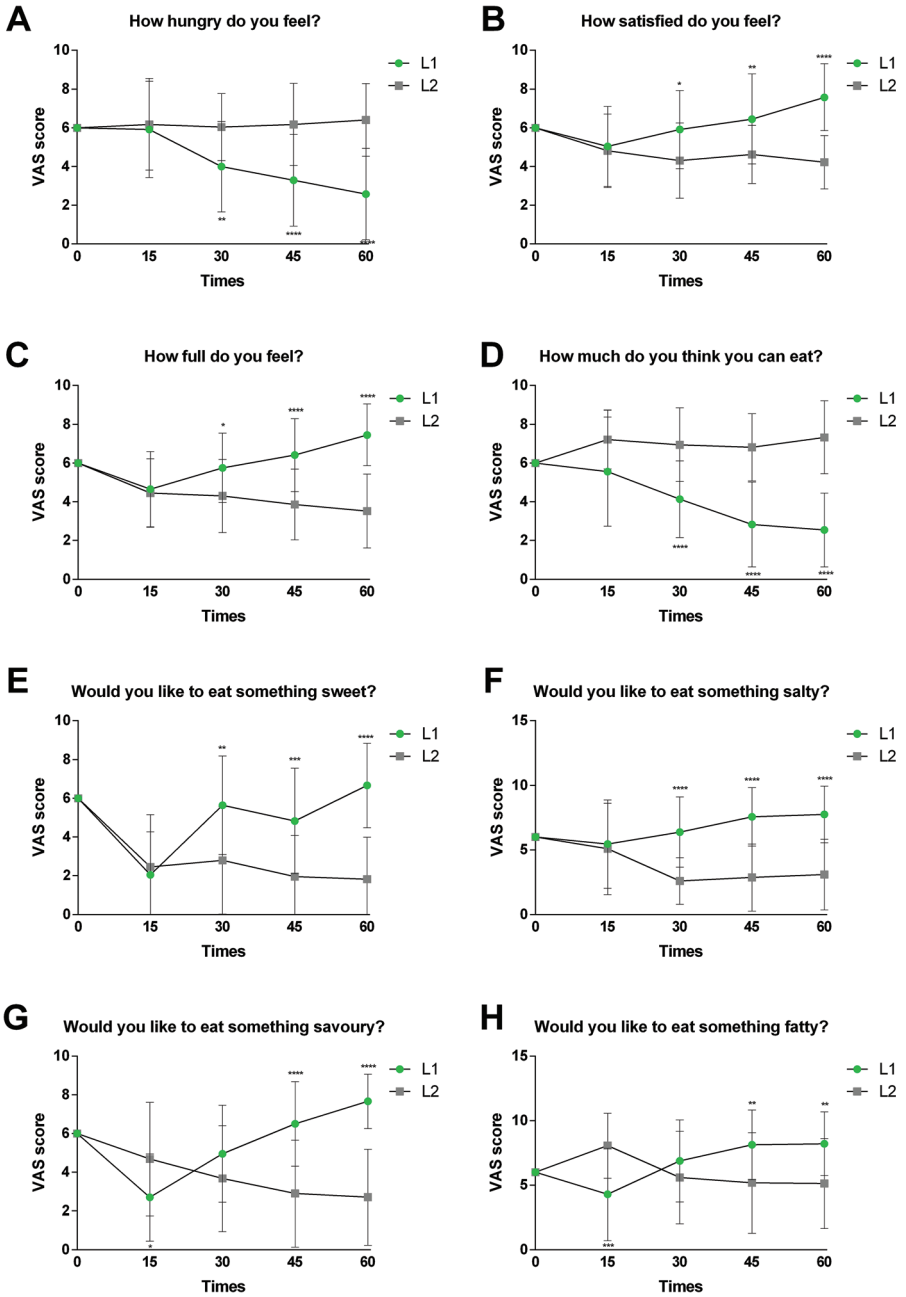

Fig. 2 Subjective scores of hunger-related sensations in the dietary supplement group (L1, n = 25) and the placebo group (L2, n = 22) after 15, 30, 45 and 60 days, expressed as means ± SD. Data represent scores of each item compared to placebo. Statistically significant differences were established at: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Item scores: 1. How hungry do you feel?: 0 "I am not hungry at all" – 10 "I have never been more hungry". 2. How satisfied do you feel?: 0 "I am completely empty" – 10 "I cannot eat another bite". 3. How full do you feel?: 0 "Not at all full" – 10 "Totally full". 4. How much do you think you can eat?: 0 "Nothing at all" – 10 "A lot". 5. Would you like to eat something sweet?: 0 "Yes, very much" – 10 "No, not at all". 6. Would you like to eat something salty?: 0 "Yes, very much" – 10 "No, not at all". 7. Would you like to eat something savoury?: 0 "Yes, very much" – 10 "No, not at all". 8. Would you like to eat something fatty?: 0 "Yes, very much" – 10 "No, not at all".

phenolic supplement significantly decreased the feeling of hunger consistently throughout the two months of intervention (Fig. 2A). The average hunger feeling decreased from 5.92 (day 15) to 2.58 (day 60) in the L1 group, whereas an increase from 6.18 (day 15) to 6.41 (day 60) was observed for the placebo group.

Furthermore, satiety increased from 5.04 (day 15) to 7.58 (day 60) in the intervention group, while it decreased from 4.82 (day 15) to 4.22 (day 60) in the placebo group (Fig. 2B). In line with this result, an increase was also observed in the answer to the question "How full do you feel?" in the intervention group (from 4.65 to 7.46 at day 60) compared to the

placebo group, where it modestly decreased (from 4.45 to 3.52 at day 60) (Fig. 2C).

After two months of intervention, a much lower score was also confirmed in response to question no. 4 "How much do you think you can eat?" in group L1 (from 5.57 to 2.54 at day 60) compared to the placebo group (from 7.22 to 7.33 at the 60) (Fig. 2D). Finally, participants in the control group showed greater preferences for sweet, salty, savoury and fatty foods, questions 5 to 8, compared to those consuming the polyphenolic supplement (Fig. 2E–H).

SF-36 questionnaire assessment

After evaluating the SF-36 questionnaires at the end of intervention, the L1 group confirmed a marked improvement in the subjective perception of the quality of life compared to the placebo group. Specifically, significantly higher scores were observed for various questions, such as "General health" in the L1 group (scoring from 40.02 to 47.5) compared to the placebo group (scoring from 37.86 to 37.97) (Fig. 3). In the same context, similar significant changes were observed for "Mental health" parameters, passing from 41.34 to 47.83 at the end of intervention in the L1 group compared to the placebo group (scoring from 47.06 to 44.45). Finally, participants in the group consuming the polyphenolic supplement significantly improved in the psychological (ranging from 46.14 to 50.27 at the end of intervention) and pain parameters (scoring from 40.81 to 47.6 at the end of intervention) compared to the placebo group (Fig. 3).

Fig. 3 Subjective scores for the SF-36 questionnaire in the dietary supplement group (L1, n = 25) and placebo group (L2, n = 22), at the beginning and two months of intervention. Data are represented using Health Outcomes Scoring Software and expressed as means ± SD. Statistically significant differences were established between the two groups after one or two months after intra-group analysis as: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Physical Health Score (PCS), Mental Health score (MCS), Physical Functioning (PF), Role Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Functioning (SF), Role Emotional (RE), Mental Health (MH). The higher scores of SF-36 correspond to the best state of health perception in each particular item. The 100 value corresponds to the higher score and the 50 value is the norm-based score (NBS).

Determination of circulating appetite related peptides and hormones

The data presented in Fig. 2 and 3 are subjective appreciations of satiety and health status. These positive sensations perceived by the subjects of the L1 group coincided with an improvement in anthropometric and vital parameters (Tables 1 and 2), although complementary analysis is required to confirm this hypothesis. To this end, the measurement of specific circulating peptides and obesity-related biomarkers can provide additional information related to the evolution of the pathology, suggesting at the same time putative mechanisms of action for the polyphenolic extracts assayed in this trial. In this vein, eight obesity-related markers were analyzed in plasma (Table 3). These peptides provide information regarding changes in adiposity, presence of insulin resistance and satiety.

It must be mentioned that certain appetite-related gut peptides (such as GLP-1, PYY and ghrelin) are secreted by the digestive track in the presence of nutrients. Since the plasma samples were obtained under fasting conditions, the levels determined for these particular gut hormones corresponded to basal values. Initially, baseline characteristics of the two groups were well matched and no significant differences were found (Table 3). Fibroblast growth factor 23 (FGF-23), a protein biomarker related to obesity and insulin resistance, showed a similar decrease in both groups, suggesting that the isocaloric diet was the most relevant factor in the reduction of this protein. Most of the other studied gut peptides and adipokines presented significant changes in the L1 group compared to the L2 group. The incretin GLP-1 increased significantly in the L1 group while it decreased in the L2 group. On the other hand, leptin significantly decreased in the L1 group at the end of intervention compared to the placebo group, where no significant changes were observed. In addition, resistin significantly decreased in the group consuming the dietary supplement, while no changes were observed in the L2 group. Finally, ghrelin presented a significant increase only in the placebo group, with no changes in the group that consumed the dietary supplement. In general, all the changes observed seem to give information that matches the positive evolution of the pathology in terms of weight reduction, blood pressure normalization, satiety control and optimal health status appreciation (Tables 1, 2 and Fig. 2, 3).

Adverse events

The consumption of the polyphenolic dietary supplement was well-tolerated. No adverse side effects were documented during the intervention period.

Table 2 Differences with respect to baseline (beginning of the intervention) in anthropometric parameters and vital signs after one and two months expressed as means ± SE. Inter-group statistical analysis was performed at each period compared to the baseline. Significance was established at: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

	Differences after the first month		Differences after two months	
Anthropometric parameters	Placebo (L2)	Supplement (L1)	Placebo (L2)	Supplement (L1)
Body weight (kg)	−1.44 ± 0.27	−2.46 ± 0.28*	−2.08 ± 0.30	−3.48 ± 0.40*
Body mass index (kg m^{-2})	−0.63 ± 0.14	−1.00 ± 0.15*	−0.83 ± 0.12	−1.37 ± 0.16*
Arm circumference (cm)	0.03 ± 0.03	−0.11 ± 0.06	−0.22 ± 0.23	−0.32 ± 0.08**
AC1 (cm)	1.18 ± 0.29	−2.00 ± 0.27*	−1.97 ± 0.37	−2.95 ± 0.36
AC2 (cm)	0.20 ± 0.54	−1.58 ± 0.25**	−0.80 ± 0.55	−2.57 ± 0.34**
Hip circumference (cm)	0.65 ± 0.23	−2.20 ± 0.32***	−1.30 ± 0.28	−3.50 ± 0.37****
Triceps skinfold thickness (mm)	−0.10 ± 0.16	−0.66 ± 0.18*	−0.15 ± 0.15	−1.64 ± 0.29****
Biceps skinfold thickness (mm)	−0.28 ± 0.14	−0.62 ± 0.22	−0.44 ± 0.19	−1.46 ± 0.29**
Abdominal skinfold thickness (mm)	−0.33 ± 0.36	−1.73 ± 0.37*	−1.33 ± 0.29	−2.84 ± 0.53*
% Body fat	0.29 ± 0.06	−0.56 ± 0.06**	−0.45 ± 0.07	−0.83 ± 0.08***
Vital signs				
Heart rate (bpm)	0.54 ± 0.28	−2.48 ± 0.89****	0.68 ± 0.36	−4.68 ± 0.96****
Systolic pressure (mm Hg)	0.60 ± 0.28	−2.30 ± 0.64****	0.70 ± 0.30	−3.50 ± 0.61****
Diastolic pressure (mm Hg)	0.32 ± 0.43	−2.12 ± 0.39****	0.00 ± 0.50	−3.92 ± 0.63****

Abbreviations used: bpm, beats per minute.

Table 3 Levels of obesity and appetite-related gut peptides and hormones at the beginning (baseline) compared to the end (month 2) of intervention, expressed as means ± SD. Significance was established at: *$p < 0.05$. Statistical analysis was performed at two months compared to the baseline for each group

	Placebo (L2)		Supplement (L1)	
	Baseline	Month 2	Baseline	Month 2
FGF-23 (pg mL^{-1})	5.97 ± 0.38	5.67 ± 0.09*	5.88 ± 0,25	5.72 ± 0.18*
GLP-1 (pg mL^{-1})	5.66 ± 1.27	4.228 ± 2.03*	5.22 ± 1.43	6.82 ± 1.78*
Ghrelin (pg mL^{-1})	30.64 ± 0.64	33.74 ± 3.48*	32.70 ± 4.82	32.89 ± 3.23
Insulin (pg mL^{-1})	4.13 ± 1.56	3.674 ± 2,03	4.57 ± 0.78	4.74 ± 0.55
Leptin (pg mL^{-1})	9669 ± 6617	5237 ± 4886	9772 ± 4455	4311 ± 2304*
C-Peptide (pg mL^{-1})	35.46 ± 6.98	33.53 ± 10.19	28.28 ± 5.11	30.01 ± 5.66
PYY (pg mL^{-1})	7.84 ± 4.15	5.82 ± 5.01	9.80 ± 0.31	9.81 ± 0.24
Resistin (pg mL^{-1})	15 593 ± 6718	10 799 ± 6624	18 101 ± 6274	12 049 ± 4022*

Discussion

Trial limitations

The limitations that the study presents stem from the fasting conditions under which the plasma samples were obtained. The obtained values for gut hormones corresponded to basal levels. Postprandial complementary analysis needs to be performed in subsequent interventions for these particular hormones.

External validity and applicability of the trial findings

The female participants in this study were chosen from a Dietetics Shopping Centre in a southeastern Spanish city. Nevertheless, we can state that the results are applicable to participants in urban environments, taking into account the fact that lifestyles and eating habits are similar in most Spanish cities. On the other hand, other factors that can influence the study variables were not taken into account, as this study is centred exclusively on the consumption of particular polyphenol extracts and their effects on circulating satiety peptides as primary variables, and anthropometric and functional parameters used as secondary variables. Regarding the studied polyphenol extracts, the results would be valid under the aforementioned terms for the brand analysed.

Discussion of results

Despite the use of calorie restrictions through diet or increased caloric expenditure through exercise, many individuals still present enormous difficulties in losing weight and/or improving metabolic parameters. Considering the accumulated evidence on the effects of plant-derived polyphenols on obesity models, we proposed that some specifically designed nutraceuticals based on polyphenolic extracts may offer additional support for these particular patients in the management of obesity.[17,20,23]

Over the years, scientific evidence has shown that polyphenolic extracts from plant sources may be valuable tools to treat obesity.[24] In this regard, our research at the pre-clinical and clinical level has shown the efficiency of the polyphenolic extracts from LC and HS.[12–16] In the present intervention

study and in the context of an isocaloric diet, we have observed that the group consuming the dietary supplement containing both polyphenolic extracts significantly reduced body weight, with a similar reduction in the body fat component compared with the placebo (Tables 1 and 2). This weight reduction was accompanied by the normalization of blood pressure and heart rate, which may be assigned to the reported capacity of the polyphenolic extracts from HS to reduce blood pressure and inflammatory plasma markers in humans.[14,25] Because of the isocaloric diet, the placebo group also presented a similar tendency in the anthropometric parameters, but the differences at the end of intervention were statistically less prominent. Altogether, these data suggest that diet is an instrumental tool in weight reduction, but the dietary supplement seems to regulate other metabolic aspects further than those activated by calorie restriction and correct certain altered parameters.

The most intriguing effect observed by the participants who consumed the dietary supplement was a modulation of satiety. This parameter is instrumental in weight management, because it is the most effective parameter to achieve a consistent long-term weight loss and to stop body fat accumulation. Under normal circumstances, caloric restriction to reduce weight causes anxiety in the consumers, who often revert to compulsive intake, leading to weight regain.[26] This situation decompensates diet balance and compromises weight reduction protocols. To this end, we have inquired trial participants about satiety using the validated VAS questionnaire. As a result, individuals consuming the dietary supplement reported significantly positive satiety scores compared to the placebo group (Fig. 2) and an improved perception of the quality of life through the SF-36 questionnaire (Fig. 3).

In short, energy balance is controlled by different components that are in charge of regulating appetite at the start and end of meals. These respond primarily to gastrointestinal signals that control satiety during meals, and to hormones secreted by the adipose tissue and hypothalamus that contribute to a more balanced feeding behavior in the long term.[2] Consequently, a selection of peptides and hormones that regulate hunger and satiety and adipokines was analyzed, which are generally altered in overweight individuals. These include: FGF-23, GLP-1, ghrelin, insulin, leptin, C-peptide, PYY and resistin. It is important to note that satiety is a complex mechanism, mediated by a variety of orexigenic and anorexigenic signals that are integrated in the hypothalamus.[27] Therefore, the analysis of only one or a few peptides may not be sufficient to understand satiety regulation. Furthermore, for a diet-induced weight loss, satiety is not the only factor involved, as fat reduction and metabolic normalization also play a role in regulating these peptides due to the variation of adiposity. Therefore, in this particular study, the changes observed in the analyzed gut hormones and obesity-related biomarkers must be taken in a more generalized, simplified context where satiety could be a supporting component for weight reduction, but where other parameters may also be involved.

In our study, blood samples were obtained under fasting conditions. Therefore, the biomarkers were analyzed in a "hunger" state, and the results must be interpreted accordingly (Table 3). For example, GLP-1 is an anorexigenic incretin produced by the intestinal L-cells that stimulates insulin secretion while also inducing satiety.[28] The results shown in Table 3 indicated that the group taking the polyphenolic dietary supplement presented higher levels of this cytokine after two months compared to the placebo group, most likely contributing to the satiety effect perceived by the consumers (Fig. 2). The other gastrointestinal peptide (PYY) did not show significant differences in any of the groups by comparing the beginning and the end of the intervention. On the other hand, the orexigenic hormone ghrelin is secreted by the stomach and induces appetite.[24] This cytokine was significantly increased only in the placebo group, which could contribute to increased food intake and stimulate appetite after calorie restriction. In normal weight individuals, plasma ghrelin concentrations rise during fasting and drop with meal ingestion proportional to the calorie content. However overweight individuals do not display the same suppression of ghrelin in response to calorie ingestion and weight loss led to increased plasma level in overweight individuals as a compensatory response.[29–31] Other studies have corroborated that the intravenous administration of ghrelin increases both appetite and food intake.[32] Our results of higher ghrelin levels at the end of the study for the placebo group clearly indicate a higher hunger sensation for this group compared to the group having the supplement, which could explain, at least in part, the higher satiety results observed in the hunger-related sensation analysis for the intervention group compared to the placebo group (Fig. 2).

Leptin can be considered as a lipostatic hormone mainly secreted by adipose tissue. Leptin exerts a variety of effects on target tissues, adapting specific functions to the amount of fat present in the organism.[33] In addition, leptin decreases appetite by stimulating the secretion of anorexigenic peptides by the hypothalamus, increasing basal metabolism, reducing lipogenesis and augmenting lipolysis to produce energy.[2] In the present study, the placebo group did not show significant changes in leptin values, while the group consuming the dietary supplement displayed a significant reduction in plasmatic leptin levels. Although high leptin levels induce satiety, the loss of body weight (mainly adipose tissue) observed by the participants may be accompanied by a decrease in leptin secretion by adipocytes. These results about leptin may be difficult to reconcile on the basis of satiety control only and should be interpreted in relation to the complex variety of functions exerted by this peptide.[33] In fact, it is well established that many overweight individuals show leptin resistance i.e., higher levels of leptin in blood are necessary to exert its effect on target tissues. In addition, we have also observed a decrease of leptin by plant-derived polyphenols in correlation to decreased triglyceride content in a hypertrophic adipocyte model.[12] We postulate that the normalization of leptin levels achieved by the weight reduction strategy could contribute to modulate the satiety sensation and the function of other leptin

targets more efficiently. However, as previously mentioned, a post-prandial analysis would have provided more accurate results of this adipokine. In this context, resistin is generally increased in overweight individuals, giving rise to insulin resistance.[34] The expression level of this adipokine was significantly lowered in the group consuming the polyphenolic supplement, but not in the placebo group. This could favor leptin's effect by controlling satiety as well as other functions. Moreover, FGF-23 was originally considered to be implicated in bone metabolism, but recently it has been shown to also play a role in obesity and cardiovascular diseases.[35] In the present study, FGF-23 was significantly decreased in both groups, suggesting that the balanced diet could play a more prominent role than the polyphenolic extract in the expression of this growth factor. Finally, insulin and C-peptide did not show significant changes in both groups.

In our intervention study, overweight participants from both groups followed an isocaloric diet for two months; so, in response to a first period of weight loss, the calorie restriction should have led to a compensatory adaptation phenomenon governed by the hypothalamus, *i.e.* "energy gap", by increasing hunger and reducing energy expenditure, as reported (Fig. 4).[7–9] According to this concept, upon dietary restriction, individuals losing weight increase orexigenic hormones and peptides (ghrelin)[36,37] and decrease anorexigenic hormones (GLP-1)[38] in order to increase hunger and to reduce energy expenditure which triggers weight regain. In response to diet, leptin and resistin also decrease, due presumably to reduced adiposity, leading to increased hunger and subsequently to weight regain.

Interestingly, the results of our trial can be explained on the basis of counteracting the "energy gap" phenomenon.[7] In our trial these two hormones, leptin and resistin, decreased in both groups, most probably in correlation to decreased adiposity. Contrary to the expected behavior upon the calorie restriction situation, in the group having the dietary supplement for two months, we could observe an increase in anorexigenic hormones (GLP-1) and a normalization of orexigenic hormones (ghrelin). Therefore, we propose that the consumption of the dietary supplement counteracted the effects associated with dietary restriction, promoting anorexigenic hormones and decreasing orexigenic hormones and restoring the balance between hunger and energy (Fig. 4). In a similar way, appropriate dietary interventions that reduce hunger (increasing dietary protein and fiber) and increase energy expenditure (exercise) are proposed for a long-term weight loss maintenance, narrowing the energy gap.[7]

Evidence derived from our research and those of others have proposed the activation of the energy sensor AMPK by polyphenols as a therapeutic target for obesity,[18,19,39] through the modulation of lipid accumulation,[17–19] the inhibition of oxidative stress and the blockage of inflammatory adipokine secretion.[12,25,40] According to these pieces of evidence, we hypothesize that the polyphenolic extracts in the combination could help to reduce adipose fat mass, by modulating AMPK activity in these tissues, with the concomitant normalization of the secreted levels of leptin and resistin. However, further research may be required to confirm the role of the combination of polyphenolic extracts in gastrointestinal peptide secretion and to prove the AMPK-activating properties *in vivo*.

We also postulate that the dietary supplement, besides regulating satiety, might also modulate some components of the TDEE, probably through its capability to modulate AMPK in

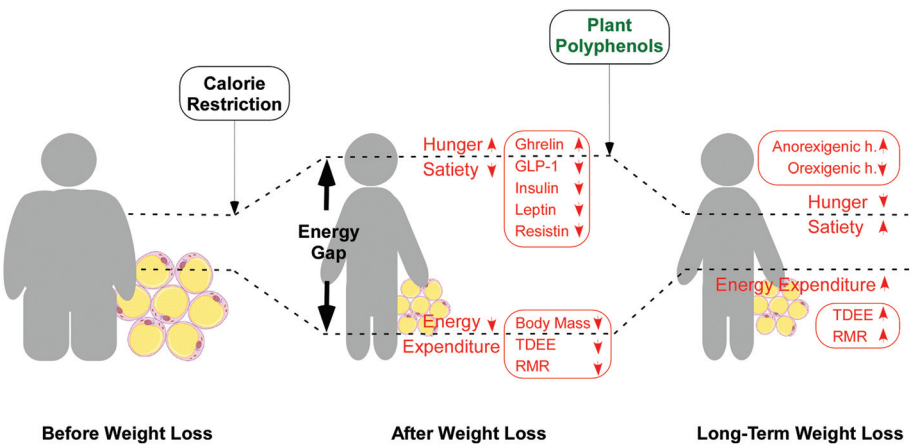

Fig. 4 Modulation of the energy gap by diet restriction and polyphenol supplementation. In response to calorie restriction and the subsequent weight loss, discordance between nutrient ingestion and energy expenditure appears, *i.e.* the "energy gap". This metabolic situation leads to the brain triggering a compensatory hormonal response that reflects both appetite-related hormones and peptide changes and energy expenditure adaptation. On one side, orexigenic hormones are enhanced resulting in increased hunger. On the expenditure side, weight loss and reduced adiposity cause a metabolic adaptation consisting of the reduction of total daily energy expenditure (TDEE), most probably due to a reduced resting metabolic rate (RMR) and decreased thermic effect of food. The increased hunger and reduced energy expenditure give rise to prompt weight regain. We propose that the consumption of some plant polyphenols counteracts these effects by promoting anorexigenic hormones and decreasing orexigenic hormones and restores the balance between hunger and energy. This may enable long-term weight loss maintenance by narrowing the energy gap upon calorie restriction (adapted from Melby, Paris, Foright, & Peth, 2017).

different tissues. Indeed, it has also been shown that the activation of AMPK has pleiotropic effects in multiple tissues.[41,42] AMPK activation in most tissues (liver, adipose tissue and muscle) promotes increased fatty acid oxidation, glucose uptake and glycolysis as well as the inhibition of fatty acid, cholesterol and glycogen synthesis. Paradoxically, the activation of hypothalamic AMPK led to increased food intake.[41,43] Whether metabolites of polyphenols derived from the dietary supplement can pass through the haematoencephalic barrier and reach AMPK directly at the hypothalamus still needs to be verified.

With regard to the putative metabolites capable of undertaking these effects, previous evidence lead us to propose some phenylpropanoids from *L. citriodora* and flavonol derivatives from *H. sabdariffa*.[44–47] In any case, these hypotheses need to be verified in further animal trials specifically designed for such measurements.

In conclusion, the consumption of 500 mg day^{-1} of the combination of *Hibiscus* and *Lippia* polyphenolic extracts in the context of an isocaloric diet for two months in overweight subjects confirmed significant reductions of body fat, blood pressure and heart rate and a more positive perception in their overall health status compared to the placebo group. In addition, trial participants exhibited a decrease in hunger and appetite and a lower attraction for fatty, sweet and salty food compared to the placebo group, concomitantly with a reduction in circulating resistin and normalization of leptin expression, while regulating ghrelin and GLP-1 levels (which were higher and lower in the placebo group, respectively). Altogether, the results of the present study demonstrate that polyphenols are capable of modulating adipohormones and gut peptide expression and control satiety and hunger states, with AMPK modulation in different tissues as a putative target, although the underlying mechanism is still unknown.

Conflicts of interest

This study was partially funded by Monteloeder S. L. Monteloeder was involved in the design of the study protocol and provided the test product samples. Employees of the sponsor were not involved in data analysis. The manuscript was prepared by Maria Herranz, Enrique Roche and Vicente Micol. Monteloeder was permitted to review the manuscript and suggest changes, but the final decision on content was exclusively retained by the corresponding author. Dr Vicente Micol is the guarantor for this article and takes responsibility for the integrity of the work as a whole. NC works for Monteloeder S. L.

Abbreviations

LC	*Lippia citriodora*
HS	*Hibiscus sabdariffa*
AMPK	AMP-activated protein kinase
TDEE	Total daily energy expenditure
RMR	Resting metabolic rate
AC	Abdominal circumferences
BMI	Body mass index
VAS	Validated visual analogue scale
PYY	Gastrointestinal peptide
FGF-23	Fibroblast growth factor 23
GLP-1	Glucagon-like peptide-1

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References

1 M. Luna-Luna, A. Medina-Urrutia, G. Vargas-Alarcon, F. Coss-Rovirosa, J. Vargas-Barron and O. Perez-Mendez, Adipose Tissue in Metabolic Syndrome: Onset and Progression of Atherosclerosis, *Arch. Med. Res.*, 2015, **46**, 392–407.
2 K. Timper and J. C. Bruning, Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity, *Dis. Models & Mech.*, 2017, **10**, 679–689.
3 C. Drenowatz, Reciprocal Compensation to Changes in Dietary Intake and Energy Expenditure within the Concept of Energy Balance, *Adv. Nutr.*, 2015, **6**, 592–599.
4 A. Vegiopoulos, M. Rohm and S. Herzig, Adipose tissue: between the extremes, *EMBO J.*, 2017, **36**, 1999–2017.
5 G. Asghari, P. Mirmiran, E. Yuzbashian and F. Azizi, A systematic review of diet quality indices in relation to obesity, *Br. J. Nutr.*, 2017, **117**, 1055–1065.
6 J. Vairavamurthy, L. J. Cheskin, D. L. Kraitchman, A. Arepally and C. R. Weiss, Current and cutting-edge interventions for the treatment of obese patients, *Eur. J. Radiol.*, 2017, **93**, 134–142.
7 C. L. Melby, H. L. Paris, R. M. Foright and J. Peth, Attenuating the Biologic Drive for Weight Regain Following Weight Loss: Must What Goes Down Always Go Back Up?, *Nutrients*, 2017, **9**, DOI: 10.3390/nu9050468.
8 Z. J. Zhao, Y. A. Liu, J. Y. Xing, M. L. Zhang, X. Y. Ni and J. Cao, The role of leptin in striped hamsters subjected to food restriction and refeeding, *Dongwuxue Yanjiu*, 2014, **35**, 262–271.

9 P. Sumithran and J. Proietto, The defence of body weight: a physiological basis for weight regain after weight loss, *Clin. Sci.*, 2013, **124**, 231–241.
10 M. Rosenbaum, J. Hirsch, D. A. Gallagher and R. L. Leibel, Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight, *Am. J. Clin. Nutr.*, 2008, **88**, 906–912.
11 J. J. Reho and K. Rahmouni, Oxidative and inflammatory signals in obesity-associated vascular abnormalities, *Clin. Sci.*, 2017, **131**, 1689–1700.
12 M. Herranz-Lopez, S. Fernandez-Arroyo, A. Perez-Sanchez, E. Barrajon-Catalan, R. Beltran-Debon, J. A. Menendez, C. Alonso-Villaverde, A. Segura-Carretero, J. Joven and V. Micol, Synergism of plant-derived polyphenols in adipogenesis: perspectives and implications, *Phytomedicine*, 2012, **19**, 253–261.
13 J. Joven, E. Espinel, A. Rull, G. Aragones, E. Rodriguez-Gallego, J. Camps, V. Micol, M. Herranz-Lopez, J. A. Menendez, I. Borras, A. Segura-Carretero, C. Alonso-Villaverde and R. Beltran-Debon, Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice, *Biochim. Biophys. Acta*, 2012, **1820**, 894–899.
14 J. Joven, I. March, E. Espinel, S. Fernandez-Arroyo, E. Rodriguez-Gallego, G. Aragones, R. Beltran-Debon, C. Alonso-Villaverde, L. Rios, V. Martin-Paredero, J. A. Menendez, V. Micol, A. Segura-Carretero and J. Camps, Hibiscus sabdariffa extract lowers blood pressure and improves endothelial function, *Mol. Nutr. Food Res.*, 2014, **58**, 1374–1378.
15 S. Fernández-Arroyo, I. C. Rodríguez-Medina, R. Beltrán-Debón, F. Pasini, J. Joven, V. Micol, A. Segura-Carretero and A. Fernández-Gutiérrez, Quantification of the polyphenolic fraction and *in vitro* antioxidant and *in vivo* anti-hyperlipemic activities of *Hibiscus sabdariffa* aqueous extract, *Food Res. Int.*, 2011, **44**, 1490–1495.
16 M. Herranz-López, E. Barrajón-Catalán, A. Segura-Carretero, J. A. Menéndez, J. Joven and V. Micol, Lemon verbena (*Lippia citriodora*), polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms, *Phytomedicine*, 2015, **22**, 605–614.
17 M. Herranz-Lopez, M. Olivares-Vicente, J. A. Encinar, E. Barrajon-Catalan, A. Segura-Carretero, J. Joven and V. Micol, Multi-Targeted Molecular Effects of *Hibiscus sabdariffa* Polyphenols: An Opportunity for a Global Approach to Obesity, *Nutrients*, 2017, **9**, DOI: 10.3390/nu9080907.
18 E. Barrajon-Catalan, M. Herranz-Lopez, J. Joven, A. Segura-Carretero, C. Alonso-Villaverde, J. A. Menendez and V. Micol, Molecular promiscuity of plant polyphenols in the management of age-related diseases: far beyond their antioxidant properties, *Adv. Exp. Med. Biol.*, 2014, **824**, 141–159.
19 J. Joven, A. Rull, E. Rodriguez-Gallego, J. Camps, M. Riera-Borrull, A. Hernandez-Aguilera, V. Martin-Paredero, A. Segura-Carretero, V. Micol, C. Alonso-Villaverde and J. A. Menendez, Multifunctional targets of dietary polyphenols in disease: a case for the chemokine network and energy metabolism, *Food Chem. Toxicol.*, 2013, **51**, 267–279.
20 J. Joven, V. Micol, A. Segura-Carretero, C. Alonso-Villaverde, J. A. Menéndez, G. Aragonès, E. Barrajón-Catalán, R. Beltrán-Debón, J. Camps, S. Cufí, S. Fernández-Arroyo, A. Fernández-Gutiérrez, E. Guillén, M. Herranz-López, I. Iswaldi, J. Lozano-Sánchez, B. Martin-Castillo, C. Oliveras-Ferraros, A. Pérez-Sánchez, E. Rodríguez-Gallego, A. Rull, D. Saura and A. Vázquez-Martín, Polyphenols and the Modulation of Gene Expression Pathways: Can We Eat Our Way Out of the Danger of Chronic Disease?, *Crit. Rev. Food Sci. Nutr.*, 2014, **54**, 985–1001.
21 A. Weltman, S. Levine, R. L. Seip and Z. V. Tran, Accurate assessment of body composition in obese females, *Am. J. Clin. Nutr.*, 1988, **48**, 1179–1183.
22 A. Boyum, Separation of White Blood Cells, *Nature*, 1964, **204**, 793–794.
23 S. Camacho, S. Michlig, C. de Senarclens-Bezencon, J. Meylan, J. Meystre, M. Pezzoli, H. Markram and J. le Coutre, Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying, *Sci. Rep.*, 2015, **5**, 7919.
24 S. Wang, N. Moustaid-Moussa, L. Chen, H. Mo, A. Shastri, R. Su, P. Bapat, I. Kwun and C. L. Shen, Novel insights of dietary polyphenols and obesity, *J. Nutr. Biochem.*, 2014, **25**, 1–18.
25 R. Beltran-Debon, C. Alonso-Villaverde, G. Aragones, I. Rodriguez-Medina, A. Rull, V. Micol, A. Segura-Carretero, A. Fernandez-Gutierrez, J. Camps and J. Joven, The aqueous extract of *Hibiscus sabdariffa* calices modulates the production of monocyte chemoattractant protein-1 in humans, *Phytomedicine*, 2010, **17**, 186–191.
26 C. Ural, H. Belli, M. Akbudak, A. Solmaz, Z. D. Bektas and F. Celebi, Relation of binge eating disorder with impulsiveness in obese individuals, *World J. Psychiatr.*, 2017, **7**, 114–120.
27 T. Amin and J. G. Mercer, Hunger and Satiety Mechanisms and Their Potential Exploitation in the Regulation of Food Intake, *Curr. Obes. Rep.*, 2016, **5**, 106–112.
28 T. A. Lutz, Gut hormones such as amylin and GLP-1 in the control of eating and energy expenditure, *Int. J. Obes. Suppl.*, 2016, **6**, S15–S21.
29 C. W. le Roux, M. Patterson, R. P. Vincent, C. Hunt, M. A. Ghatei and S. R. Bloom, Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects, *J. Clin. Endocrinol. Metab.*, 2005, **90**, 1068–1071.
30 P. J. English, M. A. Ghatei, I. A. Malik, S. R. Bloom and J. P. Wilding, Food fails to suppress ghrelin levels in obese humans, *J. Clin. Endocrinol. Metab.*, 2002, **87**, 2984.
31 D. E. Cummings, J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse and D. S. Weigle, A preprandial rise in plasma

ghrelin levels suggests a role in meal initiation in humans, *Diabetes*, 2001, **50**, 1714–1719.

32 N. M. Neary, C. J. Small, A. M. Wren, J. L. Lee, M. R. Druce, C. Palmieri, G. S. Frost, M. A. Ghatei, R. C. Coombes and S. R. Bloom, Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial, *J. Clin. Endocrinol. Metab.*, 2004, **89**, 2832–2836.

33 J. S. Flier and E. Maratos-Flier, Leptin's Physiologic Role: Does the Emperor of Energy Balance Have No Clothes?, *Cell Metab.*, 2017, **26**, 24–26.

34 X. Huang and Z. Yang, Resistin's, obesity and insulin resistance: the continuing disconnect between rodents and humans, *J. Endocrinol. Invest.*, 2016, **39**, 607–615.

35 X. Hu, X. Ma, Y. Luo, Y. Xu, Q. Xiong, X. Pan, Y. Xiao, Y. Bao and W. Jia, Associations of serum fibroblast growth factor 23 levels with obesity and visceral fat accumulation, *Clin. Nutr.*, 2018, **37**, 223–228.

36 A. B. Crujeiras, E. Goyenechea, I. Abete, M. Lage, M. C. Carreira, J. A. Martinez and F. F. Casanueva, Weight regain after a diet-induced loss is predicted by higher baseline leptin and lower ghrelin plasma levels, *J. Clin. Endocrinol. Metab.*, 2010, **95**, 5037–5044.

37 D. E. Cummings, D. S. Weigle, R. S. Frayo, P. A. Breen, M. K. Ma, E. P. Dellinger and J. Q. Purnell, Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery, *N. Engl. J. Med.*, 2002, **346**, 1623–1630.

38 P. Sumithran, L. A. Prendergast, E. Delbridge, K. Purcell, A. Shulkes, A. Kriketos and J. Proietto, Long-term persistence of hormonal adaptations to weight loss, *N. Engl. J. Med.*, 2011, **365**, 1597–1604.

39 D. Carling, AMPK signalling in health and disease, *Curr. Opin. Cell Biol.*, 2017, **45**, 31–37.

40 S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda and I. Shimomura, Increased oxidative stress in obesity and its impact on metabolic syndrome, *J. Clin. Invest.*, 2004, **114**, 1752–1761.

41 B. Kola, Role of AMP-activated protein kinase in the control of appetite, *J. Neuroendocrinol.*, 2008, **20**, 942–951.

42 B. Kola, M. Boscaro, G. A. Rutter, A. B. Grossman and M. Korbonits, Expanding role of AMPK in endocrinology, *Trends Endocrinol. Metab.*, 2006, **17**, 205–215.

43 M. Lopez, R. Nogueiras, M. Tena-Sempere and C. Dieguez, Hypothalamic AMPK: a canonical regulator of whole-body energy balance, *Nat. Rev. Endocrinol.*, 2016, **12**, 421–432.

44 R. Quirantes-Pine, M. Herranz-Lopez, L. Funes, I. Borras-Linares, V. Micol, A. Segura-Carretero and A. Fernandez-Gutierrez, 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**, 1112–1118.

45 M. Herranz-Lopez, I. Borras-Linares, M. Olivares-Vicente, J. Galvez, A. Segura-Carretero and V. Micol, Correlation between the cellular metabolism of quercetin and its glucuronide metabolite and oxidative stress in hypertrophied 3 T3-L1 adipocytes, *Phytomedicine*, 2017, **25**, 25–28.

46 S. Fernandez-Arroyo, M. Herranz-Lopez, R. Beltran-Debon, I. Borras-Linares, E. Barrajon-Catalan, J. Joven, A. Fernandez-Gutierrez, A. Segura-Carretero and V. Micol, Bioavailability study of a polyphenol-enriched extract from Hibiscus sabdariffa in rats and associated antioxidant status, *Mol. Nutr. Food Res.*, 2012, **56**, 1590–1595.

47 M. Olivares-Vicente, E. Barrajon-Catalan, M. Herranz-Lopez, A. Segura-Carretero, J. Joven, J. A. Encinar and V. Micol, Plant-derived polyphenols in human health: biological activity, metabolites and putative molecular targets, *Curr. Drug. Metab.*, 2018, **19**, DOI: 10.2174/1389200219666180220095236.

Capítulo 2

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Effect of metabolaid® on pre- and stage 1 hypertensive patients: A randomized controlled trial

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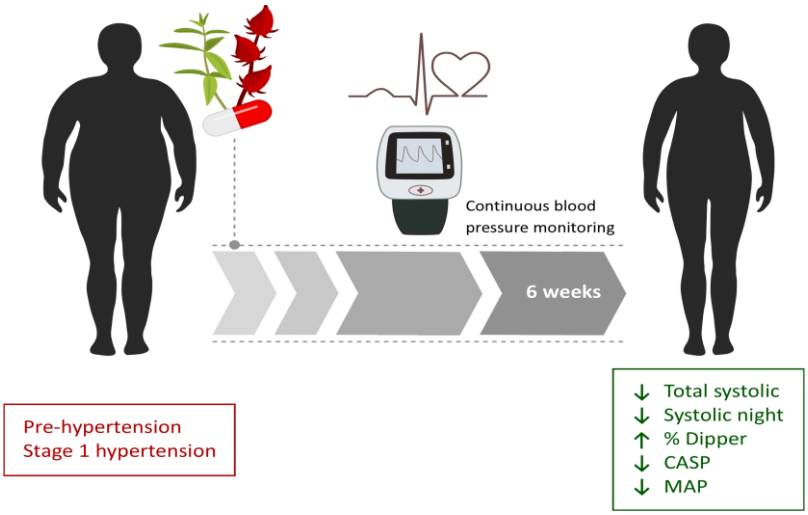

RESUMEN DE LOS RESULTADOS

En este trabajo se estudió la capacidad de un producto nutracéutico botánico basado en la combinación de los extractos de LC e HS para modular la hipertensión en personas con sobrepeso y obesidad. El extracto disminuyó significativamente la presión arterial promedio sistólica/diastólica diaria, así como la presión arterial diastólica/sistólica diurna, diastólica nocturna y el % dipper.

Se llevó a cabo un ensayo aleatorizado, doble ciego durante 6 semanas y controlado con placebo en individuos prehipertensos e hipertensos en grado 1, no medicados.

El reclutamiento inicial seleccionó un total de 563 individuos prehipertensos e hipertensos grado 1, de los cuales 479 fueron excluidos principalmente porque ya estaban siguiendo un tratamiento farmacológico.

Los individuos del grupo experimental consumieron dos cápsulas 20–30 min antes del desayuno durante todo el tiempo de intervención con 250 mg de los extractos polifenólicos cada una. Ambos grupos, fueron instruidos por una nutricionista para cambiar ciertos hábitos dietéticos: reducción de sal, reducción de grasas saturadas y reducción de alimentos azucarados. Además, recibieron indicaciones de caminar diariamente al menos 30 minutos. No se pautó ninguna dieta específica ni programa de ejercicio. Se les colocó un dispositivo BPro® a cada paciente durante 24h en diferentes momentos de la intervención como un continuo monitor de presión arterial. Durante las visitas, se registraron la presión arterial en reposo y la frecuencia cardíaca y se les realizaron mediciones antropométricas de diversos parámetros.

Los resultados mostraron una significativa disminución del porcentaje de grasa, así como en el peso corporal en pacientes del grupo suplementado. Los parámetros antropométricos en el grupo placebo no mostraron diferencias significativas en el análisis intragrupo.

Las analíticas realizadas mostraron una disminución significativa en el grupo placebo de creatinina y HDL (lipoproteínas de alta densidad) frente a disminuciones significativas en el grupo suplementado en valores como la creatinina, LDL (lipoproteínas de baja densidad), GPT (transaminasa glutámico pirúvica) o GGT (gamma glutamil transaminasa) entre otros.

El software asociado del dispositivo permitido tener una visión macroscópica precisa de los patrones de PA durante 24 h. Los resultados indicaron una mejora significativa generalizada en PA en el grupo suplementado, mejoras en la evolución semanal de la presión sistólica y diastólica total, así como en diastólica diurna, sistólica diurna, diastólica nocturna, presiones arteriales y en % dipper.

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Effect of metabolaid® on pre- and stage 1 hypertensive patients: A randomized controlled trial

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ABSTRACT

Many patients with stage 1 hypertension have difficulties to achieve lifestyle changes to avoid the progression of the disease. The use of natural alternatives with demonstrated blood pressure-regulating properties is instrumental at this stage, avoiding the side effects found in some pharmaceutical drugs. In this context, previous studies have indicated the possibility that a botanical nutraceutical product based on the combination of *Lippia citriodora* and *Hibiscus sabdariffa* extracts has hypotensive properties in overweight and obese individuals. Therefore, we aimed to evaluate the antihypertensive properties of this dietary supplement, as well as the effect on anthropometric and circulating parameters in pre-hypertensive and early stage 1 hypertensive patients (n = 84). The nutraceutical product, rich in polyphenolic compounds, has been assessed in a 6-week randomized, double-blind, placebo-controlled trial with pre-hypertensive and early stage 1 hypertensive (non-medicated) individuals. Participants consumed early in the morning in fasting conditions 2 capsules/day containing each one 250 mg of the polyphenolic extracts. Anthropometric and blood parameters as well as punctual and continuous blood pressure monitoring were determined in placebo and experimental groups. As a result and compared to baseline values, volunteers showed a significant reduction of average daily systolic/diastolic blood pressure as well as in daytime diastolic/systolic, nighttime diastolic blood pressure and in % dipper. Intergroup analysis revealed that the consumption of the plant extract resulted in a significant reduction of body fat content (−1.26%; $p < 0.05$) and nocturnal systolic blood pressure (−16.60 mmHg; $p < 0.05$) as well as an improvement of the dipper status (3.18%; $p < 0.01$). In conclusion, these results suggest that the nutraceutical acts as a main regulator of the individual's blood pressure towards healthier values, and therefore may be useful for pre-hypertensive/pre-medicated individuals.

1. Introduction

Cardiovascular diseases are the leading cause of death in industrialized countries and are strongly related to arterial hypertension (HT). Furthermore, the correlation between HT and the risk of cardiovascular disease is highly positive and independent from other risk factors. Otherwise said, the higher the blood pressure (BP), the greater the chances to have a heart attack. In this vein, the development of HT

Abbreviations: **AAMI**, Association for the Advancement of Medical Instrumentation; **AMPK**, AMP-activated protein kinase; **BHS**, British Hypertension Society; **BIA**, bioelectrical impedance analysis; **BMI**, body mass index; **BP**, blood pressure; **CASP**, central aortic systolic pressure; **DP**, diastolic pressure; **ESH**, European Society of Hypertension; **GGT**, gamma glutamyl transferase; **GPT**, glutamic-pyruvic transaminase; **HDL**, high density lipoproteins; **HS**, *Hibiscus sabdariffa*; **HT**, hypertension; **KS**, Kolmogorov-Smirnov; **LC**, *Lippia citriodora*; **LDL**, low density lipoproteins; **MAP**, mean arterial pressure; **NO**, nitric oxide; **SD**, standard deviation; **SP**, systolic pressure; **PP**, pulse pressure.

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depends on the genetic background of the individual, environmental factors and associated pathologies. Surprisingly, the majority of these risk factors, particularly environmental ones, can be prevented or treated (Oliveras & de la Sierra, 2014; Oparil & Schmieder, 2015; Samadian et al., 2016; WHO, 2007).

According to the major health organizations, such as the Task Force for Management of Arterial HT, the European Society of Cardiology and the American College of Cardiology, normal BP values are < 120 mmHg for systolic pressure and < 80 mmHg for diastolic pressure (Whelton et al., 2018). However, when an individual's systolic and diastolic pressure are in the 120–139 mmHg and 80–89 mmHg range, respectively, they are diagnosed with prehypertension. In this context, prehypertension is generally considered as an alert signal for subsequent HT development rather than a pathology by itself. If preventive measures are not applied immediately, these patients could eventually progress towards HT. In this scenario, stage 1 HT corresponds to ranges of 140–159 mmHg for systolic hypertension and 90–99 for diastolic blood pressure. Finally, stage 2 HT is considered when systolic blood pressure is ≥160 mmHg and ≥100 for diastolic blood pressure.

The high prevalence of HT in the adult population around the world is a major public health problem. The number of annual deaths worldwide, as a result of high blood pressure, is increasing (Kearney et al., 2005; Mendis, 2010). In the medical practice, when an individual is diagnosed with prehypertension or early stage 1 HT, it is generally recommended to adopt certain lifestyle habits in order to help to reduce their blood pressure before moving on towards hypotensive medications. These lifestyle interventions include more hours/week of physical activity and consuming a balanced diet. However, BP improvements are not immediate and very often affected patients are discouraged and abandon the lifestyle changes. In this critical moment, the consumption of certain natural dietary supplements with hypotensive properties may help patients to better control their BP.

This is aggravated by the fact that in the majority of cases, prehypertension or early stage 1 HT appears in overweight and obese people. Obesity is not solely an excess of adipose tissue in the organism. The pathology is generally associated with metabolic, oxidative and inflammatory alterations, reaching pandemic proportions in developed countries and increasing the expenses for Health Care Services in the Community. For this reason, metabolic syndrome is a more appropriate terminology, as it comprises increased adipose tissue mass, endothelial dysfunction, dyslipidemia, atherosclerosis, insulin resistance and HT (Furukawa et al., 2004; Luna-Luna et al., 2015).

Taking into account the high prevalence of HT, our research group studied the effect of a nutraceutical product that combines the aqueous polyphenolic extracts of *Lippia citriodora* (LC) and *Hibiscus sabdariffa* (HS). LC originates from South America, but was imported to Europe, where it is widely cultured. It possesses a lemon-like aroma and is used in herbal tea preparations, as it is known for its antispasmodic, antipyretic, sedative and digestive properties (El-Hawary et al., 2012). HS has been used traditionally in herbal drinks, in hot and cold beverages, as a flavouring agent in the food industry and as a botanical medicine (Da-Costa-Rocha et al., 2014). In our previous reports, we have observed that the resulting herbal combination (LC + HS) is capable of activating AMP-activated protein kinase (AMPK) *in vitro* and reduce intracellular lipid accumulation in cultured adipocytes through the regulation of different metabolic pathways (Herranz-López et al., 2019). The effects observed with the extract have also been corroborated in hyperlipidemic animal models (Herranz-Lopez et al., 2015; Joven et al., 2012; Lee et al., 2018). Furthermore, the combination of LC + HS has been reported to reduce body fat and improve the general health status of overweight subjects compared to the placebo group (Boix-Castejon et al., 2018; Herranz-López et al., 2019). All these effects prompted us to explore its potential use in the relief of pathologies related to obesity, including HT.

To this end, the main objective of this work was to evaluate the antihypertensive properties of the herbal combination (LC + HS), a nutraceutical product named MetabolAid®. As a second objective, data were collected in order to study the ability of this polyphenolic combination to modulate certain anthropometric parameters, as well as several circulating values in pre-hypertensive and early stage 1, non-medicated hypertensive patients participating in the study.

2. Materials and methods

2.1. Supplement formulation

MetabolAid® (patent application number P201731147) capsules were kindly provided from Monteloeder SL (Elche, Spain). The capsules contained 500 mg of a polyphenolic mixture isolated from HS calyxes and LC leaves whose composition has been previously determined by HPLC coupled to mass spectrometry (Herranz-López et al., 2019). Briefly, the supplement was composed of a combination at a weight ratio (w/w) of 65:35 of LC polyphenolic extract (25% phenylpropanoids, dry weight) and HS polyphenolic extract (10% anthocyanins, dry weight), as declared by the manufacturer. The major anthocyanins identified were delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside, and the main phenylpropanoids were verbascoside and isoverbascoside (see Fig. 1-Supplementary for detailed composition).

2.2. Participants

563 pre-hypertensive and stage 1 hypertensive patients were selected from the Raval Health Center located in the city of Elche (Spain). 479 patients were excluded because they did not meet the inclusion criteria, mainly because they were under medication. Finally, 84 patients were included in the study according to the following inclusion criteria:

a) Adults pre-hypertensive /stage 1 HT with no pharmacological treatment;
b) Participants accept to undergo hygienic-dietetic intervention;
c) Participants were willing to ingest the nutritional supplement containing the polyphenolic extract;
d) Participants signed the informed consent.

The following exclusion criteria were used:

a) Individuals under 18 years of age;
b) Individuals with BMI > 35;
c) Patients with high cardiovascular risk (stage 2 HT);
d) Patients with previous chronic diseases;
e) Presence of allergy to the compounds of the product and placebo according to the technical data sheet.

Participants were recruited by the ALUMBRA sanitary information analysis system service. To this end, the lists of patients with a history of HT were provided by the medical staff and those who met the established inclusion criteria were selected.

Before participating in the study, the researchers informed the subjects about the product and the study procedures. All subjects provided written informed consent before participating. The study was approved by the Ethics Committee of Miguel Hernández University (Elche, Spain) (reference IBM.VMM.01.17) and was conducted according to the Helsinki Declaration criteria (1983 version). The study was registered in Clinical Trials with the reference number NCT03507023.

2.3. Trial design

The study was a randomized, double-blind, placebo-controlled trial lasting 6 weeks based on previous studies from our group (Boix-Castejon et al., 2018; Herranz-López et al., 2019). After recruitment, the volunteers were randomized into the placebo/L1 (n = 41) or experimental/L2 group (n = 43) (Fig. 1). Both groups presented non-significant differences regarding BP parameters at the beginning of the study. The

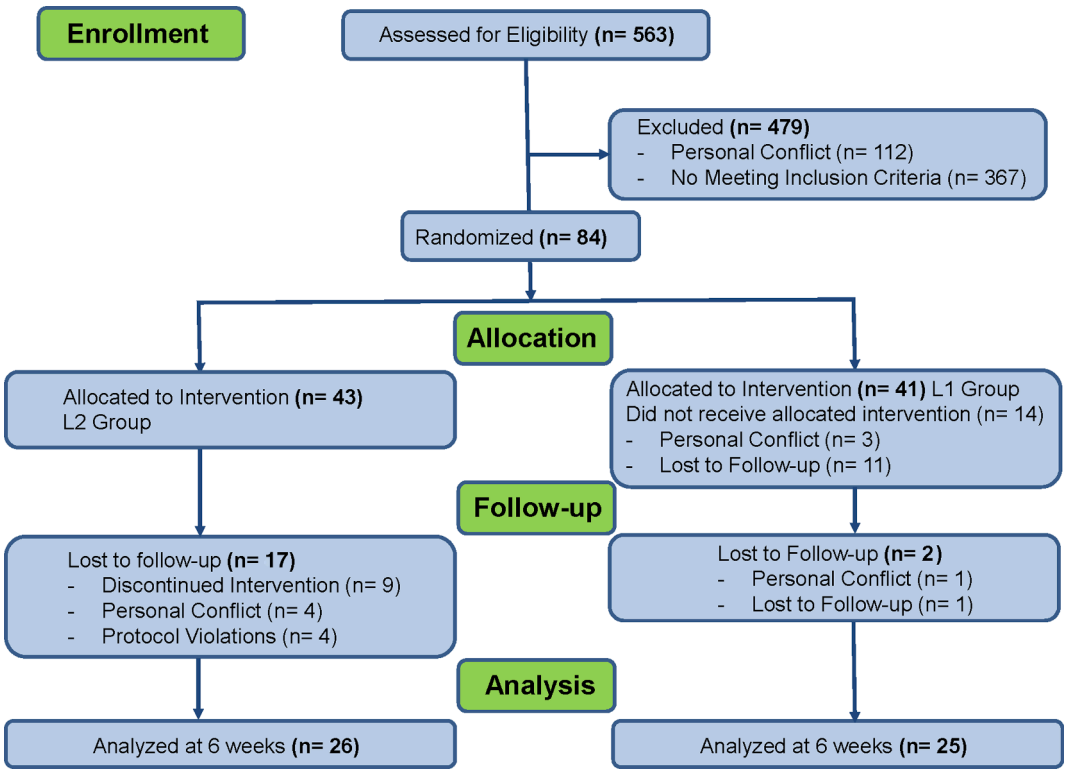

Fig. 1. Participant flow diagram. L1 corresponds to the placebo group and L2 to the dietary supplement group.

placebo group received two daily capsules of 370 mg each of crystalline microcellulose per day. The experimental group received two capsules containing each one 250 mg of Metabolaid® + 120 mg of crystalline microcellulose. Therefore, the daily consumption was 500 mg of Metabolaid®.

The MetabolAid® and placebo capsules were prepared so that they were the same size, smell and color. The volunteers were instructed to take two capsules 20–30 min before breakfast every day for 6 weeks. In addition, they received indications to change certain dietetic habits (reduction of salt, saturated fat and sugar in foods) and to walk daily for at least 30 min. No personal diet nor specific exercise programs were provided.

The compliance of the subjects with the intake of capsules and the use of the worn ambulatory device was evaluated at each visit to the Raval Health Center (Elche, Spain), or through telephone interviews every week. At the end of the study, a total of 16 and 17 participants were excluded from the placebo and experimental group, respectively (Fig. 1).

2.4. Intervention

2.4.1. Anthropometric measurements and blood analysis

Anthropometric measures were taken at the beginning of the study, week 2 and week 6 of the intervention period, including body weight and height. Percentage of fat, lean mass and body water were determined using a bioelectrical impedance analysis (BIA) device (Tanita BC-545 N, Tanita Corporation, Japan). The body mass index (BMI) was derived from body weight and height using the equation BMI = Body weight (Kg) / height^2 (m^2).

Blood analyses were performed in the same health center. Blood samples were extracted in fasting conditions, at the beginning and end of the study.

2.4.2. Blood pressure determinations

The determination of BP in the health center was carried out by trained personnel following the standards proposed by the European Society of Hypertension (Williams et al., 2018). Systolic and diastolic blood pressures at rest and heart rate were recorded at the beginning of the study, week 1, week 2, week 4 and week 6 of the intervention using a BP monitor MC3100 + validated voltage monitor (HealthStats, Singapore).

Also, a BPro® device (HealthStats, Singapore) was provided to each patient for one day at different moments of the intervention as a continuous blood pressure monitor. BPro® is validated by the BHS (British Hypertension Society), by the AAMI (Association for the Advancement of Medical Instrumentation) and by the ESH (European Society of Hypertension) (MedTach, 2019). The associated software of the device allowed to have an accurate macroscopic view of BP patterns during 24 h.

Parameters provided by the BPro® include the hourly systolic and diastolic BP measurements, from which the 24 h mean and daytime/nighttime average measurements were calculated. The percentage of Dipper/Non-Dipper was also evaluated by the BPro®. All these determinations were performed at the beginning of the study, and after 1, 2, 4 and 6 weeks of product consumption.

BP follows a circadian pattern, with higher blood pressure values during the day and lower at night (dipper pattern). On the contrary, in individuals that are non-dippers, such as those with HT, no significant decrease in night BP was observed (Islam, 2017). However, an extreme decrease of BP at night has been recently associated with other cardiovascular diseases such as cerebral ictus or vascular diabetic complications, although evidences remain weak at present (Islam, 2017; Williams et al., 2018). The % Dipper was calculated according the equation: (Average Day Systolic BP - Average Night Systolic BP) × 100% / Average Day Systolic BP). The obtained values are associated to a different prognosis. These include: ≤0% (reverse dipper); 0–10% (non-dipper); 10–15% (normal dipper) and >15% (extreme dipper). The profiles with worse prognosis are associated with the values of the reverse dipper (≤0%) and extreme dipper (>15%). Normal dipper (10–15%) is considered the healthiest pattern.

Other parameters provided by the BPro® device are: central aortic systolic pressure (CASP), pulse pressure (PP) and mean arterial pressure

(MAP). For CASP, the BPro® determines the average of the progressive motion of the pulse wave in a point by considering the heart rate of the point divided by 4. Therefore, CASP allows comparing the intracardiac pressure respect to peripheric (brachial) pressure. CASP range = 101–136 mmHg, and CASP > 117 mmHg indicates risk of angiopathy. PP indicates the flexibility of vessel walls. PP is determined as the difference between systolic pressure (SP) and diastolic pressure (DP): PP = SP-DP. PP > 50 mmHg indicates risk of atherosclerosis. Finally, MAP indicates the mean perfusion pressure into the different organs. MAP > 60 mmHg indicates a good perfusion pattern with a low risk of ischemia. On the other hand, MAP < 60 mmHg indicates an increased risk of ischemia in peripheral organs.

2.5. Statistical analysis

The statistical analysis was carried out using the Graphpad Prism software. The results were expressed as means ± standard deviation (SD). The anthropometric, biochemical and vital values (intragroup) were analyzed by the unpaired Student *t* test. On the other hand, the vital signs and the anthropometric parameters (intergroup) were analyzed by means of the paired Student *t* test. The values obtained through the BPro® devices were analyzed by one-way ANOVA. The outcome variables were evaluated to determine their compliance with the normal distribution and transformed if necessary by a Shapiro-Wilk test. The p values reported were bilateral and values of 0.05 or less (0.01, 0.001 and 0.0001) were considered statistically significant for the comparisons with the baseline (start of the study) and between the groups (placebo *vs* supplement) at the same moment of the study.

3. Results

3.1. Anthropometric parameters

The anthropometric parameters in the intragroup analysis, obtained by comparing the baseline data at the beginning of the intervention study versus the follow-up at 3 weeks, did not show significant differences in placebo group. On the other hand, the results obtained showed a significant decrease in the percentage of fat (approximately 1%), in patients of the supplemented group after six weeks of intervention (Table 1). Regarding weight, there was a significant reduction of approximately 1 kg after six weeks in the supplemented group (Table 1). In the inter-group analysis, significant differences were observed when comparing the values obtained in the placebo group versus the supplemented group for fat percentage at the end of intervention (Table 2).

Table 1
Evolution in anthropometric parameters comparing values at the beginning (t = 0) *vs* the end of the 2nd week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: **p < 0.01. Means ± SD.

	Placebo (n = 25)			Supplemented (n = 26)		
Anthropometric parameters	**t = 0**	**2nd week**	**6th week**	**t = 0**	**2nd week**	**6th week**
Weight (Kg)	78.85 ± 14.48	78.96 ± 14.49	78.78 ± 14.71	88.91 ± 15.46	88.92 ± 15.49	87.93 ± 16.18 **
Fat mass (%)	33.72 ± 8.40	33.85 ± 8.51	33.11 ± 8.54	35.15 ± 9.30	34.49 ± 9.69	33.89 ± 9.65 **
Body water (%)	48.32 ± 6.84	48.44 ± 7.21	48.40 ± 7.05	47.16 ± 5.94	47.67 ± 6.19	47.60 ± 6.37
Muscle mass (%)	51.03 ± 11.79	50.67 ± 12.31	51.44 ± 10.90	53.88 ± 8.91	54.20 ± 9.01	54.16 ± 9.03
BMI (kg/m^2)	28.58 ± 4.62	28.62 ± 4.60	28.55 ± 4.56	31.37 ± 6.35	31.38 ± 6.40	31.17 ± 6.43

Abbreviations: Body mass index (BMI), t = 0 (baseline).

3.2. Circulating parameters

Table 3 shows changes in circulating parameters at the end of intervention in the same group. The placebo group only presented significant decreases in creatinine and HDL (high density lipoproteins). Meanwhile, the supplemented group presented significant decreases in creatinine, LDL (low density lipoproteins), GPT (glutamic-pyruvic transaminase) or GGT (gamma glutamyl transferase) and red cell number. However, no significant changes were observed when comparing the evolution (6 weeks respect to beginning of intervention) of the circulating parameters between both groups (placebo *vs* supplemented) (Table 1-Supplementary).

3.3. Parameters related to blood pressure

3.3.1. Continuous monitoring by BPro® ambulatory devices

Table 4 compares the different values obtained from the continuous monitoring of the BPro® devices between patients in the placebo group and the supplemented group. Table 5 shows the intergroup comparison. The weekly results indicated a generalized significant improvement in BP in the supplemented group (Table 4). The improvements were observed in the weekly evolution of total systolic and diastolic pressure as well as in daytime diastolic, daytime systolic, nighttime diastolic blood pressures and in % dipper. Interestingly, improvements began to be evident from the second week of consumption of the supplement for total systolic BP and systolic BP at day and at night (Figs. 2 and 3-Supplementary).

The analysis of the % dipper in the placebo group showed a tendency from the beginning to maintain a non-dipper pattern (0–10%). On the other hand, in the treated group, an upward significant trend towards the normal dipper pattern was observed (Table 5) (Fig. 4-Supplementary). Regarding CASP, the BPro® values decreased in the group consuming the supplement towards the non-risk range. However, no significant CASP changes were noticed in the placebo group (Table 4). In addition, PP values improved in the supplemented group compared with the placebo, where significant changes were not noticed (Table 4). Finally, MAP values indicated that both groups displayed an optimal perfusion in peripheral organs (Table 4). Altogether, total systolic pressure, nighttime systolic pressure, % dipper, CASP and MAP in the supplemented group presented significant improvements compared to placebo (Table 5).

3.3.2. Digital pressure monitor

The data obtained with the BPro® device were corroborated with the data from the sphygmomanometer (Table 6). A significant improvement of both systolic and diastolic blood pressures was observed in the

Table 2
Differences in anthropometric parameters comparing changes during the intervention between both groups (placebo *vs* supplemented) (intergroup analysis). Significance was established at: *p < 0.05. Means ± SD.

	Differences after two weeks (Value at 2 weeks – Value at t = 0)		Differences after six weeks (Value at 6 weeks – Value at t = 0)	
Anthropometric parameters	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Weight (Kg)	0.11 ± 0.15	0.02 ± 0.14	−0.07 ± 0.25	−0.98 ± 0.33 *
Fat mass (%)	0.14 ± 0.18	−0.66 ± 0.43	−0.60 ± 0.53	−1.26 ± 0.41 *
Body water (%)	0.12 ± 0.21	0.51 ± 0.38	0.08 ± 0.22	0.44 ± 0.42
Muscle mass (%)	0.36 ± 0.25	0.32 ± 0.52	0.40 ± 0.54	0.28 ± 0.54
BMI (kg/m^2)	0.04 ± 0.06	0.01 ± 0.05	−0.03 ± 0.10	−0.20 ± 0.19

Abbreviations: Body mass index (BMI), t = 0 (baseline)

Table 3
Evolution of circulating parameters comparing beginning *vs* the end of intervention into the same group (placebo or supplemented group) (intragroup analysis). The significance was established at: *p < 0.05, **p < 0.01. Means ± SD.

	Placebo (n = 25)		Supplemented (n = 26)	
Blood parameters	t = 0	6th week	t = 0	6th week
Glucose (mg/dl)	106.3 ± 29.32	108.4 ± 24.04	102.1 ± 18.28	101.6 ± 18.29
Creatinine	0.87 ± 0.17	0.82 ± 0.17 **	0.84 ± 0.16	0.80 ± 0.17 *
Uric acid (mg/dl)	5.19 ± 1.34	5.31 ± 1.29	5.17 ± 1.24	5.27 ± 1.29
Triglycerides (mg/dl)	159.5 ± 114.6	164.2 ± 110.7	127.8 ± 66.48	127.5 ± 67.53
Total cholesterol (mg/dl)	220.0 ± 36.02	216.2 ± 37.69	209.9 ± 40.10	206.2 ± 41.61
HDL (mg/dl)	57.54 ± 16.26	54.24 ± 13.83*	55.85 ± 15.67	56.25 ± 16.66
LDL (mg/dl)	135.2 ± 35.16	133.2 ± 34.02	131.3 ± 35.22	123.2 ± 33.24 *
GOT/AST (U/L)	36.50 ± 63.09	38.15 ± 62.90	23.50 ± 7.33	22.58 ± 6.06
GPT/ALT (U/L)	32.30 ± 12.64	34.90 ± 24.52	30.21 ± 13.94	26.92 ± 11.30 *
GGT (U/L)	39.05 ± 26.11	39.95 ± 26.80	27.83 ± 18.46	26.17 ± 17.17*
Erythrocyte (x 10^6 μL)	4.92 ± 0.49	4.94 ± 0.44	4.89 ± 0.42	4.83 ± 0.43 *
Haemoglobin (g/dL)	14.69 ± 1.43	14.90 ± 1.35	14.80 ± 1.44	14.63 ± 1.41
Haematocrit (%)	44.63 ± 4.53	44.73 ± 4.11	44.50 ± 4.09	43.81 ± 4.23
MCV (fL)	87.80 ± 13.69	83.35 ± 22.80	91.15 ± 4.43	90.68 ± 3.97

Abbreviations: Gamma-glutamil transferase (GGT), glutamic-oxaloacetic transaminase/aspartate transaminase (GOT/AST), glutamic-pyruvic transaminase/alanine transaminase (GPT/ALT), high density lipoproteins (HDL), low density lipoproteins (LDL), mean corpuscular volume (MCV), t = 0 (baseline).

supplemented group starting from the second week taking the supplement. The results indicated a progressive improvement until the last measurement registered in consultation (sixth week). The most significant improvement was observed for the supplemented group compared with the placebo at the sixth week in systolic pressure (Table 7).

4. Discussion

Arterial HT is the risk factor with the greatest impact on cardiac and cerebrovascular mortality in both women and men (Messerli et al., 2007). BP control requires complex integration of regulatory mechanisms across multiple physiological systems. A sustained increase in arterial pressure therefore reflects a failure of one or more of these controls (Bolívar, 2013). This research focuses on the study of the effects of a polyphenolic dietary supplement (LC + HS) in HT, as a major component of metabolic syndrome (Savica et al., 2010), providing a new research framework.

Dietary control in hypertensive patients is very important to prevent, improve or eradicate mild HT. However, adherence to dietary guidelines is generally not maintained in the long term (Vairavamurthy et al., 2017). Previous studies have demonstrated the hypotensive effect of Metabolaid® (Boix-Castejon et al., 2018; Herranz-López et al., 2019). The results suggest that this combination of LC + HS acts as a main regulator of the individual's blood pressure. Indeed, arterial pressure showed a significant improvement in all parameters comparing baseline to week 6 (systolic, diastolic, day and night tension) only in the supplemented group. Comparing both groups, systolic night pressure and % dipper showed significant differences. This suggests a hypotensive effect that starts to be evident at night, when individuals are resting (compare % dipper *vs* non-dipper pattern). However, this improvement is not totally established during the day when individuals are more active. Altogether, this suggests a tendency of BP towards a normal circadian rhythm as a first step to correct HT. In addition, PP and CASP show a significant improvement towards a lower risk to develop angiopathy compared to placebo. The changes in HT were accompanied by a significant decrease in LDL, a circulating parameter that depends on diet and exercise. In this context, dyslipidemia and HT are pathological conditions that favour endothelium damage, particularly in the case of oxidized LDL (Hurtubise et al., 2016). Since reaching normal BP is a long process, these results suggest the necessity to reinforce the hypotensive/hypolipidemic effects of Metabolaid® by introducing a customized program of exercise and balanced diet that has not been implemented in the present study.

In this vein, we have observed that the supplemenís effects are enhanced when it is administered in the context of diet + exercise (Boix-Castejon et al., 2018; Herranz-López et al., 2019). This is supported by the observation that anthropometric parameters (weight, fat % and BMI) improved in previous reports from our laboratory (Boix-

Table 4
Evolution in BPro® measurements comparing values at the beginning (t = 0) *vs* the end of the 1st week, 2nd week, 4th week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001. Means ± SD.

	Placebo (n = 25)					Supplemented (n = 26)				
	t = 0	1st week	2nd week	4th week	6th week	t = 0	1st week	2nd week	4th week	6th week
Total Systolic (mmHg)	138.7 ± 15.63	133.2 ± 30.64	137.2 ± 20.80	139.3 ± 20.84	136.7 ± 20.77	149.5 ± 27.78	140.7 ± 17.62	135.7 ± 18.56**	136.5 ± 11.26*	130.2 ± 18.56***
Systolic day (mmHg)	143.9 ± 15.24	141.7 ± 13.98	142.5 ± 21.58	143.7 ± 22.53	140.5 ± 21.26	147.1 ± 18.27	144.6 ± 18.00	139.6 ± 16.95**	142.4 ± 14.94	136.1 ± 18.93**
Systolic night (mmHg)	131.3 ± 17.23	135.0 ± 17.09	128.9 ± 17.36	130.7 ± 19.01	133.5 ± 22.97	139.1 ± 28.53	131.8 ± 18.20	126.6 ± 17.73**	130.9 ± 13.03	122.5 ± 19.40**
% dipper	9.15 ± 5.65	4.50 ± 8.34*	8.54 ± 5.22	8.44 ± 3.75	7.30 ± 5.79	6.94 ± 6.02	8.46 ± 5.13	8.80 ± 5.00	8.45 ± 4.63	10.13 ± 4.84*
Total diastolic (mmHg)	89.76 ± 11.78	90.76 ± 10.86	86.08 ± 11.90	89.40 ± 14.82	86.36 ± 13.86	93.56 ± 13.13	91.56 ± 11.98	87.16 ± 12.45*	89.88 ± 13.89	84.36 ± 14.76**
Diastolic day (mmHg)	93.88 ± 12.34	92.85 ± 11.04	90.85 ± 13.45	93.04 ± 15.63	89.50 ± 14.78	96.26 ± 13.43	95.48 ± 12.65	90.57 ± 12.61	93.35 ± 15.84	88.26 ± 15.26*
Diastolic night (mmHg)	84.72 ± 12.34	87.36 ± 10.07	81.16 ± 10.96	83.92 ± 13.89	81.92 ± 12.75	87.60 ± 14.30	86.12 ± 11.52	81.60 ± 12.11*	84.52 ± 13.32	78.88 ± 14.79**
CASP (mmHg)	145.3 ± 23.54	142.6 ± 23.69	143.7 ± 20.52	142.2 ± 21.90	140.4 ± 21.65	149.5 ± 29.89	138.3 ± 21.40*	135.4 ± 17.04**	135.5 ± 14.21**	134.3 ± 22.07***
PP (mmHg)	59.64 ± 19.92	54.20 ± 20.56	56.40 ± 19.95	52.00 ± 14.04	56.92 ± 15.51	62.52 ± 22.89	54.52 ± 13.10	54.16 ± 12.98*	49.00 ± 15.41**	53.40 ± 17.59*
MAP (mmHg)	115.3 ± 1360	114.7 ± 14.44	112.2 ± 14.78	111.7 ± 16.19	110.6 ± 14.60*	119.1 ± 17.63	111.6 ± 12.60**	109.3 ± 13.23***	109.4 ± 12.14**	106.7 ± 16.39***

Table 5
Differences in BPro® measurements (#week-(t = 0)) comparing changes during the intervention between both groups (placebo *vs* supplemented) (intergroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001. Means ± SD.

	1st week – (t = 0)		2nd week – (t = 0)		4th week – (t = 0)		6th week – (t = 0)	
	Placebo	**Supplemented**	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Total Systolic (mmHg)	−5.50 ± 6.41	−8.78 ± 5.05	−1.53 ± 3.38	−13.74 ± 4.44*	0.54 ± 3.42	−12.96 ± 5.68	−2.04 ± 3.31	−19.30 ± 4.64**
Systolic day (mmHg)	−2.15 ± 2.72	−2.56 ± 4.18	−1.42 ± 3.33	−7.56 ± 2.38	−0.15 ± 3.77	−4.68 ± 4.51	−3.42 ± 3.36	−11.00 ± 3.32
Systolic night (mm Hg)	2.11 ± 3.54	−7.24 ± 4.26	−2.38 ± 3.28	−12.44 ± 4.27	−0.58 ± 3.24	−8.20 ± 5.12	−10.15 ± 11.33	−16.60 ± 4.64*
% dipper	−4.65 ± 1.92	1.51 ± 0.93**	−0.61 ± 1.25	1.86 ± 1.12	−0.72 ± 1.36	1.51 ± 1.23	−1.85 ± 1.20	3.18 ± 1.34**
Total diastolic (mmHg)	1.00 ± 2.35	−2.00 ± 2.57	−3.68 ± 1.96	−6.40 ± 2.64	−0.36 ± 2.68	−3.68 ± 3.30	−3.04 ± 2.45	−9.20 ± 2.90
Diastolic day (mmHg)	−1.04 ± 2.25	−0.72 ± 2.55	−3.04 ± 1.99	−5.70 ± 2.79	−0.85 ± 2.80	−2.91 ± 3.94	−4.39 ± 2.45	−8.00 ± 3.14
Diastolic night (mmHg)	2.64 ± 2.36	−1.48 ± 2.23	−4.11 ± 1.94	−6.00 ± 2.47	−4.65 ± 4.65	−3.08 ± 3.19	−2.80 ± 2.52	−8.72 ± 3.09
CASP (mmHg)	2.74 ± 3.16	−11.21 ± 5.02	−1.60 ± 3.64	−14.13 ± 4.72*	−3.13 ± 3.21	−14.08 ± 4.80	−4.87 ± 2.95	−15.29 ± 3.79*
PP (mmHg)	−5.44 ± 3.32	8.00 ± 3.91	−3.24 ± 3.04	−8.36 ± 3.64	−7.64 ± 4.06	−13.52 ± 4.83	−2.72 ± 2.79	−9.12 ± 3.99
MAP (mmHg)	−0.60 ± 1.67	−12.44 ± 2.02***	−3.16 ± 2.06	−9.72 ± 2.70	−3.60 ± 2.21	−9.84 ± 2.05*	−4.68 ± 1.97	−12.44 ± 2.02**

Table 6
Evolution in vital signs parameters using the digital pressure monitor and comparing values at the beginning (t = 0) *vs* the end of the 1 st week, 2nd week, 4th week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001. Means ± SD.

	Vital signs	t = 0	1 st week	2nd week	4th week	6th week
Placebo	**Heart rate (bpm)**	78.80 ± 15.03	78.88 ± 12.22	82.36 ± 17.65	79.76 ± 11.72	78.44 ± 12.56
n = 25	**Systolic pressure (mmHg)**	154.7 ± 21.86	152.6 ± 24.15	149.5 ± 23.30	149.3 ± 23.03*	146.9 ± 21.40*
	Diastolic pressure (mmHg)	95.36 ± 11.28	95.58 ± 11.67	92.76 ± 14.09*	93.36 ± 15.11	91.00 ± 13.22*
Supplemented	**Heart rate (bpm)**	84.76 ± 15.86	85.72 ± 17.54	86.84 ± 16.57	85.08 ± 15.26	81.08 ± 15.07
n = 26	**Systolic pressure (mmHg)**	160.8 ± 31.11	148.0 ± 18.32**	145.3 ± 18.47***	143.8 ± 13.28**	142.3 ± 22.76***
	Diastolic pressure (mmHg)	97.88 ± 12.42	93.56 ± 11.04**	91.12 ± 12.13***	92.48 ± 13.30*	88.88 ± 15.58***

Table 7
Differences respect to baseline (beginning of the intervention) of vital signs expressed as means ± Std. Error. Intergroup statistical analysis was performed at 2nd and 6th week compared to the baseline (t = 0). Significance was established at: *p < 0.05.

	Differences after two weeks (Value at 2 weeks – Value at t = 0)		Differences after six weeks (Value at 6 weeks – Value at t = 0)	
Vital signs	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Heart rate (bpm)	3.56 ± 4.61	2.08 ± 2.94	0.36 ± 2.35	−3.68 ± 2.91
Systolic pressure (mmHg)	−5.24 ± 3.06	−15.48 ± 3.98	−7.84 ± 2.97	−18.48 ± 3.86*
Diastolic pressure (mmHg)	−2.60 ± 1.87	−6.76 ± 1.55	−4.46 ± 1.80	−9.00 ± 1.98

Castejon et al., 2018; Herranz-López et al., 2019), meanwhile only fat % decreased significantly but modestly in the present study. Therefore, diet and exercise management are instrumental for weight reduction in overweight patients. It must be mentioned that participants in the present study were selected according to the HT value at the beginning of the study as the main selection criterion, but not according to their BMI. In this respect, the number of obese (IBM > 30) was balanced in both groups (n = 15 in the placebo and n = 12 in the supplemented group). In addition, participants followed only general indications from the health center, with no personal diet nor physical activity program included. For this reason, improvements in anthropometric parameters were modest or not significant. The indications provided were very general and subjected to the interpretation made by participants. This observation must alert health authorities and consider that more direct interventions need to be implemented to change unhealthy lifestyle habits in affected populations. In the same context, the tendency of circulating lipid parameters showed a modest but positive evolution towards values into the healthy range. Nevertheless, under these unfavourable conditions, the plant extract was capable of improving HT values in the supplemented group.

There are several hypothesis as to the molecular mechanisms behind the observed effects (Joven et al., 2014a, 2014b). In this context, AMPK is a major therapeutic target of the plant extract by controlling lipid accumulation in liver, adipose tissue and bloodstream (Herranz-Lopez et al., 2017). However, the evidences accumulated *in vitro* do not discard other molecular targets such as oxidative stress inhibition and the blockade of inflammatory cytokine secretion (Herranz-Lopez et al., 2012). It is well established that AMPK activation in the majority of tissues promotes activation of fatty acid oxidation and glucose uptake, decreasing at the same time fatty acid and cholesterol synthesis (Ford et al., 2012; Greig et al., 2015; López et al., 2016; Sun et al., 2015; Yeh et al., 2018). These observations support the decrease in transaminases detected in the present study in the supplemented group, suggesting a hepato-protective effect.

Previous studies have suggested that LC and HS extracts may exert a blood-pressure lowering effect, and their mechanisms of action have been elucidated to a certain extent. In the case of LC, though it is not a well-known effect, the active compounds of this plant, especially verbascoside, have been shown to have an effect in lowering blood pressure in animal models (Ragone et al., 2010). Specifically, verbascoside has been shown to reduce BP in rats at 2 h after administration. Similar studies reported a nitric oxide (NO)-mediating relaxing effect in the aorta in rats (Wong et al., 2001). Both direct administration (injection) and oral intake have been studied, with similar results. In fact, the authors report that compared to captopril, which is a drug to treat HT, verbascoside at 10 mg/kg body weight had similar effects to captopril at 5 mg/kg in lowering systolic blood pressure and was better than captopril in lowering diastolic blood pressure.

In the above mentioned study using LC extract (Ragone et al., 2010), blood pressure was lowered using increasing concentrations of an aqueous solution of LC. Based on the tests performed, the hypotensive effect was not due to muscarinic receptors nor NO release. Rather, components of the extract seem to have a direct effect on the smooth muscles, causing a vasorelaxing effect (more technically, a non-competitive contractile blockade). Also, in the heart, a negative cardiac ionotropism (i.e., a weakening of the force used to contract the heart, similar to beta-blocker medication) was detected, which could

contribute to the hypotension. The authors suggest that verbascoside was the major compound involved in the observed effect.

On the other hand, HS extracts have been shown to have several effects on blood vessel endothelial cells to induce blood pressure. These mechanisms include:

- Vasorelaxation effect (Zheoat et al., 2019): Hibiscus acid has a vasorelaxation effect in the aorta, due to the inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels. A similar effect has been observed with garcinia acid of *Garcinia cambogia*, which is an isomer of hibiscus acid.
- Increased NO production (Joven et al., 2014a, 2014b): HS polyphenols increase endothelial NO synthase (eNOS) production, with in turn increases NO. This is similar to other botanical extracts that reduce blood pressure, such as garlic and citric extracts (i.e. vitamin C).
- ACEi (Angiotensin Converting Enzyme Inhibitors) act on the angiotensin-renin-aldosterone system by inhibiting the enzyme that converts angiotensin I into angiotensin II, thus causing an increase in systemic vasodilation of peripheral blood vessels, resulting in a decrease in blood pressure. HS extracts have been shown to stimulate this pathway (Herman, 2020; Ojeda et al., 2010; Salem et al., 2020).
- Decreased blood viscosity through cyclooxygenase inhibitory activity (Christian et al., 2006).

Obviously, the compounds responsible for such activity must be the metabolites derived from the metabolic transformation of the LC and HS combination upon ingestion. Based on our own evidences obtained in cell and animal models and from others, we postulate that the putative candidate compounds that exert the hypotensive effect must be verbascoside, quercetin glucuronide and/or quercetin. In this context, 16% of the total dry weight of the dietary supplement were phenylpropanoids and verbascoside was the major compound in this family, meaning that the daily consumption of phenylpropanoids in the supplemented group was approximately 80 mg (Fernandez-Arroyo et al., 2012; Herranz-Lopez et al., 2015, 2017, Herranz-López et al., 2019, 2020; Joven et al., 2014a, 2014b). However, the identification of the active metabolites in human blood plasma and how these compounds reach their cell/tissue targets related to such effects should be further investigated. Therefore, these studies suggest that the combination of these extracts exerts a hypotensive effect through multifaceted mechanism, as reported (Medina-Remón et al., 2015). In conclusion, the LC + HS nutraceutical presented in this report offers new dietary possibilities in the treatment of pathologies associated to HT which molecular mechanisms need to be assessed in future research.

Ethics statement

All subjects provided written informed consent before participating. The protocol was in accordance with national legal requirements and the Helsinki Declaration for research on human beings and approved by the Ethics Committee of Miguel Hernández University (Elche, Spain) (reference IBM.VMM.01.17). The study was registered in Clinical Trials with the reference number NCT03507023.

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CRediT authorship contribution statement

Marina Boix-Castejón: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **María Herranz-López:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing - review & editing. **Mariló Olivares-Vicente:** Investigation, Methodology, Software. **Paula Campoy:** Data curation, Methodology. **Nuria Caturla:** Conceptualization, Funding acquisition, Visualization. **Jonathan Jones:** Conceptualization, Funding acquisition, Visualization. **Juan M. Zazo:** Funding acquisition, Investigation, Supervision. **Enrique Roche:** Conceptualization, Investigation, Validation, Visualization, Writing - review & editing. **Vicente Micol:** Conceptualization, Funding acquisition, Project administration, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104583.

References

Boix-Castejon, M., Herranz-Lopez, M., Perez Gago, A., Olivares-Vicente, M., Caturla, N., Roche, E., & Micol, V. (2018). Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: A randomized controlled trial. *Food & Function, 9*(6), 3173–3184. https://doi.org/10.1039/c8fo00367j.

Bolívar, J. J. (2013). Essential Hypertension: An Approach to Its Etiology and Neurogenic Pathophysiology. *International Journal of Hypertension, 2013*, 1–11. https://doi.org/10.1155/2013/547809.

Christian, K. R., Nair, M. G., & Jackson, J. C. (2006). Antioxidant and cyclooxygenase inhibitory activity of sorrel (Hibiscus sabdariffa). *Journal of Food Composition and Analysis, 19*(8), 778–783. https://doi.org/10.1016/j.jfca.2006.04.004.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L. - a phytochemical and pharmacological review. *Food Chemistry, 165*, 424–443. https://doi.org/10.1016/j.foodchem.2014.05.002.

El-Hawary, S. S., Yousif, M. F., Abdel Motaal, A. A., & Abd-Hameed, L. M. (2012). Bioactivities, phenolic compounds and in-vitro propagation of Lippia citriodora Kunth cultivated in Egypt. *Bulletin of Faculty of Pharmacy, Cairo University, 50*(1), 1–6. https://doi.org/10.1016/j.bfopcu.2011.12.001.

Fernandez-Arroyo, S., Herranz-Lopez, M., Beltran-Debon, R., Borras-Linares, I., Barrajon-Catalan, E., Joven, J., Fernandez-Gutierrez, A., Segura-Carretero, A., & Micol, V. (2012). Bioavailability study of a polyphenol-enriched extract from Hibiscus sabdariffa in rats and associated antioxidant status. *Molecular Nutrition & Food Research, 56*, 1590–1595. https://doi.org/10.1002/mnfr.201200091.

Ford, R. J., Teschke, S. R., Reid, E. B., Durham, K. K., Kroetsch, J. T., & Rush, J. W. (2012). AMP-activated protein kinase activator AICAR acutely lowers blood pressure and relaxes isolated resistance arteries of hypertensive rats. *Journal of Hypertension, 30*, 725–733.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., & Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation, 114*(12), 1752–1761. https://doi.org/10.1172/jci21625.

Greig, F. H., Ewart, M.-A., McNaughton, E., Cooney, C. M., & Kennedy, S. (2015). The hypotensive effect of acute and chronic AMP-activated protein kinase activation in normal and hiperlipidemic mice. *Vascular Pharmacology, 74*, 93–102. https://doi.org/10.1016/j.vph.2015.07.010.

Herman LL, P. S., Annamaraju P, Bashir K. (2020). Angiotensin Converting Enzyme Inhibitors (ACEI). In StatPearls.

Herranz-Lopez, M., Barrajon-Catalan, E., Segura-Carretero, A., Menendez, J. A., Joven, J., & Micol, V. (2015). Lemon verbena (Lippia citriodora) polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms. *Phytomedicine, 22*(6), 605–614. https://doi.org/10.1016/j.phymed.2015.03.015.

Herranz-Lopez, M., Fernandez-Arroyo, S., Perez-Sanchez, A., Barrajon-Catalan, E., Beltran-Debon, R., Menendez, J. A., Alonso-Villaverde, C., Segura-Carretero, A., Joven, J., & Micol, V. (2012). Synergism of plant-derived polyphenols in adipogenesis: Perspectives and implications. *Phytomedicine, 19*(3–4), 253–261. https://doi.org/10.1016/j.phymed.2011.12.001.

Herranz-López, M., Olivares-Vicente, M., Boix-Castejón, M., Caturla, N., Roche, E., & Micol, V. (2019). Differential effects of a combination of Hibiscus sabdariffa and Lippia citriodora polyphenols in overweight/obese subjects: A randomized

controlled trial. *Scientific Reports, 9*(1), 2999. https://doi.org/10.1038/s41598-019-39159-5.

Herranz-Lopez, M., Olivares-Vicente, M., Encinar, J. A., Barrajon-Catalan, E., Segura-Carretero, A., Joven, J., & Micol, V. (2017). Multi-Targeted Molecular Effects of Hibiscus sabdariffa Polyphenols: An Opportunity for a Global Approach to Obesity. *Nutrients, 9*(8), 907. https://doi.org/10.3390/nu9080907.

Herranz-López, M., Olivares-Vicente, M., Rodríguez Gallego, E., Encinar, J. A., Pérez-Sánchez, A., Ruiz-Torres, V., Joven, J., Roche, E., & Micol, V. (2020). Quercetin metabolites from Hibiscus sabdariffa contribute to alleviate glucolipotoxicity-induced metabolic stress in vitro. *Food and Chemical Toxicology, 144*, Article 111606. https://doi.org/10.1016/j.fct.2020.111606.

Hurtubise, J., McLellan, K., Durr, K., Onasanva, O., Nwabuko, D., & Ndisang, J. F. (2016). The different facets of dyslipidemia and hypertension in atherosclerosis. *Current Atherosclerosis Reports, 18*(12), 82. https://doi.org/10.1007/s11883-016-0632-z.

Islam, M. S. (2017). Ambulatory Blood Pressure Monitoring in the Diagnosis and Treatment of Hypertension. *Advancesin Experimental Medicine and Biology, 956*, 109–116. https://doi.org/10.1007/5584_2016_177.

Joven, J., Espinel, E., Rull, A., Aragones, G., Rodriguez-Gallego, E., Camps, J., Micol, V., Herranz-Lopez, M., Menendez, J. A., Borras, I., Segura-Carretero, A., Alonso-Villaverde, C., & Beltran-Debon, R. (2012). Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. *Biochimica et Biophysica Acta, 1820*(7), 894–899. https://doi.org/10.1016/j.bbagen.2012.03.020.

Joven, J., March, I., Espinel, E., Fernández-Arroyo, S., Rodríguez-Gallego, E., Aragonés, G., Beltrán-Debón, R., Alonso-Villaverde, C., Ríos, L., Martín-Paredero, V., Menéndez, J. A., Micol, V., Segura-Carretero, A., & Camps, J. (2014a). Hibiscus sabdariffa extract lowers blood pressure and improves endothelial pressure. *Molecular Nutrition & Food Research, 58*(6), 1374–1378. https://doi.org/10.1002/mnfr.201300774.

Joven, J., Micol, V., Segura-Carretero, A., Alonso-Villaverde, C., & Menéndez, J. A. (2014b). Polyphenols and the modulation of gene expression pathways: Can we eat our way out of the danger of chronic disease? *Critical Reviews in Food Science and Nutrition, 54*(8), 985–1001. https://doi.org/10.1080/10408398.2011.621772.

Kearney PM, W. M., Reynolds K, Muntner P, Whelton PK, He J. (2005). Global Burden of Hypertension: Analysis of Worldwide Data. The Lancet, 365(9455), 217-223. Doi: 10.1016/S0140-6736(05)17741-1.

Lee, Y.-S., Yang, W.-K., Kim, H. Y., Min, B., Caturla, N., Jones, J., Park, Y.-C., Lee, Y.-C., & Kim, S.-H. (2018). Metabolaid(®) Combination of Lemon Verbena and Hibiscus Flower Extract Prevents High-Fat Diet-Induced Obesity through AMP-Activated Protein Kinase Activation. *Nutrients, 10*(9), 1204. https://doi.org/10.3390/nu10091204.

López, M., Nogueiras, R., Tena-Sempere, M., & Diéguez, C. (2016). Hypothalamic AMPK: A canonical regulator of whole-body energy balance. *Nature Reviews Endocrinology, 12*(7), 421–432. https://doi.org/10.1038/nrendo.2016.67.

Luna-Luna, M., Medina-Urrutia, A., Vargas-Alarcon, G., Coss-Rovirosa, F., Vargas-Barron, J., & Perez-Mendez, O. (2015). Adipose Tissue in Metabolic Syndrome: Onset and Progression of Atherosclerosis [Review]. *Archives of Medical Research, 46*(5), 392–407. https://doi.org/10.1016/j.arcmed.2015.05.007.

Medina-Remón, A., Tresserra-Rimbau, A., Pons, A., Tur, J. A., Martorell, M., Ros, E., Buil-Cosiales, P., Sacanella, E., Covas, M. I., Corella, D., Salas-Salvadó, J., Gómez-Gracia, E., Ruiz-Gutiérrez, V., Ortega-Calvo, M., García-Valdueza, M., Arós, F., Saez, G. T., Serra-Majem, L., Pinto, X., Vinyoles, E., Estruch, R., & Lamuela-Raventos, R. M. (2015). PREDIMED Study Investigators. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutrition, Metabolism & Cardiovascular Diseases, 25*(1), 60–67. https://doi.org/10.1016/j.

MedTach. (2019). BPro Technology Validation, 24 hour Smart Watch ABPM Blood Pressure Monitor. Retrieved from https://www.medtach.com/bpro-reports.html/. Accessed February 18, 2019.

Mendis, S. (2010). World Health Organisation; 2010. Retrieved from http://www.who.int/nmh/publications/ncd_report2010/en/. Accessed April 24, 2019.

Messerli, F. H., Williams, B., & Ritz, E. (2007). Essential hypertension. *Lancet, 370*(9587), 591–603. https://doi.org/10.1016/S0140-6736(07)61299-9.

Ojeda, D., Jiménez-Ferrer, E., Zamilpa, A., Herrera-Arellano, A., Tortoriello, J., & Alvarez, L. (2010). Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. *Journal of Ethnopharmacology, 127*(1), 7–10. https://doi.org/10.1016/j.jep.2009.09.059.

Oliveras, A., & de la Sierra, A. (2014). Resistant hypertension: Patient characteristics, risk factors, co-morbidities and outcomes. *Journal of human hypertension, 28*(4), 213–217. https://doi.org/10.1038/jhh.2013.77.

Oparil, S., & Schmieder, R. E. (2015). New approaches in the treatment of hypertension. *Circulation Research, 116*(6), 1074–1095. https://doi.org/10.1161/circresaha.116.303603.

Ragone, M. I., Sella, M., Pastore, A., & Consolini, A. E. (2010). Sedative and cardiovascular effects of Aloysia citriodora Palau, on mice and rats. *Latin American Journal of Pharmacy, 29*(1), 79–86.

Salem, M. A., Michel, H. E., Ezzat, M. I., Okba, M. M., El-Desoky, A. M., Mohamed, S. O., & Ezzat, S. M. (2020). Optimization of an Extraction Solvent for Angiotensin-Converting Enzyme Inhibitors from Hibiscus sabdariffa L. Based on Its UPLC-MS/MS Metabolic Profiling. *Molecules, 25*(10), 2307. https://doi.org/10.3390/molecules25102307.

Samadian, F., Dalili, N., & Jamalian, A. (2016). Lifestyle Modifications to Prevent and Control Hypertension. *Iranian Journal of Kidney Diseases, 10*(5), 237–263.

Savica, V., Bellinghieri, G., & Kopple, J. D. (2010). The effect of nutrition on blood pressure. *Annual Review of Nutrition, 30*, 365–401. https://doi.org/10.1146/annurev-nutr-010510-103954.

Sun, G. Q., Li, Y. B., Du, B., & Meng, Y. (2015). Resveratrol via activation of AMPK lowers blood pressure in DOCA-salt hypertensive mice. *Clinical and Experimental Hypertension, 37*(8), 616–621. https://doi.org/10.3109/10641963.2015.1036060.

Vairavamurthy, J., Cheskin, L. J., Kraitchman, D. L., Arepally, A., & Weiss, C. R. (2017). Current and cutting-edge interventions for the treatment of obese patients. *European Journal of Radiology, 93*, 134–142. https://doi.org/10.1016/j.ejrad.2017.05.019.

Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E., Jr., Collins, K. J., Dennison Himmelfarb, C., DePalma, S. M., Gidding, S., Jamerson, K. A., Jones, D. W., MacLaughlin, E. J., Muntner, P., Ovbiagele, B., Smith, S. C., Jr., Spencer, C. C., Stafford, R. S., Taler, S. J., Thomas, R. J., Williams, K. A., Sr., Williamson, J. D., … Wright, J. T., Jr. (2018). 2017 AAC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journals of the American College of Cardiology, 71*(6), 1269–1324. https://doi.org/10.1161/HYP.0000000000000066.

WHO. (2007). Prevention of cardiovascular disease: guidelines for assessment and management of total cardiovascular risk. Retrieved from https://apps.who.int/iris/handle/10665/43685. Accessed April 14, 2020.

Williams, B., Mancia, G., Spiering, W., Agabiti Rosei, E., Azizi, M., Burnier, M., Clement, D. L., Coca, A., de Simone, G., Dominiczak, A., Kahan, T., Mahfoud, F., Redon, J., Ruilope, L., Zanchetti, A., Kerins, M., Kjeldsen, S. E., Kreutz, R., Laurent, S., Lip, G. Y. H., McManus, R., Narkiewicz, K., Ruschitzka, F., Schmieder, R. E., Shlyakhto, E., Tsioufis, C., Aboyans, V., & Desormais, I. (2018). 2018 ESC/ESH Guidelines for the management of arterial hypertension. *European Heart Journal, 39*(33), 3021–3104. https://doi.org/10.1093/eurheartj/ehy339.

Wong, I. Y. F., He, Z.-D., Huang, Y., & Chen, Z.-Y. (2001). Antioxidative activities of phenylethanoid glycosides from Ligustrum purpurascens. *Journal of Agricultural and Food Chemistry, 49*(6), 3113–3119. https://doi.org/10.1021/jf0100604.

Yeh, T. C., Shin, C. S., Chen, H. H., Lai, C. C., Sun, G. C., Tseng, C. J., & Cheng, P. W. (2018). Resveratrol regulates blood pressure by enhancing AMPK signaling to downregulate a Rac1-derived NADPH oxidase in the central nervous system. *Journal of Applied Physiology, 125*(1), 40–48. https://doi.org/10.1152/japplphysiol.00686.2017.

Zheoat, A. M., Gray, A. I., Igoli, J. O., Ferro, V. A., & Drummond, R. M. (2019). Hibiscus acid from Hibiscus sabdariffa (Malvaceae) has a vasorelaxant effect on the rat aorta. *Fitoterapia, 134*, 5–13. https://doi.org/10.1016/j.fitote.2019.01.012.

Capítulo 3

Phytomedicine, 2023, 113, 154735.

Plant compounds for obesity treatment through neuroendocrine regulation of hunger: A systematic review.

Marina Boix-Castejón, Enrique Roche, Mariló Olivares-Vicente, Francisco Javier Álvarez-Martínez, María Herranz-López, Vicente Micol.

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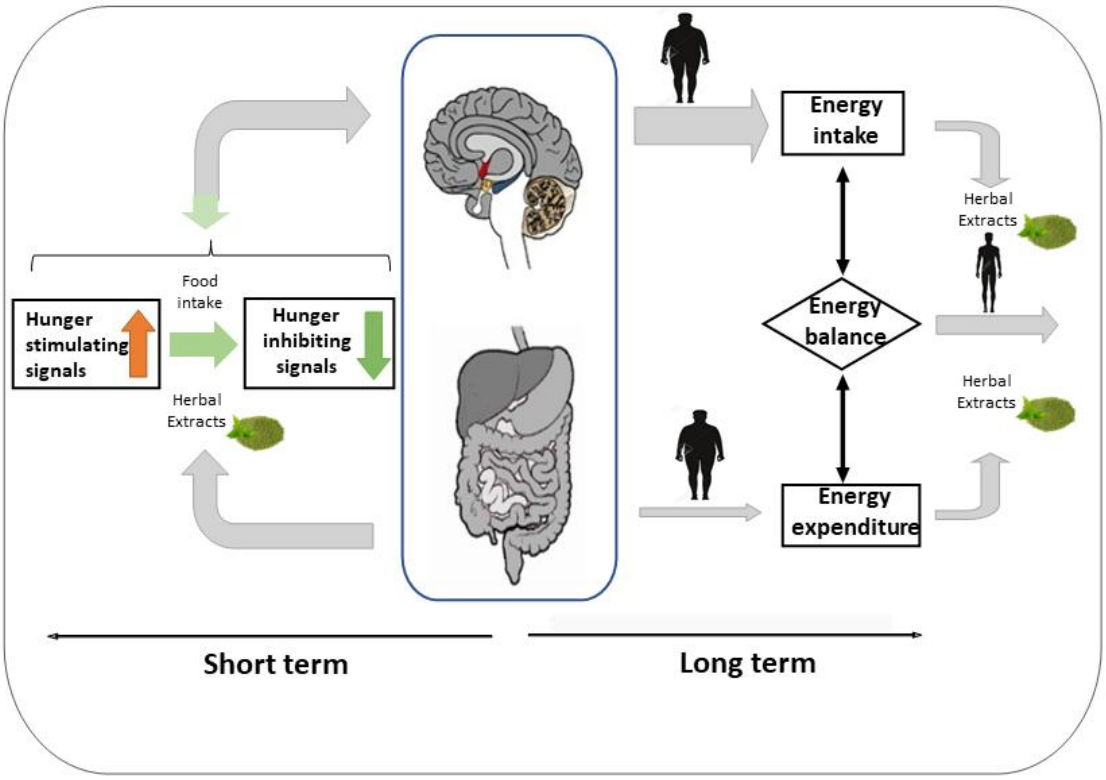

RESUMEN DE LOS RESULTADOS

La evidencia acumulada sobre los múltiples efectos contra la obesidad tanto en modelos celulares y animales, así como en humanos es cada vez más amplia.

La presente revisión, recoge los resultados publicados hasta el momento sobre los estudios centrados en compuestos naturales con efecto saciante o supresor del apetito. Se realizó una búsqueda de las hormonas orexigénicas y anorexigénicas para recoger y conocer las características de su fisiología. Los artículos revisados fueron contribuciones entre los años 2010 y 2021 obtenidos de la base de datos Medline vía Pubmed.

Los resultados de la revisión indican que tan solo unos pocos compuestos establecen de manera integral una posible correlación entre el efecto del ingrediente sobre el hambre o la saciedad y los cambios corporales y fisiológicos. Además, se ha comprobado que no existe una metodología común para evaluar el apetito, el hambre o la saciedad, se requerirían estudios clínicos más sistemáticos en las futuras investigaciones.

Cabe recalcar que son pocos los estudios con resultados concluyentes, por lo que serían necesarios ensayos más grandes (mayor número de participantes) y rigurosos (metodología protocolizada) para evaluar los efectos de los compuestos polifenólicos. Tras realizar la revisión, se ha comprobado que existen varios factores necesarios para poder dar consistencia a los estudios.

Un factor clave es la duración de las intervenciones, no existe uniformidad en la duración de los estudios. Son necesarios estudios más extensos para poder obtener resultados a largo plazo ya que la gran mayoría de las intervenciones son a corto plazo.

Las dosis óptimas de algunos compuestos como el extracto de Garcinia, no son rigurosas mostrando contraindicaciones entre estudios y no pudiendo confirmar resultados concluyentes por la gran variabilidad de resultados obtenidos.

Es necesaria mayor precisión para establecer condiciones de estudio más similares y un uso de la metodología más generalizado.

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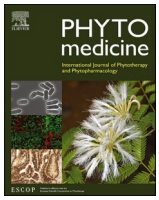

Review

Plant compounds for obesity treatment through neuroendocrine regulation of hunger: A systematic review

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ABSTRACT

Background: Food intake behavior is influenced by both physiological and psychological complex processes, such as appetite, satiety, and hunger. The neuroendocrine regulation of food intake integrates short- and long-term acting signals that modulate the moment of intake and energy storage/expenditure, respectively. These signals are classified as orexigenic, those that activate anabolic pathways and the desire of eating, and anorexigenic, those that activate the catabolic pathways and a sensation of satiety. Appetite control by natural vegetal compounds is an intense area of research and new pharmacological interventions have been emerging based on an understanding of appetite regulation pathways. Several validated psychometric tools are used to assess the efficacy of these plant ingredients. However, these data are not conclusive if they are not complemented with physiological parameters, such as anthropometric evaluations (body weight and composition) and the analysis of hormones related to adipose tissue and appetite in blood.

Purpose: The purpose of this manuscript is the critical analysis of the plant compounds studied to date in the literature with potential for the neuroendocrine regulation of hunger in order to determine if the use of phytochemicals for the treatment of obesity constitutes an effective and/or promising therapeutic tool.

Methods: Relevant information on neuroendocrine regulation of hunger and satiety for the treatment of obesity by plant compounds up to 2022 in English and/or Spanish were derived from online databases using the PubMed search engine and Google Scholar with relevant keywords and operators.

Results: Accordingly, the comparison performed in this review between previous studies showed a high degree of experimental heterogeneity. Among the studies reviewed here, only a few of them establish comprehensively a potential correlation between the effect of the ingredient on hunger or satiety, body changes and a physiological response.

Conclusions: More systematic clinical studies are required in future research. The first approach should be to decode the pattern of circulating hormones regulating hunger, satiety, and appetite in overweight/obese subjects. Thereafter, studies should correlate brain connectivity at the level of the hypothalamus, gut and adipose tissue with the hormone patterns modulating appetite and satiety. Extracts whose mode of action have been well characterized and that are safe, can be used clinically to perform a moderate, but continuous, caloric restriction in overweight patients to lose weight excess into a controlled protocol.

Abbreviations: AgRP, Aguti-related protein; AMPK, adenosine 5'-monophosphate-activated protein kinase; AP, anthropometric parameters; BF, Body fat; BG, β-glucan; BMI, Body mass index; BW, Body weight; CCK, Cholecystokinin; CNS, Central nervous system; CRH, Corticotropin releasing hormone; EGCG, Epigallocatechin gallate; GC, Green coffee; GIMM, Gastrointestinal microbiome modulator; GIP, Glucose-dependent insulinotropic polypeptide; GIT, Gastrointestinal track; GLP-1, glucagon-like peptide-1; HCA, Hydroxycitric acid; MCH, Melanin Concentrating Hormone; NPY, Neuropeptide Y; ORX, Orexin; OXM, Oxynthomodulin; PI-II, Proteinase inhibitor II; POMC, Pro-opiomelanocortin, PP, Pancreatic polypeptide; PYY, Peptide tyrosine-tyrosine; TG, triglycerides; VAS, Visual analog scale; WPI, Whey protein isolate.

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Introduction

Being overweight and obesity have become health problems of epidemic proportions at the European and global levels (Aranceta-Bartrina et al., 2005; Kim et al., 2015; WHO, 2015). Both conditions are associated with a metabolic disorder of multifactorial origin related to other chronic degenerative or inflammatory diseases (Ntrigiou V. N.I. et al., 2019), resulting from an energy imbalance between food intake and energy expenditure. Therefore, two main causes give rise to weight gain: overeating and sedentarism. Both have a different participation in obesity and overweight development, but overeating seems to play a trigger role resulting first in weight gain that impairs optimal body motion, which is essential for energy expenditure. Despite scientific efforts to understand the mechanisms that leads to overeating, only a few methods of weight control are effective in the long term. One implies that calorie restriction is difficult to implement with obese patients in cases of overeating but is the most effective strategy in the long term.

Food intake behavior is both physiologically and psychologically influenced and involves different related biological processes, such as appetite, satiety, and hunger. The psychological desire to eat is called appetite and is linked to sensory and pleasure experiences. Once the sensation of filling occurs, the feeding brake called satiety appears, inhibiting eating for a period of time. The term hunger defines the physiological need to eat food (Blundell, 1991). In this process, the central nervous system (CNS) plays a key role as an integrator of most of the actions that maintain energy balance, regulating energy intake, expenditure and storage (Huynh et al., 2016). Nevertheless, food intake control is not a process that occurs exclusively in the CNS; other peripheral signals and extrahypothalamic brain regions are involved (Myers et al., 2012; Waterson et al., 2015). When this complex neuroendocrine circuit system fails, pathologies appear to disturb the metabolic balance between intake and expenditure. Regarding food intake, obesity can result from a strong desire to eat, inappropriate food choices and/or a weak inhibition of eating when fat body stores have been filled.

The neuroendocrine regulation of food intake integrates many signals that can be grouped according to several criteria, as summarized in Fig. 1. Short-term signals act at the moment of food intake, while long-term signals are involved in nutrient storage and expenditure from body compartments, mainly adipose tissue. In this context, food intake is modulated by signals that control the starting and ending of meals through hunger/appetite regulation. In the long term, other signals are responsible for the filling of body stores accompanied by the appearance of a satiety sensation. Hunger and satiety sensations are regulated by several signals that contribute to maintaining or gaining body weight. These signals can be subdivided into i) orexigenic, those that activate anabolic pathways through an intrinsic desire to ingest food due to a strong sensation of hunger and appetite and promote the inhibition of energy expenditure, and ii) anorexigenic, those that activate the catabolic pathways, stimulating signals of gastrointestinal filling and hypothalamic satiety.

Plants produce a large amount of bioactive molecules with therapeutic potential in many pathologies, such as cardiovascular disease, diabetes, metabolic syndrome, cancer and obesity (Konstantinidi and Koutelidakis, 2019). In this context, certain compounds presenting biological activity through different in vitro and in vivo models have been the basis for the development of specific pharmacological formulations. Over time, scientific evidence has shown that the consumption of some specific plant bioactive compounds contributes to improving and treating metabolic disorders related to obesity. In this regard, there is a growing interest in the study of the mechanisms involved and the development of dietary supplements focused on the management of body weight and obesity. Some bioactive compounds, such as plant-derived polyphenols, have been shown to regulate energy metabolism and body weight through specific molecular metabolic pathways, including AMPK activation or the secretion of appetite hormones that control caloric intake (Camacho et al., 2015; Herranz-López et al., 2015,

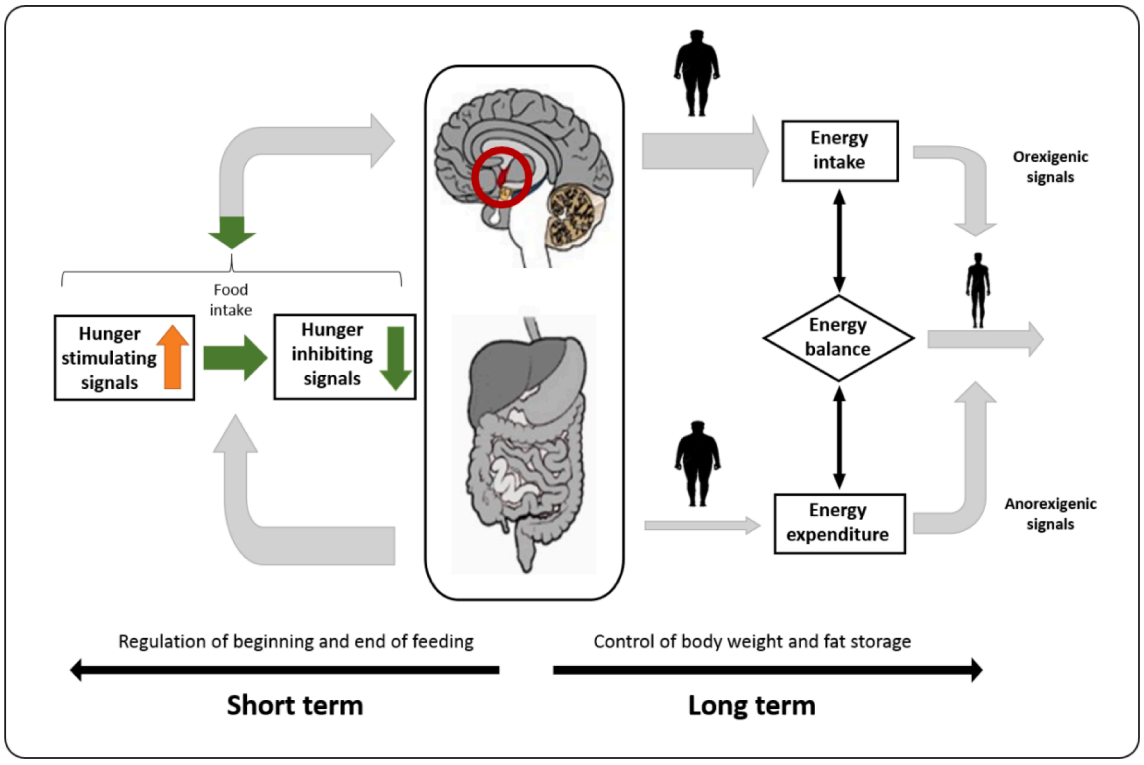

Fig. 1. Short-term (coming mainly from the GIT) and long-term (coming mainly from adipose tissue) regulatory signals of feeding and satiety. Short-term signals control hunger and appetite regulation at the moment of food intake. Long-term signals modulate energy storage to maintain body functions and promote physical activity. The central nervous system and hypothalamus (in red) play a key role as integrators of most of the signals that maintain energy balance, regulating energy intake, expenditure and storage. The disruption of one of these axes leads to obesity development.

2019, 2017; Jimenez-Sanchez et al., 2017; Joven et al., 2014b; Konstantinidi and Koutelidakis, 2019; Olivares-Vicente et al., 2018, 2019). In the long term, obese patients who adopt diets based on caloric restriction have a consequent energy deficit and weight loss, which leads to hunger and reduced energy expenditure. This so-called "energy gap" (Boix-Castejon et al., 2018) is an important barrier to the progress of body weight regulation. In this context, the appetite-suppressing property of certain plant compounds could be useful as a complementary strategy to energy-restricted diets.

The practical application of this manuscript is the critical analysis of the plant compounds studied to date in the literature with potential for the neuroendocrine regulation of hunger in order to determine if the use of phytochemicals for the treatment of obesity constitutes an effective and/or promising therapeutic tool. This knowledge is essential for the possible development of new nutraceutical strategies for the treatment of obesity and associated disorders.

Methodology

In this review, studies focusing on the use of plant compounds bearing satiating and/or appetite-suppressing effects, including information regarding their potential mechanism of action in healthy and overweight or obese individuals, were identified and summarized. A bibliographic search was also carried out on hormones with orexigenic and anorexigenic activity to better understand their physiology. Articles were extracted from the Medline database using the PubMed search engine and Google Scholar with relevant keywords and operators. The following search terms were used: “satiety”, “appetite”, “hunger”, “supplement”, “hormones”, “bioactive ingredient”, “nutraceutical”, “adipohormones”, “weight”, “obesity”, “polyphenol”,” compound”, ”phytochemical”, “CCK”, “GLP-1″, “ghrelin”, “PYY”, and “plant extracts”. No restrictions concerning the type of study were applied. Inclusion criteria for the review were as follows: (1) the study examined the effect of plant compounds on body weight or appetite regulation in humans or animal models; (2) the study was published in a peer-review journal; and (3) the study was published in English and/or Spanish. Contributions up to 2022 were included. 1488 records were identified, from which we reviewed 352 full-text documents after screening. Two reviewers independently screened the titles and abstracts of the identified studies for inclusion in the review. Full-text articles were obtained for all potentially relevant studies and were independently assessed for eligibility by the two reviewers. Finally, 106 papers were included in this review. The PRISMA 2020 flow diagram with the management of the collected information can be seen below in Fig. 2.

Hormones with orexigenic and anorexigenic activity

As previously stated, anorexigenic hormones suppress appetite and decrease food consumption, while orexigenic hormones stimulate appetite and increase food intake. Anorexigenic hormones include leptin, which is produced by adipose cells. Leptin acts on receptors in the hypothalamus to suppress appetite and decrease food intake (Perry et al., 2019). Other anorexigenic hormones include insulin, which is produced by the pancreas (Qiu et al., 2018), and peptide YY (PYY), which is produced by cells in the intestine (Hamamah and Covasa, 2022). Both insulin and PYY act on receptors in the hypothalamus to decrease appetite and food intake. The main anorexigenic hormones involved in appetite regulation are briefly described in Table 1. The role

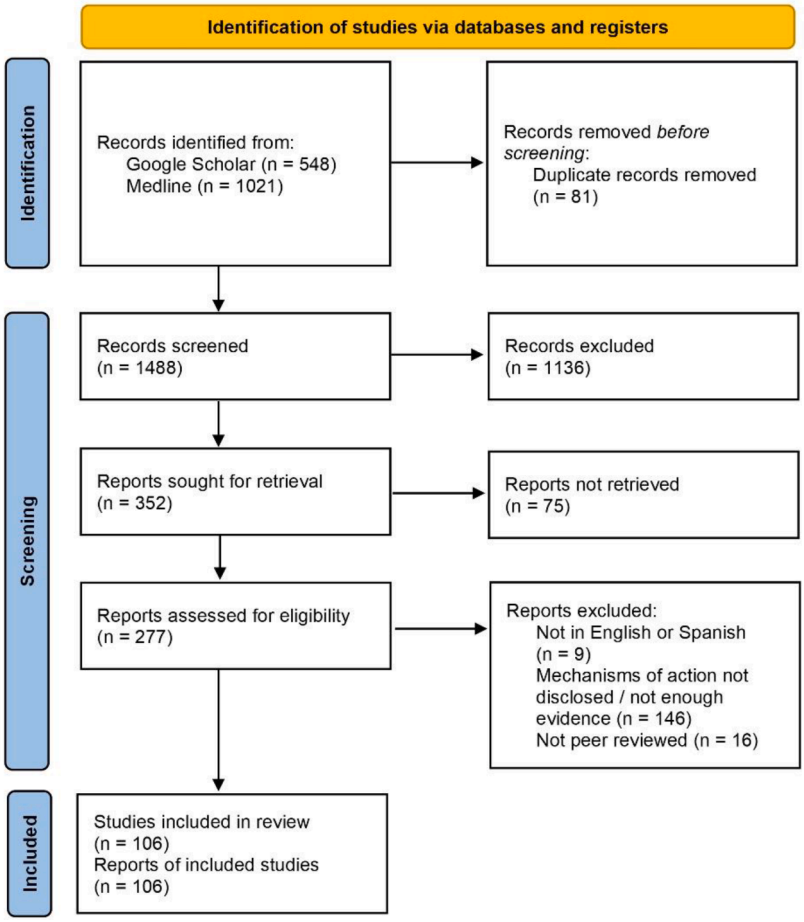

Fig. 2. PRISMA 2020 flow diagram.

Table 1
Main peptide hormones with anorexigenic activity, their synthesis, targets and functions.

Anorexigenic	Synthesis	Target	Function
Glucagon-like peptide-1 (GLP-1)	Small intestine (l cells), mainly ileum and colon. CNS areas	Receptors in the GIT, heart, vessels, kidney, muscle, and lung. At the CNS level, it acts in the hypothalamus	It regulates glucose homeostasis by increasing insulin synthesis and secretion and inhibiting glucagon production. It slows gastric emptying (Carranza Quispe, 2016; Jaimes et al., 2005; Näslund E et al., 2004; Verdich et al. C, 2001)
Insulin	Endocrine pancreas. CNS areas.	Receptors at the CNS level, liver, skeletal muscles, and adipose tissue	It regulates anabolic metabolism. It favors the formation of fatty tissue and increases leptin production. It decreases NPY expression (Mitchell and Begg, 2021)
Obestatin	GIT	AgRP neurons of the arcuate nucleus of the hypothalamus	It participates in the process of gastric emptying, insulin release and pancreatic β cell survival (Alvarez-Crespo et al., 2009; Zhang et al., 2005)
Resistin	Adipose tissue	Liver	It participates in energy homeostasis and modulates insulin resistance (Heilbronn et al., 2004; Rajala et al., 2004)
Oxynthomodulin (OXM)	Small intestine (l cells). CNS areas	GLP-1 receptors and hypothalamus	It reduces circulating ghrelin (Cohen et al., 2003), causing a reduction in the feeling of hunger. It decreases gastric emptying and inhibits gastric secretion
Peptide Tyrosine-Tyrosine (PYY)	Small intestine (l cells) from the distal ileum and colon. CNS regions	Y receptors in the CNS at the level of the hypothalamus	It increases satiety (postpones the consumption of the next meal) and delays gastric emptying (Batterham et al., 2003; Nicolaïdis, 2008). It inhibits NPY secretion
Cholecystokinin (CK)	Small intestine CNS areas	CCK-1 receptors, in the gastrointestinal tract, and CCK-2 receptors, in the nervous system	It inhibits ghrelin and stimulates the release of PYY. It is involved in the secretion of pancreatic enzymes and in thermoregulation processes. It delays gastric emptying (Cummings and Overduin, 2007; Frühbeck, 2005; Gibbs and McHugh, 1976)
Pancreatic polypeptide (PP)	Endocrine and exocrine pancreas and in distal regions of the gastrointestinal tract such as colon or rectum	Y receptors in the CNS at the level of the hypothalamus	It modulates satiety and controls energy homeostasis. PP is secreted after food ingestion correlating with the amount of calories ingested (Alvarez-Crespo et al., 2009)
Pro-opiomelanocortin (POMC)	CNS: arcuate nucleus of hypothalamus, anterior pituitary	Different areas of the brain	It signals satiety and reduces food intake. It controls body energy status by integrating signals from peripheral ghrelin, leptin, and insulin to regulate feeding and energy expenditure (Vohra et al., 2022)
Glucose-dependent insulinotropic polypeptide (GIP)	K cells of the small intestine, located mainly in duodenum, and in smaller amounts in jejunum and ileum	Adipocytes	It stimulates insulin secretion, increases the sensation of gastric fullness (Daousi et al., 2009). Controversial results regarding food intake
Leptin	Mainly expressed and produced by adipose tissue	Hypothalamus stimulating POMC and inhibiting NPY production	It regulates energy expenditure and eating behavior. Increased adiposity is related to leptin production (lipostatic action). Leptin suppresses appetite by stimulating anorexigenic signals (POMC) and decreasing orexigenic signals (NPY) (Frühbeck, 2005)
Adiponectin	Adipose tissue. CNS	It can cross the blood brain barrier and binds to receptors located in the CNS (Bassi et al., 2012)	It regulates energy homeostasis. It stimulates the oxidation of fatty acids. It improves insulin sensitivity. It stimulates food intake and decreases energy expenditure during fasting by acting on the CNS (Kubota et al., 2007)
Amylin	Pancreatic β cells	Hypothalamus, interacting with peptides such as leptin, CCK or GLP-1	It reduces gastric intake, promoting a reduction in body weight and improving glycemic control (Zhang et al., 2016)
Corticotropin releasing hormone (CRH)	Hypothalamic paraventricular nucleus	Pituitary	Regulates energy balance and modulates the response to stress (Frühbeck, 2005)

Other abbreviations used: AgRP, aguti-related protein; CNS, central nervous system; GIT, gastrointestinal track; NPY, neuropeptide Y.

of some of them has been known for decades, and the theoretical postulates about the processes of hunger and satiety regulation have been expanded and modified over several scientific reports. However, the physiological relevance of these hormones and the mechanisms that regulate their secretion and action have not been precisely determined (Yoshihiro Suzuki, 2014). Numerous drugs can reduce body weight, including those derived from hormones produced and secreted by the gastrointestinal tract (i.e., glucagon-like peptide-1/GLP-1) or adipose tissue (i.e., leptin). In particular, these two hormones have been extensively studied and seem to have clear anti-obesity action, reducing body weight and regulating glucose metabolism (Müller et al., 2018).

Orexigenic hormones act on receptors in the brain, particularly in the hypothalamus, to regulate energy balance and hunger. They include ghrelin, which is produced by cells in the stomach and hypothalamus. Ghrelin primarily stimulates receptors in the hypothalamus to promote appetite and food intake. Table 2 describes the main orexigenic hormones involved in appetite regulation.

Psychometric assessment of appetite, hunger, satiety, and fullness in clinical trials

First, appetite modulation by plant ingredients and compounds can be assessed based on subjective aspects obtained through validated questionnaires. One of these tools is the visual analog scale (VAS), a psychometric response scale able to quantify the feeling of hunger, satiety, desire to consume food or the feeling of fullness. In addition, VAS assesses the amount of food consumed over a period of time using a food consumption log. However, these data are not conclusive if they are not supported by complementary physiological parameters, such as body weight control, specific anthropometric evaluations (body composition), bioimpedance or adipohormone analysis. The effects of various bioactive phytochemicals have been evaluated by means of the VAS, but very few reports complete the obtained information by analyzing anorexigenic/orexigenic circulating hormones. Therefore, a comparison between studies is difficult due to the high degree of heterogeneity. More systematic clinical studies are required in future research.

The first heterogeneity results from the use of the terms “hunger,

Table 2
Main peptide hormones with orexigenic activity, their synthesis, targets and functions.

Orexigenic	Synthesis	Target	Function
Ghrelin	Parasympathetic nervous system, GIT and oxyntic glands of the stomach (Cummings et al., 2001). It is also synthesized in a lesser extent throughout the intestine and in the CNS	Hypothalamus. Receptors in stomach, intestine, pancreas, adipose tissue, cardiovascular system, testis, ovary and muscle	It induces appetite and accelerates gastric emptying by increasing plasma levels before meals (Goldstein et al., 2011; González-Jiménez and Schmidt Río-Valle, 2012; Tschop et al., 2000) Excitatory effect on neurons producing NPY and AgRP; and inhibitory effect on neurons that produce POMC
Neuropeptide Y (NPY)	GIT and arcuate nucleus of the hypothalamus	Y receptors in the CNS at the level of the hypothalamus	It promotes feeding, reduces energy expenditure, regulates energy balance, controlling whole-body energy homeostasis (Beck, 2006; Loh et al., 2015, 2017)
Orexin (ORX)/ Hypocretin	Neurons located mainly in the perifornical area of the posterolateral hypothalamus	Adipose tissue	It contributes to early hyperphagia (Linehan et al., 2020), stimulates feeding, controls energy regulation and participates in the neuroendocrine regulation of GIT (Ebrahim et al., 2002; Nuñez et al., 2009)
Melanin Concentrating Hormone (MCH)	Hypothalamus	MCH receptor 1 in brain and other areas of the CNS	MCH overexpression promotes hyperphagia, weight gain, and lipogenesis (Carranza Quispe, 2016). It participates in energy balance and emotional control (Torterolo et al., 2010)
Agouti-related protein (AgRP)	Hypothalamus	NPY neurons	It blocks the MC3 and MC4 receptors of melanocortin, thus preventing its anorectic effect (Frühbeck, 2005)

Other abbreviations used: CNS, central nervous system; GIT, gastrointestinal track; POMC, pro-opiomelanocortin.

appetite and satiety". This ambiguity causes misinterpretations when comparing different studies. A consensus definition of these terms is instrumental to compare the results obtained from different reports. Furthermore, the methodology for evaluating appetite, satiety, hunger, or food consumption is not lineal. Moreover, conformity in the use of VAS is lacking for the evaluation of subjective sensations, such as indicators of regulation, feelings of hunger or satiety and prospective consumption or desire to consume some type of specific or general food. In addition, methods to validate individual states of mind and motivations vary between studies (Blundell et al., 2010). Due to these limitations, the record of food consumption ad libitum has been proposed as an alternative and more precise methodology to assess satiety.

In addition, the length of the studies varied, challenging subsequent analysis. Numerous studies show significant effects on appetite suppression, hunger suppression, or increased satiety, but most are short-term trials. This observation suggests that the ingestion of certain nutraceuticals over longer periods should be further investigated. Moreover, new studies that strictly control the macronutrient content of participants are warranted to draw conclusions regarding food preferences. Overall, scientific consensus is necessary to standardize experimental conditions between studies and establish a more general methodology.

Recently, neuroimaging technology has added a new perspective in the research of human brain-gut interactions. In this context, Zanchi et al. (Zanchi et al., 2017) demonstrated a direct link between changes in the plasma concentrations of certain hormones and changes in the brain regions that form part of the neural circuit of appetite. To date, the main discrepancy in the different studies lies in the variability of the experimental designs.

Knowledge of the mechanism of action of endocrine signals could provide a candidate pathway to develop new therapies for the prevention and even treatment of metabolic syndrome. In-depth studies of appetite regulation demonstrate the complexity of the participating mechanisms. This new knowledge clearly supports that pathologies such as being overweight or obese, are not only metabolic disorders but also neurological imbalances.

Phytochemicals with anorexigenic activity: molecular mechanism

The scientific literature is extensive in relation to medicinal plants or plant-based nutraceuticals that have shown benefits in preventing metabolic syndrome, improving insulin sensitivity, or improving glucose tolerance. However, data on nutraceuticals acting specifically as appetite modulators are scarce (Zuñiga et al., 2017). To date, few studies have confirmed a clear decrease in appetite or hunger or an increase in satiety or fullness using plant extracts or nutraceuticals compared to a placebo. Nevertheless, molecular mechanisms have been studied in more detail in cell or animal models. Therefore, the results obtained in these systems need to be interpreted with caution due to their difficulty to be extrapolated to humans (Timper et al., 2017). Table 3 lists the plant ingredients and phytochemicals with anorexigenic activity together with their active components, effects, mechanisms of action and methods of measuring their effect.

The extracts shown in Table 3 have provided evidence for a potential modulation of the feeling of plenitude that likely act in the appetite centers of the brain. The putative mechanism of action might be similar to that of gut hormones by inducing signals of satiety and fullness, which regulates energy homeostasis in humans (Murphy and Bloom, 2006). Examples of hunger neuroendocrine regulators presented in Table 3 include EGCG that increased adiponectin levels, maintaining glucose, insulin and leptin levels, achieving a delayed gastric emptying in healthy women (Fernandes et al., 2018). Consumption of MetabolAid®, composed of polyphenolic extracts of *H. sabdariffa* and *L. citriodora*, generated improvement of anthropometric measurements, decreased blood pressure and heart rate and a more positive perception in the overall health status by increasing anorexigenic hormones (GLP-1) and decreasing orexigenic hormones (ghrelin) (Herranz-López et al., 2019). Slendesta®, a standardized potato extract, achieved lower postprandial hunger, desire to eat, and prospective consumption, as well as significantly higher postprandial fullness by increasing plasma levels of CCK (Zhu et al., 2017). These plant ingredients exert their metabolic effect through the increase of anorexigenic hormones present in Table 1 or by decreasing the levels of orexigenic hormones present in Table 2. The state of energy balance is sent to key brain regions, such as the hypothalamus and brainstem, using central and peripheral signals. In addition, some of these ingredients show a decrease in hunger ratings and an increase in satiety hormone levels. Overall, energy intake and expenditure are homeostatically regulated, leading to a cascade of reactions that reduces the sensation of hunger. This cascade results in a significant reduction of appetite sensation or a stimulation in early satiety, leading to a decrease in body weight.

Some of the studies shown in Table 3 did not exhibit conclusive results, and larger and more rigorous trials are needed to objectively assess the effects of the proposed polyphenolic compounds (Brum et al., 2016; Onakpoya et al., 2011b; Rebello et al., 2012). For example, the dosage for Garcinia extracts and HCA are not conclusive in the reports, and to date, the correct dose of HCA is unknown because each study has concluded different optimal doses (Onakpoya et al., 2011b).

Table 3
Main plant ingredients and phytochemicals with anorexigenic activity, their herbal parts used, extraction solvents, experimental models, routes of administration, active components, effects on appetite/satiety, body changes, other relevant effects, target/mechanisms of action and appetite/weight measurements used.

Plant/Extract (*scientific name*)	Herbal part used	Extraction solvent	Dosage	Experimental models	Route of administration	Description/ Active components	Effect on Appetite/ Satiety	Body Changes	Other relevant effects	Target/ Mechanism	Appetite/ Weight measurements	References
Baobab extract (*Adansonia digitata* l. (*Bombacaceae*))	Fruit	Not disclosed	15 g	Human	Oral	Smoothie of baobab extract	↓Hunger	Possible effect on weight maintenance.	No significant difference in calorie intake at an ad libitum meal	Unclear	Subjective ratings with VAS	(Garvey et al., 2017)
Oat β-glucan (BG) (*Avena sativa* l. (*Poaceae*))	Seed	Not applicable	3–4 g	Human	Oral	Varying doses of β-glucan in extruded breakfast cereals	↑Satiety	No other effects	↓Post-prandial glycemia	↑ The viscosity of the meal bolus in the stomach and delays gastric emptying	Glycemia	(Tosh, 2013)
Caraway extract (*Carum carvi* l. (*Apiaceae*))	Seed	Water	30 ml per day	Human	Oral	Tannins, alkaloids and terpenoids. Carvone and limonene. Aqueous extract from the seeds	↓Appetite	↓AP ↓Carbohydrate intake	Indigestion and pneumonia treatment. Galactagogue and carminative. Management of functional dyspepsia	↑ The secretion of gastric juice and promotes bile release.	Calorie, macronutrient intake and AP. VAS and an ad libitum pizza test	(Kazemipoor et al., 2016; Mahboubi, 2019)
Whey protein isolate (WPI) and cocoa polyphenols (*Theobroma cacao* l. (*Sterculiaceae*))	Cocoa seed	Not disclosed	340 g beverage	Human	Oral	Food formulation with bioactive ingredients:WPI is rich in leucine. Cocoa is rich in polyphenols	↓Hunger ↑Satiety	No other effects	Improve markers of metabolic syndrome	↑ Adiponectin levels and regulates insulin receptor expression	Glycemia, adiponectin levels and hunger ratings	(Campbell et al., 2016)
Coffee extract (*Coffea arabica* l. (*Rubiaceae*))	Fruit	Water	3–4 cups per day	Human	Oral	Coffee bean extracts	↓Hunger	↓BW ↓BF	Antioxidant activity	↑Plasmatic PYY	Biomarkers of oxidative stress response in blood. BW and intake of energy	(Bakuradze et al., 2011; Greenberg, 2012; Schubert et al., 2014)
Garcinia extract (*Garcinia cambogia*Desr. (*Clusiaceae*), *Garcinia indica* (Thouars) Choisy (*Clusiaceae*), *and Garcinia atroviridis* Griff. ex T.Anderson (*Clusiaceae*))	Not disclosed	Not disclosed	1–2.8 g of HCA per day	Human	Oral	Hydroxycitric acid (HCA)	↓Appetite	↓BW ↓BF		Inhibition of the lipogenic enzyme ATP-citrate lyase and increased release of serotonin in the brain, resulting in appetite suppression.	BW	(Onakpoya et al., 2011b)
Green tea extract (*Camellia sinensis* (l.) Kuntze (*Theaceae*))	Not disclosed	Not disclosed	752 mg of EGCG	Human	Oral	EGCG	↑Fullness ↑Satiety	↓BW ↓BMI ↓BF	EGCG reduces TBK1 activity, reducing TLR4, which impacts insulin resistance, inflammation, and hepatic lipid storage in obesity-related symptoms of high-fat diets. This downregulatess the production of TBK1-	Hormonal modulation. Acute EGCG supplementation is able to delay gastric emptying	Gastric emptying, VAS, insulinemia, glycemia, adiponectin and leptin levels	(Fernandes et al., 2018; Li et al., 2022)

(*continued on next page*)

Table 3 (*continued*)

Plant/Extract (*scientific name*)	Herbal part used	Extraction solvent	Dosage	Experimental models	Route of administration	Description/ Active components	Effect on Appetite/ Satiety	Body Changes	Other relevant effects	Target/ Mechanism	Appetite/ Weight measurements	References
									targeted genes, including TNF- and IL-6.			
Decaffeinated green coffee (GC) phenolic extract alone or combined with oat β-glucans (GC/BG)	Not disclosed	Not disclosed	5 g per day of GC/BG	Human	Oral	B-CAN™: 5 g/ day of 70% oat BG and 600 mg/ day of GC polyphenols	↓Hunger GC/BC is more efficient than GC	No other effects		↓ Maximum ghrelin levels. GC/ BG more efficient than GC	Subjective ratings with VAS. In a subgroup of participants blood levels of CK, PYY, GLP-1, ghrelin and leptin	(Redondo-Puente et al., 2021)
Gastrointestinal microbiome modulator (GIMM)	Not applicable	Not applicable	4 g of inulin, blueberry extract equivalent to two cups of whole blueberries, and 2.5 g of oat β-glucan	Human	Oral	Inulin, BG, blueberry anthocyanins, and blueberry polyphenols	↑Satiety		Improved blood glucose tolerance	↑ In fasting plasma PYY and ↓ in plasma ghrelin. Effects on serum glucose may be through insulin-independent pathways	Blood levels of gut microbiota, satiety hormones, glycemic control and lipid determination. Subjective ratings.	(Rebello et al., 2015)
MetabolAid® (*Hibiscus sabdariffa* l. (*Malvaceae*) + *Lippia citriodora* (Paláu) Kunth (*Verbenaceae*))	Not disclosed	Not disclosed	500 mg per day	Oral	Human	Combination of polyphenolic extracts	↑Satiety	↓BW ↓AP	Modulating the plasma concentrations of adipohormones, through the release of adiposity factors and intestinal peptides that control appetite and satiety	Activation of the AMPK pathway	Blood pressure, BW, AP and VAS. Serum adipohormones	(Arluison et al., 2004; Barrajon-Catalan et al., 2014; Boix-Castejon et al., 2018; Fernández-Arroyo et al., 2011; Herranz-López et al., 2019; Joven et al., 2014a; Turnley et al., 1999)
Psyllium	Not applicable	Not applicable	6.8 g once or twice a day	Human	Oral	Soluble fiber obtained from *Plantago major subvar. ovata Pilg.* (*Plantaginaceae*).	↓Hunger ↑Satiety ↑Fullness	↓BMI ↓BF	Reduction in plasma TG	↑ In fiber intake is linked to a ↓ in energy intake.	Hunger and desire to eat. AP, lipids, TG, BW and BF	(Brum et al., 2016; Pal et al., 2011)
Red pepper, chili, cayenne, or paprika extracts (*Capsicum annuum* l. (*Solanaceae*))	Not disclosed	Not disclosed	1.03 g of red chili pepper (2.56 mg capsaicin) per meal	Human	Oral	Capsaicin	↑Fullness ↑Satiety	↓BW ↓Energy intake ↑Energy expenditure	↑ Fat oxidation and thermogenesis.	Effect might be related to ↑ in sympathetic nervous system activity.	VAS. Energy expenditure, core body and skin temperature, and subjective ratings	(Janssens and Westerterp-Plantenga , 2014) (Ludy, 2011)
Slendesta®: Standardized potato (*Solanum tuberosum*l. (*Solanaceae*)) extract	Tuber	Water	15 mg of proteinase inhibitor II (PI-II)	Human	Oral	The active compound is a proteinase inhibitor II (PI-II)	↓Food consumption ↑Satiety ↑Fullness	↓BW	↓Epididymal fat. Histomorphological changes of fat and pancreas.	↑ CCK secretion ↑ UCP1 and beige-specific genes causing elevated energy expenditure.	VAS, and blood levels of CCK, insulinemia and glycemia	(Zhang et al., 2022; Zhu et al., 2017)
Satiereal® (*Crocus sativus* l. (*Iridaceae*))	Stigma	Not disclosed	176.5 mg extract per day	Human	Oral	Extract from saffron stigma.	↓Caloric intake ↑Satiety	↓BW ↓Snacking frequency		Suggested mood-improving effect	BW and subjective ratings	(Gout et al., 2010)

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Table 3 (*continued*)

Plant/Extract (*scientific name*)	Herbal part used	Extraction solvent	Dosage	Experimental models	Route of administration	Description/ Active components	Effect on Appetite/ Satiety	Body Changes	Other relevant effects	Target/ Mechanism	Appetite/ Weight measurements	References
White bean + Artichoke (*Phaseolus vulgaris* l. (*Leguminosae*) + *Cynara scolymus* l. (*Asteraceae*))	*P. vulgaris*: beans. *C. scolymus*: flowering buds.	Not disclosed	100 mg *P. vulgaris* extract + 200 mg *C. scolymus* extract 3 times a day.	Human	Oral	Crocetin and safranal α-amylase inhibitor	↑Satiety	↓AP ↓BMI ↓BW ↓BF	↓Carbohydrate absorption. Hypoglycemic effect. Improvements in glucose and lipid profiles		Lipidemia and glycemia VAS BW, AP and BF	(Barrett and Udani, 2011; Onakpoya et al., 2011a; Rondanelli et al., 2011)
Sorghum (*Sorghum bicolor* (l.) Moench (*Poaceae*)) flaked biscuits	Grain	Not applicable	50 g per day	Human	Oral	Sorghum whole grain	↑Satiety compared to bread	No other effects		↑ Postprandial GLP-1 and GIP levels. Hormonal modulation	VAS, glycemia, insulinemia and blood levels of GIP, GLP-1, PYY, and ghrelin	(Stefoska-Needham et al., 2016)

Other abbreviations and symbols used: AP, anthropometric parameters; BF, body fat; BG: β-glucan; BMI, body mass index; BW, body weight; CCK: Cholecystokinin; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; PYY, peptide tyrosine-tyrosine; TBK1: TANK-binding kinase 1; TG, triglycerides; TLR4: toll-like receptor 4; UCP1: uncoupling protein 1; VAS: visual analog scale; (↑) increase; (↓) decrease.

Additionally, some results show no solid evidence or show contradictions (Logan et al. , 2006; Rebello et al., 2012). Therefore, larger, well-controlled randomized clinical trials are needed, particularly in the case of Garcinia and red pepper.

Another factor necessary to increase consistency between studies is the length of interventions. Most of the studies are short-term; therefore, longer studies are needed to evaluate the long-term effect of polyphenolic extracts (Zhu et al., 2017). Inconsistencies in the composition of the sources from which polyphenols were obtained have been noticed when comparing different studies. For instance, extracts containing BG from different sources provide similar benefits, but each product requires individual testing to assess the possible role of other compounds present in the extract (Tosh, 2013). Additionally, studies using coffee extracts to decrease appetite (Campbell et al., 2016; Kazemipoor et al., 2016; Mahboubi, 2019) are incomplete, as they suggest that one or more noncaffeine ingredients that remain to be identified may have the potential to decrease body weight (Bakuradze et al., 2011; Greenberg , 2012; Redondo-Puente et al., 2021; Schubert et al., 2014).

Other reports show no significant differences when comparing appetite/satiety-related hormones (Redondo-Puente et al., 2021) or in insulin sensitivity, plasma satiety hormones, or serum lipid concentrations between the intervention groups (Rebello et al., 2015). Moreover, the subjective feeling of satiety in response to nutraceutical supplements derived from plant extracts, such as nopal (El-Mostafa et al., 2014) or baobab (Garvey et al., 2017), has been studied without assessing the levels of circulating hormones. The considerable variability and inadequacy of the presented randomized clinical trials necessitate larger and more rigorous designs to objectively assess the effects of polyphenolic extracts on body weight reduction (Onakpoya et al., 2011a). In some studies present in Table 3, adverse events were reported, including nausea, gastric intolerance or headache (Gout et al., 2010; Onakpoya et al., 2011b). Therefore, the optimum dosage needs to be assessed.

Plant preparations are generally safer than synthetic anti-obesity medications. This is attributed to the variety of metabolites contained in these formulations in addition to their active substances, which cause a variety of targeted reactions and can control side effects (Kazemipoor et al., 2015). In addition, most of the agents with anorexigenic capacity included in Table 3 come from plant ingredients or phytochemicals that are regularly consumed in the human diet and are known not to present toxicity at the levels used. However, this can depend on the specific plant extract. Garcinia extracts have been used as weight loss supplement in a variety of clinical trials (Onakpoya et al., 2011b). Their action can be attributed to hydroxycitric acid (HCA), a derivative of citric acid. The anti-obesity effects of HCA are known to be caused by a number of mechanisms. HCA inhibits the enzyme adenosine triphosphatase-citrate-lyase, an enzyme for the extra-mitochondrial catalysis of citrate into oxaloacetate and acetyl coenzyme A (acetyl-CoA), a component of the synthesis of fatty acids. Thus, HCA limits the availability of two carbon groups needed for the production of lipids and cholesterol by decreasing the acetyl-CoA and subsequently the malonyl-CoA pool (Tomar et al., 2019). However, there might be a potential causal association between consumption of Garcinia products and development of acute liver injury (Crescioli et al., 2018). HCA has shown to increase the amount of hepatic collagen, lipid peroxidation, mRNA levels of oxidative stress-related genes (such as glutathione peroxidase and superoxide dismutase), and inflammatory responses (Kim et al., 2013) (such as tumor necrosis factor and monocyte chemoattractant protein-1). Therefore, further research should be done in this clinical setting to identify, isolate, and analyze the toxicological effects of plant active principles.

The plant-based nutraceuticals described in Table 3 may exert anti-obesity effects due to their active components. These active components may have a variety of mechanisms of action that can contribute to weight loss or weight management. For example, leucine has shown to promote a decrease in adiposity by inhibiting lipogenesis, promoting lipolysis and fatty acid oxidation, and greatly increasing leptin release in

adipocytes via the mTOR signaling pathway. Dietary leucine lowers hyperglycemia, lowers the rate of fat formation, lowers body fat, and raises insulin sensitivity, all of which are brought on by the high fat diet (Zhang et al., 2020). EGCG inhibits TLR4 signaling by specifically inhibiting TANK binding kinase 1 (TBK1) activity and consequently downregulating the expression of TBK1-targeted genes, including TNF-α, and IL-6. TNF-α is crucial for lipid metabolism and hepatocyte cell death in the emergence of obesity (Li et al., 2022). Inulin can alter the gut microbiota of obese individuals, increase the abundance of bifidobacteria and *Akkermansia muciniphila* in obese individuals, and improve metabolic disorders. Short-chain fatty acids in the colon, such as acetic acid, propionic acid, and butyric acid, change in response to inulin administration. Dietary treatment with these metabolites has been demonstrated to considerably reduce body weight gain by boosting beige adipogenesis, mitochondrial biogenesis, and triglyceride hydrolysis and fatty acid oxidation in adipose tissue (Wu et al., 2022).

The anti-obesity properties of capsaicin may be mediated via a variety of mechanisms. By increasing the expression of PPARγ and UCP-1 in preadipocytes and adipocytes, capsaicin can suppress adipogenesis (Szallasi, 2022). As a result, it will promote the release of adiponectin and lead to an increase in body fat storage. Capsaicin can cause an increase in UCP-1 and PGC-1α expression and brown adipose tissue activity (Takeda and Dai, 2022). It can also decrease insulin resistance, promote satiety, and reduce appetite. Finally, capsaicin can influence the function of the gastrointestinal tract and gut microbiota stimulating GLP-1 secretion and increasing the population of the gut bacterium *Akkermansia muciniphila* (Zheng et al., 2017),

Recently, PPI II increased the expression of the uncoupling protein 1 (UCP1) protein and gene and beige-specific genes, including Cd137, Cited1, Tbx1, and Tmem26 in vitro. PPI II treatment for three months in diet-induced obesity mice increased the levels of the UCP1 protein in white adipose tissue, causing elevated energy expenditure, thus preventing obesity and improving glucose tolerance (Zhang et al., 2022). α-Amylase inhibitors are effective in reducing postprandial hyperglycemia by slowing the digestion of carbohydrates and absorbing postprandial glucose. The formation and accumulation of triacylglycerol are inhibited by reducing postprandial hyperglycemia via preventing glucose uptake into adipose tissue (Kim et al., 2020). Additionally, α -amylase inhibitors might alter the gut microbiota, potentially boosting the diversity of species such as Bacteroidetes and Akkermansia as well as short fatty-acid-producing bacteria (Peddio et al., 2022).

Data from Table 3 demonstrate a lack of rigorous studies on the effects of nutraceuticals based on plant ingredients. Among the studies reviewed that claimed an effect of the ingredient on hunger or satiety for the management of obesity, only a few of them (Boix-Castejon et al., 2018; Fernandes et al., 2018; Rebello et al., 2012; Zhu et al., 2017) have measured in some extent anthropometric parameters, food intake and appetite in correlation with the levels of gut or adipohormones. Therefore, the study of nutraceuticals that can modulate key adipohormones release may be an opportunity for the development of new lines of research in the treatment of obesity and other metabolic disorders. To this end, appetite control is a new field with more active research. The growing demand for new products of natural origin for weight control and appetite suppression has generated new lines of investigation in the field. Furthermore, certain in vitro studies can be extrapolated to human intervention conditions. Overall, more human clinical studies are needed.

Multiple peripheral signals control energy homeostasis, but all are integrated in the hypothalamus, specifically in the arcuate nucleus, which is mainly responsible for the control of food intake. This neurological center is especially sensitive to biochemical messengers, such as leptin, insulin and ghrelin. Other regions are responsible for eating behavior control and brain responses directly to the presence of nutrients, such as glucose, amino acids or fatty acids. We found that most studies have focused on the mechanisms involved in the regulation of energy homeostasis mediated by adenosine 5′-monophosphate-activated protein kinase (AMPK) in tissues, such as muscle, liver or adipose tissue (Hardie et al., 2012; Herranz-López et al., 2015; Jimenez-Sanchez et al., 2017). Nevertheless, the role of this enzyme is not limited to this homeostasis. Several studies indicate that AMPK plays a fundamental role in the regulation of appetite and that the enzyme is widely expressed in hypothalamic areas that control food intake (Huynh et al., 2016; Kola et al., 2006; Minokoshi et al., 2004). Increased energy expenditure leads to energy intake through appetite stimulation, simultaneously inhibiting energy expenditure through various metabolic pathways, including those regulated by AMPK. The inhibition of AMPK expression in the hypothalamus reduces food intake and body weight. Kola et al. suggested AMPK kinase as a mediator of orexigenic effects (Kola et al., 2006). Evidence has shown that the brain-intestine axis is responsible for neuronal functions, including the control of eating behavior. In conclusion, changes in the plasma concentrations of various hormones, such as ghrelin, GLP-1, PYY, CCK, leptin and insulin, as well as nutrients, such as glucose, are key influencers of the function of regions of the brain that regulate appetite and satiety in which AMPK seems to be expressed.

Concluding remarks

The use of plant extracts for weight control is increasingly noticeable. Various plant ingredients have been shown to be potentially effective in alleviating the signs and pathologies resulting from obesity, such as hyperlipidemia, hypercholesterolemia and hypertension. However, most recent research has opened a challenging area of research indicating that some herbal supplements seem to play an important role in food intake disorders, especially in hyperphagia, which is also related to obesity. Caloric restriction, under normal conditions in the face of weight reduction, can be associated with anxiety states accompanied by a significant increase in appetite in the individual, which is mitigated by uncontrolled eating. Therefore, the investigation of nutraceuticals that can modulate satiety or appetite for the treatment of obesity would become a very interesting and novel area of research. Nevertheless, new studies are needed to precisely determine the participating molecular mechanisms and the physiological effects and psychological response elicited by plant extracts and their bioactive compounds. Among all the studies reviewed that claimed an effect of the ingredient on hunger or satiety, only a few of them have rigorously determined the effect of the ingredient on food intake, appetite or body weight in correlation with the levels of gut or adipohormones.

The main limitations of this study lie in the general lack of studies that perform quantitative measurements of anorexigenic and/or orexigenic hormones during the different stages of clinical interventions. Future research should decipher the plasmatic pattern of hormones that regulate hunger, satiety and appetite in overweight and/or obese subjects in different phases, including fasting and the postprandial state, and compare these patterns with those in healthy subjects. In turn, subsequent studies should assess brain connectivity at the level of the hypothalamus, gut and adipose tissue and correlate this connectivity with the hormone patterns involved in the regulation of appetite and satiety. Following these study guidelines will provide a more solid understanding of the effect of phytochemicals on the neuroendocrine regulation of hunger.

Translating these findings to clinical practice will be the challenge in the future treatment of obesity. At the moment, the extracts seem to work best in overweight people. An efficient use of the extracts in patients with obesity needs further research, since this group presents a strong hormonal and metabolic dysregulation. Safe extracts whose mode of action have been well characterized, may be prescribed into a context of diet and exercise, which are the variables that will determine effective weight loss. It is important to identify the active ingredients of the extracts to ensure their effectiveness during treatments. In addition, it will be necessary to prescribe the extract more in line with the profiles of appetite hormones that appear dysregulated in the patient,

personalizing the treatment. The diet should be balanced and slightly hypocaloric, to generate no anxiety in the patient during the weight loss process. It is important to educate the patient to autonomously control their nutritional status. Once the goal of normal weight has been reached by the patient, taking the extracts should not be necessary, although this is still an aspect to investigate. In conclusion, future research in the use of appetite-controlling plant extracts might improve existing protocols for safe and effective weight reduction in cases of overweight and obesity.

Author contributions

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Disclosure statement

The authors report that there are no competing interests to declare.

CRediT authorship contribution statement

M. Boix-Castejón: Data curation, Investigation, Methodology, Writing – original draft. **E. Roche:** Data curation, Visualization, Writing – review & editing. **M. Olivares-Vicente:** Data curation, Visualization, Writing – review & editing. **F.J. Álvarez-Martínez:** Investigation, Software, Writing – review & editing. **M. Herranz-López:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **V. Micol:** Funding acquisition, Resources, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alvarez-Crespo, M., González-Matías, L., Lozano, M., Pérez, M., Vigo Gago, E.M., Paz, S., Mallo, F., 2009. Las Hormonas Gastrointestinales en el control de la Ingesta de Alimentos [Gastrointestinal Hormones in the Control of Food Intake], 56. Endocrinología y nutrición: órgano de la Sociedad Española de Endocrinología y Nutrición, pp. 317–330.

S.-M.L. Aranceta-Bartrina, J., Foz-Sala, M., Moreno-Esteban, B., 2005. Prevalencia de obesidad en España [Prevalence of obesity in Spain] Med. Clin. (Barc) 460–466.

Arluison, M., et al., 2004. Immunocytochemical localization of the glucose transporte 2 (GLUT2) in the adult rat brain. Chem Neuroana 28, 143–146.

B.N. Bakuradze, T., Janzowski, C., Lang, R., Hofmann, T., Stockis, J.P., Albert, F.W., Stiebitz, H., Bytof, G., Lantz, I., Baum, M., Eisenbrand, G., 2011. Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: results from an intervention study Mol. Nutr. Food Res. 55 (5), 793–797.

Barrajon-Catalan, E., Herranz-Lopez, M., Joven, J., Segura-Carretero, A., Alonso-Villaverde, C., Menendez, J.A., Micol, V., 2014. Molecular promiscuity of plant polyphenols in the management of age-related diseases: far beyond their antioxidant properties. Adv. Exp. Med. Biol. 824, 141–159.

Barrett, M.L., Udani, J.K., 2011. A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): a review of clinical studies on weight loss and glycemic control. Nutr. J. 10 (24).

d.C.J. Bassi, M., Hall, J.E., da Silva, A.A., 2012. Chronic effects of centrally administered adiponectin on appetite, metabolism and blood pressure regulation in normotensive and hypertensive rats Peptides 37, 1–5.

C.M. Batterham, R.L., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., et al., 2003. Inhibition of food intake in obese subjects by peptide YY3-36 N. Engl. J. Med. 349, 941–948.

Beck, B., 2006. Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. Philos. Trans. R. Soc. Lond. Ser. B, Biol. Sci. 361, 1159–1185.

Blundell, J., 1991. The biology of appeite. Clin. Appl. Nutre 21–23.

Blundell, J., de Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., van der Knaap, H., Westerterp, M., 2010. Appetite control: methodological aspects of the evaluation of foods. Obes. Rev. 11 (3), 251–270.

Boix-Castejon, M., Herranz-Lopez, M., Perez Gago, A., Olivares-Vicente, M., Caturla, N., Roche, E., Micol, V., 2018. Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial. Food Funct. 9, 3173–3184.

Brum, J.M., Gibb, R.D., Peters, J.C., Mattes, R.D., 2016. Satiety effects of psyllium in healthy volunteers. Appetite 105, 27–36.

Camacho, S., Michlig, S., de Senarclens-Bezencon, C., Meylan, J., Meystre, J., Pezzoli, M., Markram, H., le Coutre, J., 2015. Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying. Sci. Rep. 5, 7919.

Campbell, C.L., Foegeding, E.A., Harris, G.K., 2016. Cocoa and whey protein differentially affect markers of lipid and glucose metabolism and satiety. J. Med. Food 19, 219–227.

Carranza Quispe, L.E., 2016. Fisiología del apetito y el hambre. [Physiology of Appetite and Hunger]. Enfermería Investiga, Investigación, Vinculación, Docencia y Gestión 1.

Cohen, M.A., Ellis, S.M., Le Roux, C.W., Batterham, R.L., Park, A., Patterson, M., Frost, G. S., Ghatei, M.A., Bloom, S.R., 2003. Oxyntomodulin suppresses appetite and reduces food intake in humans. J. Clin. Endocrinol. Metab. 88, 4696–4701.

Crescioli, G., Lombardi, N., Bettiol, A., Marconi, E., Risaliti, F., Bertoni, M., Menniti Ippolito, F., Maggini, V., Gallo, E., Firenzuoli, F., Vannacci, A., 2018. Acute liver injury following Garcinia cambogia weight-loss supplementation: case series and literature review. Intern. Emerg. Med. 13, 857–872.

Cummings, D.E., Overduin, J., 2007. Gastrointestinal regulation of food intake. J. Clin. Invest. 117, 13–23.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S., 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50, 1714–1719.

Daousi, C., Wilding, J.P., Aditya, S., Durham, B.H., Cleator, J., Pinkney, J.H., Ranganath, L.R., 2009. Effects of peripheral administration of synthetic human glucose-dependent insulinotropic peptide (GIP) on energy expenditure and subjective appetite sensations in healthy normal weight subjects and obese patients with type 2 diabetes. Clin. Endocrinol. (Oxf.) 71, 195–201.

H.R. Ebrahim, I.O., Kopelman, M.D., Sharief, M.K., Williams, A.J., 2002. The hypocretin/orexin system J. R. Soc. Med. 95, 227–230.

El-Mostafa, K., El-Kharrassi, Y., Badreddine, A., Andreoletti, P., Vamecq, J., El Kebbaj, M. S., Latruffe, N., Lizard, G., Nasser, B., Cherkaoui-Malki, M., 2014. Nopal cactus (Opuntia ficus-indica) as a source of bioactive compounds for nutrition, health and disease. Molecules (Basel, Switzerland) 19, 14879–14901.

Fernandes, R.C., Araújo, V.A., Giglio, B.M., Marini, A.C.B., Mota, J.F., Teixeira, K.S., Monteiro, P.A., Lira, F.S., Pimentel, G.D., 2018. Acute epigallocatechin 3 Gallate (EGCG) supplementation delays gastric emptying in healthy women: a randomized, double-blind, placebo-controlled crossover study. Nutrients 10.

Fernández-Arroyo, S., Rodríguez-Medina, I.C., Beltrán-Debón, R., Pasini, F., Joven, J., Micol, V., Segura-Carretero, A., Fernández-Gutiérrez, A., 2011. Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of Hibiscus sabdariffa aqueous extract. Food Res. Int. 44, 1490–1495.

Frühbeck, J., 2005. Regulación de la ingesta alimentaria: una perpesticva clínica [Regulation of food intake: a clinical perspective]. Endocrinol. Nutr. 52 (08).

Garvey, R., Clegg, M., Coe, S., 2017. The acute effects of baobab fruit (Adansonia digitata) on satiety in healthy adults. Nutr. Health 23, 83–86.

F.J. Gibbs, J., McHugh, P.R., 1976. Cholecystokinin-decreased food intake in Rhesus monkeys Am. Physiol. Soc. 230, 15–18.

Z.T. Goldstein, J.L., Li, R.L., Sherbet, D.P., Liang, G., Brown, M.S., 2011. Surviving starvation: essential role of the ghrelin-growth hormone axis Cold Spring Harbor. Symp. Quant. Biol. 76, 121–127.

González-Jiménez, E., Schmidt Río-Valle, J., 2012. Regulation of dietary intake and energy balance; factors and mechanisms involved. Nutr. Hosp. 27 (6).

Gout, B., Bourges, C., Paineau-Dubreuil, S., 2010. Satiereal, a Crocus sativus L extract, reduces snacking and increases satiety in a randomized placebo-controlled study of mildly overweight, healthy women. Nutr. Res. 30, 305–313 (New York, NY).

Greenberg, J.A., 2012. Coffee, hunger, and peptide YY. J. Am. Coll. Nutr. 31 (3), 160–166.

Hamamah, S., Covasa, M., 2022. Gut microbiota restores central neuropeptide deficits in germ-free mice. Int. J. Mol. Sci. 23.

Hardie, D.G., Ross, F.A., Hawley, S.A., 2012. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nature reviews. Nat. Rev. Mol. Cell Biol. 13, 251–262.

R.J. Heilbronn, L.K., Janderova, L., Albu, J.B., Kelley, D.E., Ravussin, E., Smith, S.R., 2004. Relationship between serum resistin concentrations and insulin resistance in

nonobese, obese, and obese diabetic subjects J. Clin. Endocrinol. Metab. 89 (4), 1844–1848.
Herranz-López, M., Barrajón-Catalán, E., Segura-Carretero, A., Menéndez, J.A., Joven, J., Micol, V., 2015. Lemon verbena (*Lippia citriodora)* polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms. Phytomedicine 22, 605–614.
Herranz-López, M., Olivares-Vicente, M., Boix-Castejon, M., Caturla, N., Roche, E., Micol, V., 2019. Differential effects of a combination of Hibiscus sabdariffa and Lippia citriodora polyphenols in overweight/obese subjects: a randomized controlled trial. Sci. Rep. 9, 2999.
Herranz-López, M., Olivares-Vicente, M., Encinar, J.A., Barrajon-Catalan, E., Segura-Carretero, A., Joven, J., Micol, V., 2017. Multi-targeted molecular effects of hibiscus sabdariffa polyphenols: an opportunity for a global approach to obesity. Nutrients 9, 907.
Huynh, M.K.Q., Kinyua, A.W., Yang, D.J., Kim, K.W., 2016. Hypothalamic AMPK as a regulator of energy homeostasis. Neural Plast. 12.
Jaimes, L., Cabrera-Wrooman, A., Vilches, A., Guzmán, C., Camacho-Arroyo, I., 2005. Péptidos anorexigénicos y su participación en la conducta alimentaria [Anorexigenic peptides and their participation in eating behavior]. Endocrinol. Nutr. 13 (2), 67–74.
H.R. Janssens, P.L., Westerterp-Plantenga, M.S., 2014. Capsaicin increases sensation of fullness in energy balance, and decreases desire to eat after dinner in negative energy balance Appetite 77, 44–49.
Jimenez-Sanchez, C., Olivares-Vicente, M., Rodriguez-Perez, C., Herranz-Lopez, M., Lozano-Sanchez, J., Segura Carretero, A., Fernandez-Gutierrez, A., Micol, V., 2017. AMPK modulatory activity of olive–tree leaves phenolic compounds: bioassay-guided isolation on adipocyte model and in silico approach. PLoS One. https://doi.org/10.1371/journal.pone.0173074.
Joven, J., March, I., Espinel, E., Fernandez-Arroyo, S., Rodriguez-Gallego, E., Aragones, G., Beltran-Debon, R., Alonso-Villaverde, C., Rios, L., Martin-Paredero, V., Menendez, J.A., Micol, V., Segura-Carretero, A., Camps, J., 2014a. Hibiscus sabdariffa extract lowers blood pressure and improves endothelial function. Mol. Nutr. Food Res. 58, 1374–1378.
Joven, J., Micol, V., Segura-Carretero, A., Alonso-Villaverde, C., Menéndez, J.A., Aragonès, G., Barrajón-Catalán, E., Beltrán-Debón, R., Camps, J., Cufí, S., Fernández-Arroyo, S., Fernández-Gutiérrez, A., Guillén, E., Herranz-López, M., Iswaldi, I., Lozano-Sánchez, J., Martin-Castillo, B., Oliveras-Ferraros, C., Pérez-Sánchez, A., Rodríguez-Gallego, E., Rull, A., Saura, D., Vázquez-Martín, A., 2014b. Polyphenols and the modulation of gene expression pathways: can we eat our way out of the danger of chronic disease? Crit. Rev. Food Sci. Nutr. 54, 985–1001.
Kazemipoor, M., Cordell, G.A., Sarker, M.M.R., Radzi, C.W.J.B.W.M., Hajifaraji, M., En Kiat, P., 2015. Alternative treatments for weight loss: safety/Risks and effectiveness of anti-obesity medicinal plants. Int. J. Food Prop. 18, 1942–1963.
Kazemipoor, M., Hamzah, S., Hajifaraji, M., Radzi, C.W., Cordell, G.A., 2016. Slimming and appetite-suppressing effects of caraway aqueous extract as a natural therapy in physically active women. Phytother. Res. 30, 981–987.
Kim, D.H., Park, Y.H., Lee, J.S., Jeong, H.I., Lee, K.W., Kang, T.H., 2020. Anti-obesity effect of DKB-117 through the inhibition of pancreatic lipase and α-Amylase activity. Nutrients 12.
Kim, S.H., et al., 2015. Obesity and cardiovascular disease: friend or foe? Eur. Heart J. 37, 3560–3568.
Kim, Y.J., Choi, M.S., Park, Y.B., Kim, S.R., Lee, M.K., Jung, U.J., 2013. Garcinia Cambogia attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. World J. Gastroenterol. 19, 4689–4701.
Kola, B., Boscaro, M., Rutter, G.A., Grossman, A.B., Korbonits, M., 2006. Expanding role of AMPK in endocrinology. Trends Endocrinol. Metab. 17 (5), 205–215.
Konstantinidi, M., Koutelidakis, A., 2019. Functional foods and bioactive compounds: a review of its possible role on weight management and Obesity's metabolic consequences. Medicines 6, 94.
Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., Kozono, H., Takamoto, I., Okamoto, S., Shiuchi, T., Suzuki, R., Satoh, H., Tsuchida, A., Moroi, M., Sugi, K., Noda, T., Ebinuma, H., Ueta, Y., Kondo, T., Araki, E., Ezaki, O., Nagai, R., Tobe, K., Terauchi, Y., Ueki, K., Monokoshi, Y., Kadowaki, T., 2007. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell Metab. 6, 55–68.
Li, X., Zheng, L., Zhang, B., Deng, Z.Y., Luo, T., 2022. The structure basis of phytochemicals as metabolic signals for combating obesity. Front Nutr 9, 913883.
Linehan, V., Fang, L.Z., Parsons, M.P., Hirasawa, M., 2020. High-fat diet induces time-dependent synaptic plasticity of the lateral hypothalamus. Mol. Metab. 36.
M.T. Logan, C.M., Wallace, J.M., Robson, P.J., Welch, R.W., Dunne, A., Livingstone, M. B., 2006. Investigation of the medium-term effects of Olibra trade mark fat emulsion on food intake in non-obese subjects Eur. J. Clin. Nutr. 60, 1081–1091.
Loh, K., Herzog, H., Shi, Y.C., 2015. Regulation of Energy Homeostasis by the NPY System. Trends in endocrinology and metabolism: TEM 26, pp. 125–135.
Loh, K., Zhang, L., Brandon, A., Wang, Q., Begg, D., Qi, Y., Fu, M., Kulkarni, R., Teo, J., Baldock, P., Brüning, J.C., Cooney, G., Neely, G.G., Herzog, H., 2017. Insulin controls food intake and energy balance via NPY neurons. Mol. Metab. 6, 574–584.
Ludy, M.J.M., 2011. The effects of hedonically acceptable red pepper doses on thermogenesis and appetite. Physiol. Behav. 102, 251–258.
Mahboubi, M., 2019. Caraway as important medicinal plants in management of diseases. Nat. Prod. Bioprospect. 9, 1–11.
Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferre, P., Birnbaum, M.J., Stuck, B.J., Kahn, B.B., 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428, 569–574.
Mitchell, C.S., Begg, D.P., 2021. The regulation of food intake by insulin in the central nervous system. J. Neuroendocrinol. 33, e12952.
C.C. Müller, T.D., Finan, B., DiMarchi, R.D., Tschöp, M.H., 2018. Anti-obesity therapy: from rainbow pills to polyagonists Pharmacol. Rev. 70, 712–746.
Murphy, K.G., Bloom, S.R., 2006. Gut hormones and the regulation of energy homeostasis. Nature 444, 854–859.
Myers, M.G., et al., 2012. Central nervous system control of metabolism. Nature 357–363.
K.N. Näslund, E., Mansten, S., Adner, N., Holst, J.J., Gutniak, M., et al., 2004. Prandial subcutaneous injections of glucagon-like peptide-1 cause weight loss in obese human subjects Br. J. Nutr. 91, 439–446.
Nicolaïdis, S., 2008. Prenatal imprinting of postnatal specific appetites and feeding behavior. Metabolism 57 (117), 13–23.
R.N. Ntrigiou, V.N.I., Dimou, C., Koutelidakis, A., 2019. Functional food consumption correlates with anthropometric characteristics and body composition in healthy adults Curr. Top. Nutraceutical. Res. 18, 279–288.
R.-A.M. Nuñez, A., Andrés, I.D., Garzón, M., 2009. Hypocretin/Orexin neuropeptides: participation in the control of sleep-wakefulness cycle and energy homeostasis Curr. Neuropharmacol. 70, 50–59.
Olivares-Vicente, M., Barrajon-Catalan, E., Herranz-Lopez, M., Segura-Carretero, A., Joven, J., Encinar, J.A., Micol, V., 2018. Plant-derived polyphenols in human health: biological activity, metabolites and putative molecular targets. Curr. Drug Metab. 19, 351–369.
Olivares-Vicente, M., Sánchez-Marzo, N., Encinar, J.A., de la, Luz, Cádiz-Gurrea, M., Lozano-Sánchez, J., Segura-Carretero, A., Arraez-Roman, D., Riva, C., Barrajón-Catalán, E., Herranz-López, M., Micol, V., 2019. The potential synergistic modulation of AMPK by Lippia citriodora compounds as a target in metabolic disorders. Nutrients 11.
Onakpoya, I., Aldaas, S., Terry, R., Ernst, E., 2011a. The efficacy of Phaseolus vulgaris as a weight-loss supplement: a systematic review and meta-analysis of randomised clinical trials. Br. J. Nutr. 106, 196–202.
Onakpoya, I., Hung, S.K., Perry, R., Wider, B., Ernst, E.J., 2011b. The use of Garcinia extract (Hydroxycitric Acid) as a weight loss supplement: a systematic review and meta-analysis of Randomised clinical trials. J. Obes.
Pal, S., Khossousi, A., Binns, C., Dhaliwal, S., Ellis, V., 2011. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. Br. J. Nutr. 105, 90–100.
Peddio, S., Padiglia, A., Cannea, F.B., Crnjar, R., Zam, W., Sharifi-Rad, J., Rescigno, A., Zucca, P., 2022. Common bean (Phaseolus vulgaris L.) α-amylase inhibitors as safe nutraceutical strategy against diabetes and obesity: an update review. Phytother. Res. 36, 2803–2823.
Perry, R.J., Resch, J.M., Douglass, A.M., Madara, J.C., Rabin-Court, A., Kucukdereli, H., Wu, C., Song, J.D., Lowell, B.B., Shulman, G.I., 2019. Leptin's hunger-suppressing effects are mediated by the hypothalamic-pituitary-adrenocortical axis in rodents. Proceedings of the National Academy of Sciences of the United States of America 116, 13670–13679.
Qiu, J., Wagner, E.J., Rønnekleiv, O.K., Kelly, M.J., 2018. Insulin and leptin excite anorexigenic pro-opiomelanocortin neurones via activation of TRPC5 channels. J. Neuroendocrinol. 30.
Q.Y. Rajala, M.W., Patel, H.R., Takahashi, N., Banerjee, R., Pajvani, U.B., Sinha, M.K., Gingerich, R.L., Scherer, P.E., Ahima, R.S., 2004. Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting Diabetes 53 (7), 1671–1679.
Rebello, C.J., Burton, J., Heiman, M., Greenway, F.L., 2015. Gastrointestinal microbiome modulator improves glucose tolerance in overweight and obese subjects: a randomized controlled pilot trial. J. Diabetes Complicat. 29, 1272–1276.
Rebello, C.J., Martin, C.K., Johnson, W.D., O'Neil, C.E., Greenway, F.L., 2012. Efficacy of Olibra: a 12-week randomized controlled trial and a review of earlier studies. J. Diabetes Sci. Technol. 6, 695–708.
Redondo-Puente, M., Mateos, R., Seguido, M.A., García-Cordero, J., González, S., Tarradas, R.M., Bravo-Clemente, L., Sarriá, B., 2021. Appetite and satiety effects of the acute and regular consumption of green coffee phenols and green coffee Phenol/Oat β-Glucan nutraceuticals in subjects with overweight and obesity. Foods 10, 2511.
A. Rondanelli, M.G., Orsini, F., Opizzi, A., Villani, S., 2011. Appetite control and glycaemia reduction in overweight subjects treated with a combination of two highly standardized extracts from phaseolus vulgaris and cynara scolymus Phytother. Res. 25, 1275–1282.
G.G. Schubert, M.M., Horner, K., King, N., Leveritt, M., Sabapathy, S., Desbrow, B., 2014. Coffee for morning hunger pangs. An examination of coffee and caffeine on appetite, gastric emptying, and energy intake Appetite 83, 317–326.
Stefoska-Needham, A., Beck, E.J., Johnson, S.K., Chu, J., Tapsell, L.C., 2016. Flaked sorghum biscuits increase postprandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. Mol. Nutr. Food Res. 60, 1118–1128.
Szallasi, A., 2022. Exercise in a Pill" (or Just Another Fad)? Pharmaceuticals 15 (Basel, Switzerland).
Takeda, Y., Dai, P., 2022. Capsaicin directly promotes adipocyte browning in the chemical compound-induced brown adipocytes converted from human dermal fibroblasts. Sci. Rep. 12, 6612.
Timper, K., et al., 2017. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. Dis. Models Mech. 10, 679–689.
Tomar, M., Rao, R.P., Dorairaj, P., Koshta, A., Suresh, S., Rafiq, M., Kumawat, R., Paramesh, R., V, B.U., Venkatesh, K.V., 2019. A clinical and computational study on anti-obesity effects of hydroxycitric acid. RSC Adv. 9, 18578–18588.
Torterolo, P., Castro, Santiago., Falconi, V., Lagos, Patricia., 2010. Hormona concentradora de melanina (MCH): neuropéptido hipotalámico que facilita la generación del sueño [Melanin-concentrating hormone (MCH): hypothalamic

neuropeptide that facilitates sleep generation]. Revista Mexicana de Neurociencia 11 (1), 46–51.
Tosh, S.M., 2013. Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. Eur. J. Clin. Nutr. 67, 310–317.
Tschop, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407, 908–913.
Turnley, A.M., Stapleton, D., Mann, R.J., Witters, L.A., Kemp, B.E., Bartlett, P.F., 1999. Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. J. Neurochem. 72, 1707–1716.
T.S. Verdich, C., Buemann, B., Lysgård Madsen, J., Juul Holst, J., Astrup, A., 2001. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety —effect of obesity and weight reduction Int. J. Obes. 25, 1206–1214.
Vohra, M.S., Benchoula, K., Serpell, C.J., Hwa, W.E., 2022. AgRP/NPY and POMC neurons in the arcuate nucleus and their potential role in treatment of obesity. Eur. J. Pharmacol. 915, 174611.
Waterson, M.J., et al., 2015. Neuronal regulation of energy homeostasis: beyond the hypothalamus and feeding. Cell Metab. 22, 962–970.
WHO, 2015. Obesity and overweight (WHO Fact sheet 311), Wold Health Organization.
Wu, Z., Du, Z., Tian, Y., Liu, M., Zhu, K., Zhao, Y., Wang, H., 2022. Inulin accelerates weight loss in obese mice by regulating gut microbiota and serum metabolites. Front. Nutr. 9.
Yoshihiro Suzuki, K.N., 2014. Changes in mRNA expression ofarcuate nucleus appetite-regulatingpeptides during lactation in rats. J. Mol. Endocrinol. 52, 97–109.
Zanchi, D.A., Egloff, L., Haller, S., Mählmann, L., Lang, U.E., Drewe, J., Beglinger, C., Schmidt, A., Borgwardt, S., 2017. The impact of gut hormones on the neural circuit of appetite and satiety: a systematic review. Neurosci. Biobehav. Rev. 80, 457–475.
Zhang, J., Ren, P.G., Avsian-Kretchme, r O., Luo, C.W., Rauch, R., Klein, C., et al., 2005. Obestatin peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310 (5750), 996–999.
Zhang, L., Li, F., Guo, Q., Duan, Y., Wang, W., Zhong, Y., Yang, Y., Yin, Y., 2020. Leucine supplementation: a novel strategy for modulating lipid metabolism and energy homeostasis. Nutrients.
Zhang, N., Liu, J., Wang, M., Guo, X., Fan, B., Wang, F., 2022. Potato protease inhibitor II prevents obesity by inducing browning of white adipose tissue in mice via β3 adrenergic receptor signaling pathway. Phytother. Res. 36, 3885–3899.
P.Y. Zhang, X.X., Huang, Y.M., Zhao, H.L., 2016. Neuroendocrine hormone amylin in diabetes World J. Diabetes 7, 189–197.
Zheng, J., Zheng, S., Feng, Q., Zhang, Q., Xiao, X., 2017. Dietary capsaicin and its anti-obesity potency: from mechanism to clinical implications. Biosci. Rep. 37.
Zhu, Y., Lasrado, J.A., Hu, J., 2017. Potato protease inhibitor II suppresses postprandial appetite in healthy women: a randomized double-blind placebo-controlled trial. Food Funct 8, 1988–1993.
Zuñiga, L., González-Ortiz, M., Martínez-Abundis, E., 2017. Effect of gymnema sylvestre administration on metabolic syndrome, insulin sensitivity, and insulin secretion. J. Med. Food 20 (8), 750–754.

Discusión

DISCUSIÓN

Las cifras de sobrepeso y obesidad en España y Europa se han vuelto alarmantes. El estudio SEEDO (Sociedad Española para el Estudio de la Obesidad) realizó en 1997 la primera estimación sobre la incidencia de estas patologías en el Estado Español. Tras diversos estudios, se ha llegado a la conclusión que en los últimos años las tasas de sobrepeso y obesidad en los países desarrollados [64] ha aumentado tan drásticamente que es considerado un tema de interés sanitario prioritario. Esto ha incrementado el interés en nuevos enfoques terapéuticos y nuevas herramientas de tratamiento a causa del notable aumento del riesgo de morbimortalidad que conlleva la obesidad.

En base a lo arriba mencionado, este trabajo de tesis tiene como objetivo proponer un nuevo enfoque terapéutico para el síndrome metabólico a través de la fitoterapia.

El abordaje de este trastorno debe partir de una sinergia entre las medidas farmacológicas y los cambios en el estilo de vida y hábitos dietéticos. La patogenia del sobrepeso y la obesidad inducen claramente, como factores de riesgo que son, a comorbilidades importantes en el conjunto de patologías conocidas como síndrome metabólico.

El síndrome metabólico es uno de los principales problemas de salud pública de nuestro tiempo. La prevalencia mundial está en constante aumento y se estima que alrededor del 25% de la población adulta lo padece. Las disfunciones metabólicas asociadas, como la obesidad, la resistencia a la insulina, la hipertensión, la dislipidemia y la aterosclerosis y la intolerancia a la glucosa, son factores de riesgo para desarrollar DM2, cáncer y ECV.

TRATAMIENTO MULTIDISCIPLINAR Y ESTILO DE VIDA

El tratamiento de la obesidad, así como de otras comorbilidades asociadas a ésta, pasan principalmente por intervenciones en el estilo de vida. A día de hoy se establece que el mejor protocolo y recomendación para su abordaje es un enfoque multidisciplinar que aborde un cambio en el estilo de vida. Pero la evidencia de los últimos años sugiere que una intervención intensiva junto con un suplemento adecuado podría reducir de forma significativa los factores de riesgo cardiovascular y mejorar las comorbilidades asociadas a la obesidad.

No existe un consenso único sobre el tratamiento más idóneo. El síndrome metabólico se sustenta en un marco multifactorial, incluyendo características genéticas y factores determinantes exógenos. Entre estos últimos, cabe destacar ciertas conductas alimentarias y hábitos dietéticos como el consumo de alimentos y bebidas ultraprocesadas, así como condicionantes sociales, como el nivel sociocultural y socioeconómico, el entorno y el sedentarismo.

La patogenia de la obesidad es el elemento de partida en el desarrollo del síndrome metabólico. Abarca numerosos factores que influyen en su desarrollo. A pesar del uso de restricciones calóricas a través de planificaciones nutricionales o bien a través del ejercicio mediante un aumento del gasto calórico, siguen existiendo dificultades para la pérdida de peso. Por ello, es necesario cada vez más, una personalización del problema (Medicina Personalizada) para lograr una mejora de la composición corporal y los parámetros metabólicos.

Hoy en día la restricción calórica, aunque no hay una única pauta dietética apropiada para todos los individuos con sobrepeso y/o obesidad, parece demostrar ser el principal punto en común en todos los planteamientos terapéuticos realizados hasta la fecha.

EXTRACTOS POLIFENÓLICOS DE HIERBALUISA E HIBISCO

La evidencia científica acumulada en modelos animales y celulares por nuestro grupo de trabajo ha demostrado que los extractos polifenólicos de LC e HS podrían ser utilizados para complementar el tratamiento de algunas patologías incluidas dentro del síndrome metabólico. Teniendo en cuenta investigaciones previas, observamos que algunos nutracéuticos basados en extractos polifenólicos pueden participar como coadyuvantes en el tratamiento de la patología [20, 21, 37].

Los extractos de HS y LC han demostrado gran potencial terapéutico en los estudios realizados en la presente Tesis, en pacientes obesos y con sintomatología de síndrome metabólico, mostrando una reducción en la pérdida de peso y en la acumulación de grasa abdominal, una reducción y una mejoría de la hiperglucemia e hiperlipidemia, una reducción de los niveles de presión arterial, así como una capacidad modulatoria del apetito.

Cabe destacar, que uno de los elementos diferenciadores de este trabajo a la hora de determinar la efectividad del compuesto, ha sido la evaluación de los cambios en la composición corporal mediante técnicas antropométricas, así como la mejora del estado de salud total mediante cuestionarios validados, analíticas sanguíneas y una monitorización continua de la tensión arterial, que como se ha puesto de relieve son parámetros que no se han evaluado en su conjunto en estudios similares [65].

EVOLUCIÓN DE LOS PARÁMETROS TRAS LAS INTERVENCIONES

CAMBIOS EN LA COMPOSICÓN CORPORAL: PÉRDIDA DE PESO Y CAMBIOS EN PARÁMETROS ANALÍTICOS

En la primera intervención, observamos una reducción significativa del peso corporal con una reducción similar en los rangos de grasa corporal en aquellos individuos que consumieron el extracto polifenólico frente al grupo placebo. El grupo suplementado presentó una mayor disminución de pliegues cutáneos, así como de perímetros corporales. Debido a que los individuos de ambos grupos llevaban una dieta isocalórica se observó una tendencia similar en los parámetros antropométricos al inicio del estudio frente al grupo suplementado. Ambos grupos disminuyeron en grasa corporal, pero al final de la intervención el grupo suplementado perdió significativamente más grasa corporal que los del grupo placebo.

Al mismo tiempo, también se observaron cambios en los parámetros circulantes en el grupo suplementado. Al final de la intervención, presentaban mejoras en creatinina, LDL (lipoproteínas de baja densidad), GPT (transaminasa glutámico-pirúvica) o GGT (gamma glutamil transferasa) y número de glóbulos rojos. Sin embargo, el grupo placebo sólo presentó descensos significativos en dos parámetros (creatinina y HDL).

PRESIÓN ARTERIAL

La normalización de la presión arterial semanal en aquellos pacientes con el extracto de LC e HS frente al placebo fue significativa. Los resultados indicaron una mejora generalizada en la presión arterial, tanto en la sistólica, como en la diastólica total. Así, las

mejoras se centraron en la presión arterial diastólica diurna, diastólica nocturna y en el porcentaje reductor.

Sin un control dietético más allá de una modificación de hábitos como reducción de sal en las comidas o reducción de grasas y azúcares, se corroboró lo anteriormente citado. Por ello, la herramienta nutricional es fundamental, pero el suplemento dietético parece regular otros cambios metabólicos más allá de los que se puedan derivar de una restricción calórica. El consumo de polifenoles llevó al grupo suplementado hacia valores de no riesgo, demostrando una mejora significativa en los parámetros de PAC.

MODULACIÓN DE LA SACIEDAD

Uno de los parámetros observados más llamativo fue la modulación de la saciedad. La regulación de la saciedad a largo plazo es fundamental para llevar un eficiente control en la pérdida de peso. Las dietas más restrictivas suelen venir asociadas con una sensación de ansiedad por la reducción en la ingesta y el incremento del apetito/hambre que acompaña a la restricción calórica. Sin embargo, tras la intervención fue significantemente positiva la mejora en las puntuaciones de saciedad, así como en las percepciones de calidad de vida realizadas con el cuestionario SF-36.

Se llevó a cabo una selección de péptidos y hormonas reguladoras del hambre y la saciedad, que generalmente suelen verse alteradas en individuos con sobrepeso/obesidad. Los resultados de estos biomarcadores pueden no ser suficientes para comprender el complejo mecanismo de la regulación de la saciedad, ya que la saciedad no es el único factor involucrado en una pérdida de peso. No obstante, aun no siendo un factor determinante, la modulación de estos biomarcadores podría ser un componente de gran apoyo en el tratamiento de la obesidad.

Así mismo, en las escalas análogas subjetivas analizadas se observaron diferencias con respecto a la sensación de apetito, hambre y saciedad en el grupo que tomaba el suplemento. Estos resultados, se correlacionan positivamente con los resultados obtenidos en las determinaciones de los péptidos circulantes relacionados con el apetito y la saciedad.

Conclusiones

CONCLUSIONES

1. En sujetos con sobrepeso el consumo de 500 mg día de la combinación de extractos polifenólicos de *Hibiscus sabdariffa* y *Lippia citriodora* confirmaron reducciones significativas de grasa corporal, mejoras en parámetros circulantes y presión arterial, además de una percepción más positiva en el estado de salud general. El consumo de estos extractos polifenólicos ha demostrado una disminución en la sensación de apetito y una menor atracción por alimentos grasos, dulces y salados.

2. La evidencia sugiere una activación del sensor de energía AMPK por polifenoles como diana terapéutica frente a la obesidad, ayudando a reducir la masa grasa adiposa.

3. La combinación de extractos polifenólicos de *Hibiscus sabdariffa* y *Lippia citriodora* es capaz de modular los niveles de adipohormonas y péptidos intestinales, controlando la sensación de saciedad. Además, parece modular algunos componentes del gasto de energía total diario, mostrando una reducción de la resistina circulante y una normalización de los niveles de leptina, grelina y GLP-1.

Proyección Futura

PROYECCIÓN FUTURA

1. El conocimiento de los mecanismos fisiológicos que modulan los péptidos reguladores del hambre y la saciedad, es imprescindible para el desarrollo de nuevas estrategias ante la obesidad. La identificación de biomarcadores gastrointestinales que modulen la homeostasis energética podría contribuir al desarrollo de nuevas moléculas, como análogos de péptidos gastrointestinales, y contribuir en el diseño de una vía alternativa para el tratamiento de la obesidad y las enfermedades relacionadas con ella.

2. Es imprescindible definir mejor las herramientas de tratamiento y evaluación de la obesidad, no se dispone de herramientas terapéuticas suficientes ni adecuadas para el tratamiento, siendo necesario un abordaje multidisciplinar.

3. Se corrobora la necesidad de contar con suplementos efectivos que mejoren la calidad de vida y ayuden en el tratamiento de la obesidad cuando otras intervenciones no resultan suficientes. El conocimiento de la composición de los extractos estudiados permitiría el desarrollo de terapias individualizadas.

Referencias

REFERENCIAS

1. *World Health Organization. Obesity and Overweight; World Health Organization: Geneva, Switzerland.* Accessed on 4 Jun 2021]; Available from: http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.
2. World Health Organization. Regional Office for, E., *WHO European Regional Obesity Report 2022*. 2022, Copenhagen: World Health Organization. Regional Office for Europe.
3. *NCD Risk Factor Collaboration*. Available from: http://ncdrisc.org/index.html.
4. *NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults.* Lancet, 2017. **16**: p. 2627-2642.
5. Today, N.F.N., *Body Mass Index: Obesity, BMI, and Health: A Critical Review.* Nutrition today, 2015. **50**(3): p. 117-128.
6. *WHO, Obesity: preventing and managing the global epidemic. Report of a WHO Consultation (WHO Technical Report Series 894), in WHO. 2000, World Health Organization*.
7. L., H.P., *A comprehensive definition for metabolic syndrome. .* Disease models & mechanisms, 2009. **2**(5-6): p. 231–237.
8. A, E., *The Definition and Prevalence of Obesity and Metabolic Syndrome.* Advances in experimental medicine and biology, 2017. **960**: p. 1-17.
9. Kearney PM, W.M., Reynolds K, Muntner P, Whelton PK, He J, *Global Burden of Hypertension: Analysis of Worldwide Data.* The Lancet, 2005. **365**(9455): p. 217-223.
10. Mendis, S. *World Health Organisation; 2010*. Global status report on non communicable diseases 2010. 2010]; Available from: http://www.who.int/nmh/publications/ncd_report2010/en/. Accessed April 24, 2019.
11. Whelton, P.K., Carey, R. M., Aronow, W. S., Casey, D. E., Jr, Collins, K. J., Dennison Himmelfarb, C., DePalma, S. M., Gidding, S., Jamerson, K. A., Jones, D. W., MacLaughlin, E. J., Muntner, P., Ovbiagele, B., Smith, S. C., Jr, Spencer, C. C., Stafford, R. S., Taler, S. J., Thomas, R. J., Williams, K. A., Sr, Williamson,

J. D., Wright, J. T., Jr, *2017 Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.* Journals of the American College of Cardiology, 2018. **71**(6): p. 1269–1324.

12. Giles, T.D., Berk, B. C., Black, H. R., Cohn, J. N., Kostis, J. B., Izzo, J. L., Jr, & Weber, M. A, *Expanding the definition and classification of hypertension.* Journal of clinical hypertension 2005. **7**(9): p. 505-512.
13. Gorostidi, M., et al., *[2022 Practice guidelines for the management of arterial hypertension of the Spanish Society of Hypertension].* Hipertens Riesgo Vasc, 2022. **39**(4): p. 174-194.
14. Hall, J.E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E., *Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms.* Circulation research, 2015. **116**(6): p. 991–1006.
15. Di Dalmazi G, V.V., Pasquali R, Pagotto U. , *The unrelenting fall of the pharmacological treatment of obesity.* Endocrine, 2013. **44**(3): p. 598-609.
16. Ioannides-Demos LL, P.J., McNeil JJ. , *Pharmacotherapy for obesity. .* Drugs, 2005. **65**(10): p. 1391-418.
17. Stuby, J., Gravestock, I., Wolfram, E., Pichierri, G., Steurer, J., & Burgstaller, J. M. (2019). Appetite-Suppressing and Satiety-Increasing Bioactive Phytochemicals: A Systematic Review. Nutrients, 11(9), 2238. https://doi.org/10.3390/nu11092238, *Appetite-Suppressing and Satiety-Increasing Bioactive Phytochemicals: A Systematic Review.* Nutrients, 2019. **11**(9): p. 2238.
18. Wing, R., Phelan, S., *Long-term weight loss maintenance.* The American journal of clinical nutrition, 2005. **82**(1): p. 222S–225S.
19. Konstantinidi, M. and A. Koutelidakis, *Functional Foods and Bioactive Compounds: A Review of Its Possible Role on Weight Management and Obesity's Metabolic Consequences.* Medicines, 2019. **6**(3): p. 94.
20. Herranz-López, M., et al., *Multi-Targeted Molecular Effects of Hibiscus sabdariffa Polyphenols: An Opportunity for a Global Approach to Obesity.* Nutrients, 2017. **9**(8): p. 907.
21. Camacho, S., et al., *Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying.* Sci Rep, 2015. **5**: p. 7919.

22. Sen, T., & Samanta, S. K., *Medicinal plants, human health and biodiversity: a broad review.* Advances in biochemical engineering/biotechnology, 2015. **147**: p. 59–110.
23. Álvarez-Martínez, F.J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V., *Antimicrobial Capacity of Plant Polyphenols against Gram-positive Bacteria: a Comprehensive Review.* Current medicinal chemistry, 2018. **27**(15): p. 2576–2606.
24. Mopuri, R., & Islam, M. S., *Medicinal plants and phytochemicals with anti-obesogenic potentials: A review.* Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2017. **89**: p. 1442–1452.
25. Liu R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Advances in nutrition (Bethesda, M., 4(3), 384S–92S. https://doi.org/10.3945/an.112.003517, *Health-promoting components of fruits and vegetables in the diet.* Advances in nutrition, 2013. **4**(3): p. 384S–92S.
26. RH, L., *Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals.* The American journal of clinical nutrition, 2003. **78**(3): p. 517S-520S.
27. Piccolella, S., Crescente, G., Candela, L., & Pacifico, S., *Nutraceutical polyphenols: New analytical challenges and opportunities.* Journal of pharmaceutical and biomedical analysis, 2019. **175**.
28. Santos-Sánchez, N.F., Salas-Coronado, R., Hernández-Carlos, B., Villanueva-Cañongo, C., *Shikimic Acid Pathway in Biosynthesis of Phenolic Compounds.* IntechOpen, 2018.
29. Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J., & Queipo-Ortuño, M. I. , *Benefits of polyphenols on gut microbiota and implications in human health.* The Journal of nutritional biochemistry, 2013. **24**(8): p. 1415–1422.
30. Pandey, K.B., & Rizvi, S. I., *Plant polyphenols as dietary antioxidants in human health and disease.* Oxidative medicine and cellular longevity, 2009. **2**(5): p. 270–278.
31. Herranz-Lopez, M., et al., *Synergism of plant-derived polyphenols in adipogenesis: perspectives and implications.* Phytomedicine, 2012. **19**(3-4): p. 253-61.
32. Joven, J., et al., *Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice.* Biochimica et Biophysica Acta, 2012. **1820**(7): p. 894-9.

33. Joven, J., et al., *Hibiscus sabdariffa extract lowers blood pressure and improves endothelial function.* Molecular Nutrition & Food Research 2014. **58**: p. 1374-8.
34. Fernández-Arroyo, S., et al., *Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of Hibiscus sabdariffa aqueous extract.* Food research international, 2011. **44**(5): p. 1490-1495.
35. Boix-Castejon, M., Herranz-Lopez, M., Perez Gago, A., Olivares-Vicente, M., Caturla, N., Roche, E., Micol, V., *Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial.* Food & Function, 2018. **9**(6): p. 3173-3184.
36. Herranz-López, M., et al., *Lemon verbena (Lippia citriodora) polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms.* Phytomedicine, 2015. **22**(6): p. 605-614.
37. Joven, J., et al., *Polyphenols and the modulation of gene expression pathways: can we eat our way out of the danger of chronic disease?* Critical reviews in food science and nutrition, 2014. **54**(8): p. 985-1001.
38. *Lippia citriodora.* Available from: https://www.pexels.com/es-es/buscar/lippia%20citriodora/.
39. *Hibiscus sabdariffa.* Available from: https://stock.adobe.com/es/search/images?load_type=search&native_visual_search=&similar_content_id=&is_recent_search=&search_type=usertyped&k=hibiscus.
40. Riaz, G. and R. Chopra, *A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L.* Biomed Pharmacother, 2018. **102**: p. 575-586.
41. Wright, C.I., et al., *Herbal medicines as diuretics: a review of the scientific evidence.* J Ethnopharmacol, 2007. **114**(1): p. 1-31.
42. Amos, A. and B. Khiatah, *Mechanisms of Action of Nutritionally Rich Hibiscus sabdariffa's Therapeutic Uses in Major Common Chronic Diseases: A Literature Review.* J Am Coll Nutr, 2021: p. 1-8.
43. Gabriel, M., et al., *Potential use of dietary fibre from Hibiscus sabdariffa and Agave tequilana in obesity management.* Journal of Functional Foods, 2016. **21**: p. 1-9.
44. Adebayo, G., *Ethnobotanical study of plants used in treating hypertension in Edo State of Nigeria.* Journal of Ethnopharmacology, 2012. **144**(1): p. 1-10.
45. Beltran-Debon, R., et al., *The acute impact of polyphenols from Hibiscus sabdariffa in metabolic homeostasis: an approach combining metabolomics and gene-expression analyses.* Food Funct, 2015. **6**(9): p. 2957-66.

46. Olivares-Vicente, M., et al., *Plant-Derived Polyphenols in Human Health: Biological Activity, Metabolites and Putative Molecular Targets.* Curr Drug Metab, 2018. **19**(4): p. 351-369.
47. Chang, H.C., et al., *Hibiscus sabdariffa extract inhibits obesity and fat accumulation, and improves liver steatosis in humans.* Food Funct, 2014. **5**(4): p. 734-9.
48. Villalpando-Arteaga, E.V., 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 Funct, 2013. **4**(4): p. 618-26.
49. Alarcon-Aguilar, F.J., et al., *Effect of Hibiscus sabdariffa on obesity in MSG mice.* J Ethnopharmacol, 2007. **114**(1): p. 66-71.
50. Pascual, M.E., et al., *Lippia: traditional uses, chemistry and pharmacology: a review.* J Ethnopharmacol, 2001. **76**(3): p. 201-14.
51. Duarte, M.C., et al., *Anti-Candida activity of Brazilian medicinal plants.* J Ethnopharmacol, 2005. **97**(2): p. 305-11.
52. Sánchez-Marzo, N., et al., *Relationships Between Chemical Structure and Antioxidant Activity of Isolated Phytocompounds from Lemon Verbena.* Antioxidants (Basel), 2019. **8**(8).
53. Bilia, A.R., et al., *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): p. 463-70.
54. Quirantes-Pine, R., 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): p. 1112-8.
55. Funes, L., et al., *Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract.* Food Chemistry, 2009. **117**(4): p. 589-598.
56. Ejtahed, H.S., 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, 2019. **180**(3): p. 165-176.
57. Ciulu, M., M.I. Cádiz-Gurrea, and A. Segura-Carretero, *Extraction and Analysis of Phenolic Compounds in Rice: A Review.* Molecules, 2018. **23**(11).
58. Waterson, M.J., et al., *Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding.* Cell Metabolism, 2015. **22**: p. 962-970.

59. Myers, M.G., et al., *Central nervous system control of metabolism.* Nature, 2012: p. 357-363.

60. Marina, B.-C., et al., *Effect of metabolaid® on pre- and stage 1 hypertensive patients: A randomized controlled trial.* Journal of Functional Foods, 2021. **84**: p. 104583.

61. Melby, C.L., Paris, H. L., Foright, R. M., & Peth, J., *Attenuating the Biologic Drive for Weight Regain Following Weight Loss: Must What Goes Down Always Go Back Up?* Nutrients, 2017. **9**(5): p. 468.

62. Müller TD, C.C., Finan B, DiMarchi RD, Tschöp MH., *Anti-Obesity Therapy: from Rainbow Pills to Polyagonists. .* Pharmacological reviews, 2018. **70**(4): p. 712-746.

63. Trujillo, J.M., W. Nuffer, and B.A. Smith, *GLP-1 receptor agonists: an updated review of head-to-head clinical studies.* Ther Adv Endocrinol Metab, 2021. **12**: p. 2042018821997320.

64. Aranceta, J., et al., *Prevention of overweight and obesity from a public health perspective.* Nutr Rev, 2009. **67 Suppl 1**: p. S83-8.

65. Jensen, M.D., et al., *2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society.* J Am Coll Cardiol, 2014. **63**(25 Pt B): p. 2985-3023.