Efecto de la dieta mediterránea y sus compuestos bioactivos en la prevención y prognosis de enfermedades crónicas

Sara Hurtado Barroso

Universidad de Barcelona

Facultad de Farmacia y Ciencias de la Alimentación

EFECTO DE LA DIETA MEDITERRÁNEA Y SUS COMPUESTOS BIOACTIVOS EN LA PREVENCIÓN Y PROGNOSIS DE ENFERMEDADES CRÓNICAS

Sara Hurtado Barroso

2019

Universidad de Barcelona
Facultad de Farmacia y Ciencias de la Alimentación

Programa de Doctorado

Alimentación y Nutrición

2015-2019

EFECTO DE LA DIETA MEDITERRÁNEA Y SUS COMPUESTOS BIOACTIVOS EN LA PREVENCIÓN Y PROGNOSIS DE ENFERMEDADES CRÓNICAS

Memoria presentada por Sara Hurtado Barroso para optar al título de doctora por la Universidad de Barcelona, dirigida por:

Dra. Rosa M. Lamuela Raventós

Dra. Olha Khymenets

Sara Hurtado Barroso

Sara Hurtado Barroso

2019

FINANCIACIÓN

Ministerio de Educación y Formación Profesional.
Beca predoctoral FPU14/01715

Ministerio de Ciencia, Innovación y Universidades.
AGL2013-49083-C3-1-R
AGL2016-75329-R
AEI/FEDER, UE

Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición.
CB12/03/30020

Generalitat de Catalunya.
53 05012 2016
2017 SGR 196

Universitat de Barcelona. Facultat de Farmàcia i Ciencies de l'Alimentació

Campus de l'Alimentació de Torribera

Institut de Recerca en Nutrició i Seguretat Alimentària

Fundació Bosch i Gimpera

AGRADECIMIENTOS

No puedo finalizar esta etapa sin agradecer a los que me han rodeado y apoyado. En primer lugar, gracias a **Rosa** por darme la oportunidad de formar parte del grupo "Antioxidantes", por enseñarme a crecer y enfrentar obstáculos y por conseguir despertar en mi más amor por la nutrición del que ya sentía. A **Olha**, por formar parte de esta tesis y por el apoyo. Gracias al gran **team "Antioxidante"** y en especial a las post-doc (**Anna 3R**, **Pao**, **Miri**, **Anna V.** y **Carol**) por vuestra ayuda y por vuestra empatía. También especial mención a **Sime**, por estar para todo y más. Y a **Marta**, porque me encanta el meta-team que hemos formado juntas. Gracias a todos los que pasaron por el grupo, y en especial a quienes dejaron su huella en mí, convirtiéndose en amigas: **Vicky**, **Marcia**, **Fer** y **Mariel**.

También fuera del grupo "Antioxidantes" hay mucho que agradecer. A todo el **departamento**, y en especial a los **torriberos** y a los **gorditos de farmacia**, por lo vivido dentro y fuera de la uni. A **Raúl**, por la oportunidad de aprender algo nuevo y que realmente me ha gustado mucho. Y especialmente, gracias a mis queridas **mexican-amigas** por los geniales momentos juntas y porque, además de con una tesis, casi termino con un pasaporte mexicano. Gracias **Fer**, por demostrar ser una gran amiga, que está en los buenos y en los malos momentos, y además tremendamente buena consejera. Porque esta tesis ha sido mucho más llevadera con nuestros audios y nuestras quedadas para trabajar juntas. Y a **Elisa**, la burguesa-cantábrica más resalada que las pesetas, ha sido una suerte conocernos. Gracias al **grupo del CIBER** que participó en el encuentro en Menorca y fue de gran ayuda en esta última etapa.

Gracias a todos aquellos que he tenido la oportunidad de conocer en Barcelona y que forman parte de **mi pequeña gran familia aquí**, especialmente **Neus** y **Adri**. A mis **amigas extremeñas** repartidas por el mundo, porque la distancia nos impide vernos, pero no querernos.

A **mi familia**, por apoyar y entender mis decisiones y porque a pesar de los kms os siento muy cerquita siempre. A **mis peludos de cuatro patas**, y en especial a **Bob**, por acompañarme en la escritura de esta tesis (aunque fuera durmiendo) y por sacarme de paseo. Y gracias a **Jesús**, mi compañero de vida, porque tan bonito es compartir las alegrías contigo como sentir tu apoyo y crecer juntos. Gracias por tu paciencia en los momentos críticos y por hacer fácil lo difícil.

Gracias a todos y cada uno de los que directa o indirectamente han estado presente durante esta etapa, la tesis.

Índice

FIGURAS

TABLAS

ABREVIATURAS / ABREVIATIONS

4-HBA: ácido 4-hidroxibenzoico / 4-hydroxybenzoic acid

ABC: transportadores dependientes de adenosín-trifosfato / adenosine triphosphate-binding cassette

AICR: American Institute for Cancer Research

ARE: elemento de respuesta antioxidante / antioxidant response element

ASTs: arilsulfotransferasas / aryl sulphotransferases

BCFA: ácidos grasos de cadena ramificada/ branched-chain fatty acid

BCO1: β-caroteno oxigenesa 1 / β-carotene oxygenese 1

BCO2: β-caroteno oxigenesa 2 / β-carotene oxygenese 2

CBG: β-glucosidasa citosólica / cytosolic β-glucosidase

CD: dieta convencional / conventional diet

CD36: determinante de grupo 36 / cluster of differentiation 36

COMTs: catecol-O-metiltransferasas / catechol-O-methyltransferases

COX-2: ciclooxigenasa-2 / cyclooxygenase-2

CRP: proteína C-reactiva / C-reactive protein

CYP: citocromo / cytochrome

DM: Dieta Mediterránea

DMAPP: dimetilalil difosfato / dimethylallyl diphosphate

ECV: enfermedades cardiovasculares

ENT: enfermedades no transmisibles

FFAR2: receptor de ácidos grasos libres 2 / free fatty acid receptor 2

FFAR3: receptor de ácidos grasos libres 3 / free fatty acid receptor 3

FFQ: cuestionario de frecuencia de consumo de alimentos / Food Frequency Questionnaire

GAE: equivalentes de ácido gálico / gallic acid equivalent

GGPP: geranil geranil pirofosfato / geranyl geranyl pyrophosphate

GPRs: receptores acoplados a proteínas G / G-protein-coupled receptors

HCAR2: receptor de ácido hidroxicarboxílico / hydroxycarboxylic acid receptor

HDAC: histona desacetilasa / histone deacetylase

HDL: lipoproteína de alta densidad / high density lipoprotein

HR: hazard ratio

ICAM-1: molécula de adhesión intercelular-1 / intercelular adhesion molecule-1

IL-1β: interleuquina 1β / interleukin 1β

IL-6: interleuquina 6 / interleukin 6

i-NOS: óxido nítrico sintasa inducible / inducible nitric oxide synthase

IPP: isopentenil difosfato / isopentenyl diphosphate

LAD: dieta baja en antioxidantes / low antioxidant diet

LDL: lipoproteína de baja densidad / low density lipoprotein

LPH: lactasa-floricina hidrolasa / lactase-phlorizin hydrolase

LRAT: lecitin-retinol-aciltransferasa / lecithin-retinol-acyltransferasa

NPC1L1: proteína Niemann-Pick C1-Like 1 / Niemann-Pick C1 like 1

NF-κB: factor nuclear potenciador de las cadenas ligeras kappa de las células B activadas / nuclear factor kappa-light-chain-enhancer of activated B cells

NHL: linfoma no Hodgkin / non-Hodgkin lymphoma

NO: óxido nítrico / nitric oxide

Nrf2: factor nuclear eritroide 2 relacionado con el factor 2 / nuclear factor erythroid 2-related factor 2

OD: dieta ecológica / organic diet

OMS: Organización Mundial de la Salud

PGI2: prostaglandina I2 (prostaciclina) / prostacyclin

PSY: fitoeno sintetasa / phytoene synthase

RDH: retinol deshidrogenasa / retinol deshydrogenase

ROS: especies reactivas del oxígeno / reactive oxygen species

RR: relative risk

SGLT1: transportador de glucosa activo dependiente de sodio 1 / sodium-dependent glucose transporter 1

SR-B1: receptores de membranas como el de clase B tipo I / scavenger receptor class B type 1

SULTs: sulfotransferasas / sulphotransferases

TNF-α: factor de necrosis tumoral α / tumor nuclear factor α

TPE: excreción de polifenoles totales / total polyphenol excretion

TXA2: tromboxano A2 / thromboxane A2

UD: dieta habitual / usual diet

UGT: uridina-5′-difosfato glucuronosiltransferasas / uridine-5′-diphosphate glucuronosyltransferase

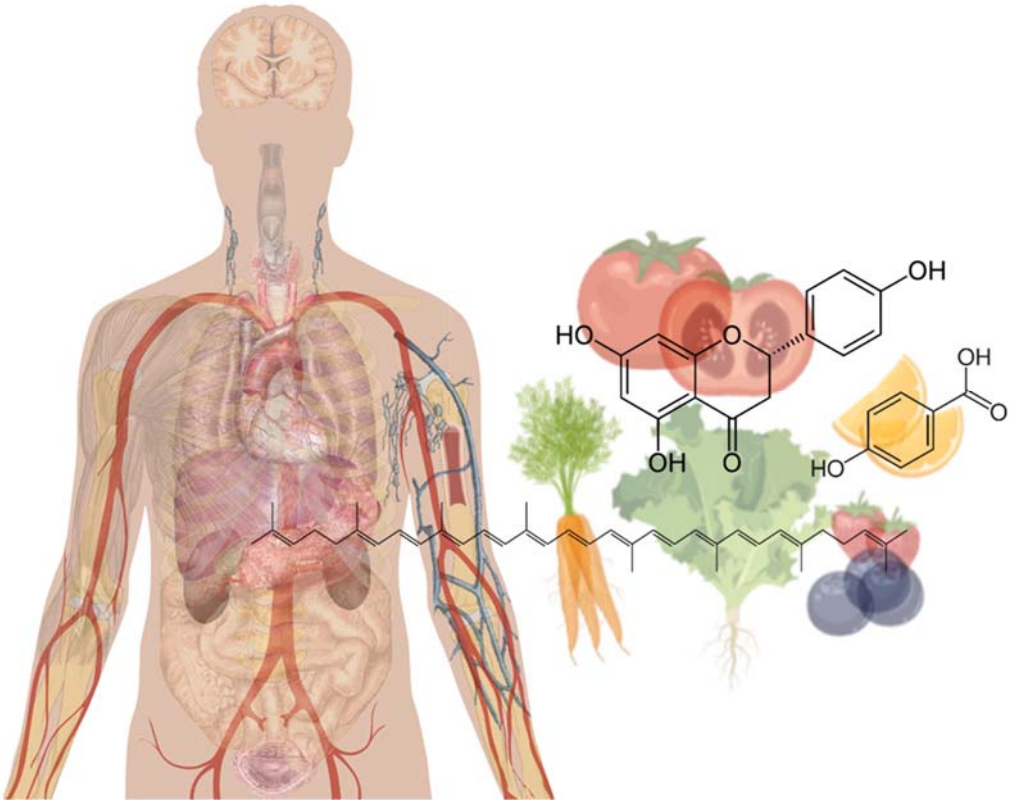
VCAM-1: molécula de adhesión vascular-1 / vascular adhesion molecule-1

VFA: ácidos grasos volátiles / volatile fatty acid

VLDL: lipoproteínas de muy baja densidad / very low density lipoprotein

WCRF: World Cancer Research Fund

RESUMEN



1. RESUMEN

La dieta mediterránea, rica en hortalizas y frutas, tiene efectos beneficiosos en la salud debido en parte a su alto contenido en compuestos bioactivos, entre ellos los carotenoides y los polifenoles. Estos compuestos que provienen de la dieta y tienen propiedades biológicas se conocen comúnmente como fitoquímicos, debido a que son producidos por el metabolismo de las plantas. Las concentraciones de fitoquímicos que son ingeridas y metabolizadas por el cuerpo humano dependen de muchos factores como la matriz alimentaria, el procesado, las interacciones entre alimentos, el método de cultivo, etc. Los polifenoles y carotenoides son compuestos procedentes del metabolismo secundario de las plantas y poseen propiedades fisiológicas relevantes en el organismo humano. Se ha observado que son más accesibles cuando se consumen con una matriz lipídica, y además el procesamiento térmico también parece afectar positivamente a los carotenoides. En cuanto al tipo de cultivo, algunas investigaciones han sugerido un mayor contenido de fitoquímicos en alimentos obtenidos mediante métodos de agricultura ecológica debido a que las plantas están más expuestas a situaciones de estrés por el uso limitado de pesticidas y fertilizantes con respecto a los convencionales.

Por otra parte, los productos del metabolismo de la microbiota intestinal también regulan las actividades biológicas y pueden disminuir el riesgo de algunas enfermedades crónicas como las cardiovasculares y el cáncer. En las últimas décadas, ha aumentado el número de publicaciones que aborda el tema de la interacción entre la dieta, la microbiota y la salud, especulándose la asociación de la ingesta de determinados alimentos y nutrientes con la composición y actividad de la microbiota, lo cual se relaciona con la presencia de ciertas enfermedades crónicas (enfermedades no trasmisibles).

No obstante, gran parte de los investigadores llevan a cabo estudios en poblaciones de riesgo, con el fin de evaluar la asociación entre los compuestos bioactivos y la prevención primaria de ciertas enfermedades. Por lo tanto, en el desarrollo de la presente tesis se propuso i) estudiar el efecto de los compuestos bioactivos de la dieta sobre algunos marcadores relacionados con la inflamación sistémica de bajo grado e implicados en la salud vascular en hombres sanos; ii) evaluar el efecto de consumir una dieta ecológica a corto plazo sobre el nivel de compuestos bioactivos, minerales y metales pesados; iii) analizar las interacciones entre los carotenos plasmáticos (utilizados como biomarcadores del consumo de hortalizas y frutas), los ácidos grasos volátiles procedentes de la microbiota y el perfil lipídico en sujetos sanos; y iv) evaluar la evidencia sobre la asociación del consumo de hortalizas y frutas y la prognosis de cáncer .

Los resultados de estas investigaciones corroboraron i) los efectos negativos a corto plazo de consumir una dieta baja en compuestos bioactivos en algunos de los biomarcadores vasculares, el óxido nítrico y el cociente tromboxano A_2/ prostaglandina I_2, en sujetos sanos. Además, se ha sugerido que el consumo habitual de *sofrito* puede tener efectos positivos sobre la proteína C-

reactiva y el factor de necrosis tumoral-α, evitando así la inflamación sistémica. ii) Dentro de un patrón dietético saludable, la ingesta de alimentos cultivados mediante agricultura ecológica podría aportar una mayor concentración de algunos compuestos fenólicos, sin embargo, son necesarios más estudios que corroboren estos resultados. iii) En cuanto a la interacción dieta-microbiota-salud, se sugiere que los carotenos ingeridos mediante la dieta podrían tener un impacto sobre el metabolismo microbiano, favoreciendo la liberación de algunos ácidos grasos volátiles, lo cual parece estar relacionado con la regulación de la síntesis del colesterol. iv) Además del efecto cardioprotector que ejercen los compuestos bioactivos incluso en sujetos sanos, el consumo de hortalizas y frutas también se relaciona con una disminución de la mortalidad total en los supervivientes de cáncer de cabeza y cuello y de ovario.

En conclusión, los resultados de las investigaciones que forman parte de esta tesis corroboran las propiedades beneficiosas del consumo de una dieta rica en hortalizas y frutas, gracias a su contenido en compuestos bioactivos, en la prevención de procesos inflamatorios y en la mejora de la supervivencia del cáncer de cabeza y cuello y de ovario (**Figura 1**).

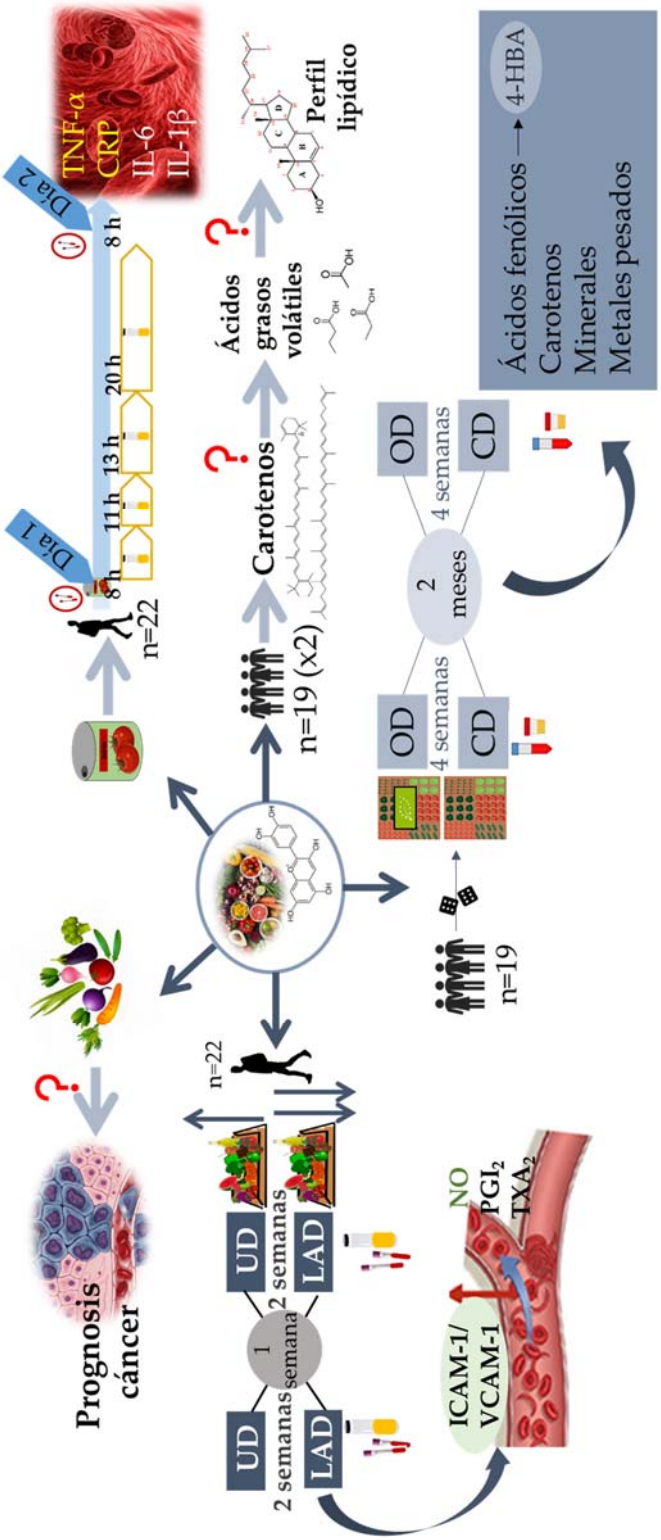


Figura 1. Resumen de los estudios incluidos en la presente tesis.

1. ABSTRACT

The Mediterranean diet, rich in vegetables and fruits, has beneficial effects on health due in part to its high content of bioactive compounds, including carotenoids and polyphenols. These compounds are commonly known as phytochemicals because they are produced by the metabolism of plants and come into humans through the diet. The amounts of phytochemicals that are ingested with food and absorbed and metabolised by the human body depend on many factors such as the food matrix, the processing, the interactions between foods, the method of cultivation etc. Polyphenols and carotenoids are plant secondary metabolites with relevant physiological properties in the human organism. They have been found to be more accessible when consumed with a lipid matrix and, in addition, thermal treatment seems to positively affect carotenoids' bioaccessibility and bioavailability. Regarding cultivation, some research has suggested that foods produced by organic farming contain a higher amount of phytochemicals because plants are more exposed to stressful conditions due to the limited use of pesticides and fertilisers compared to conventional forms of farming.

On the other hand, the metabolites originated by the intestinal microbiota also regulate biological activities and may reduce the risk of some chronic diseases such as cardiovascular disease and cancer. In recent decades, the number of publications that address the issue of interaction between diet, microbiota and health has increased, speculating on the association of the intake of certain foods and nutrients with the composition and activity of the microbiota, which in turn can be related to the development of certain chronic diseases (non-communicable diseases).

So far, a large part of the research on the association between bioactive compound consumption and the primary prevention of certain diseases has been carried out mainly in risk populations. Therefore, within the present thesis it was proposed i) to study the effect of bioactive compounds of the diet on some markers related to low-grade systemic inflammation and vascular health in healthy men; ii) to evaluate the short-term effect of consumption of an organic diet on the level of bioactive compounds, minerals and heavy metals; iii) to analyse the interactions among plasma carotenes (used as biomarkers of consumption of vegetables and fruits), volatile fatty acids of the microbiota origin and lipid profile in healthy subjects; and iv) to evaluate the evidence of the association of the consumption of vegetables and fruits and the prognosis of certain cancers.

The results of these investigations corroborated i) the short-term negative effects of consumption of a diet low in bioactive compounds on some of the vascular

biomarkers, nitric oxide and the ratio of thromboxane A_2/prostaglandin I_2 in healthy subjects. In addition, it has been suggested that the consumption of sofrito may have positive effects on the prevention of systemic inflammation through interaction with the C-reactive protein and tumour necrosis factor-α. ii) Within a healthy dietary pattern, the consumption of organically cultivated food could provide a higher concentration of some phenolic compounds; however, more studies are needed to support these results. iii) Regarding the diet-microbiota-health interaction, it is suggested that carotenes ingested through the diet could have an impact on microbial metabolism, favouring the release of some volatile fatty acids, which seems to be related to the regulation of cholesterol synthesis. iv) In addition to the cardioprotective effect exerted by bioactive compounds even in healthy subjects, the consumption of vegetables and fruits is also related to a decrease in total mortality in survivors of head and neck and ovarian cancer.

In conclusion, the results of the investigations that form this thesis corroborate that following a diet rich in bioactive compounds from vegetables and fruits may contribute to the prevention of inflammatory processes and to the improvement of the survival rates of head and neck and ovarian cancer (**Figure 1**).

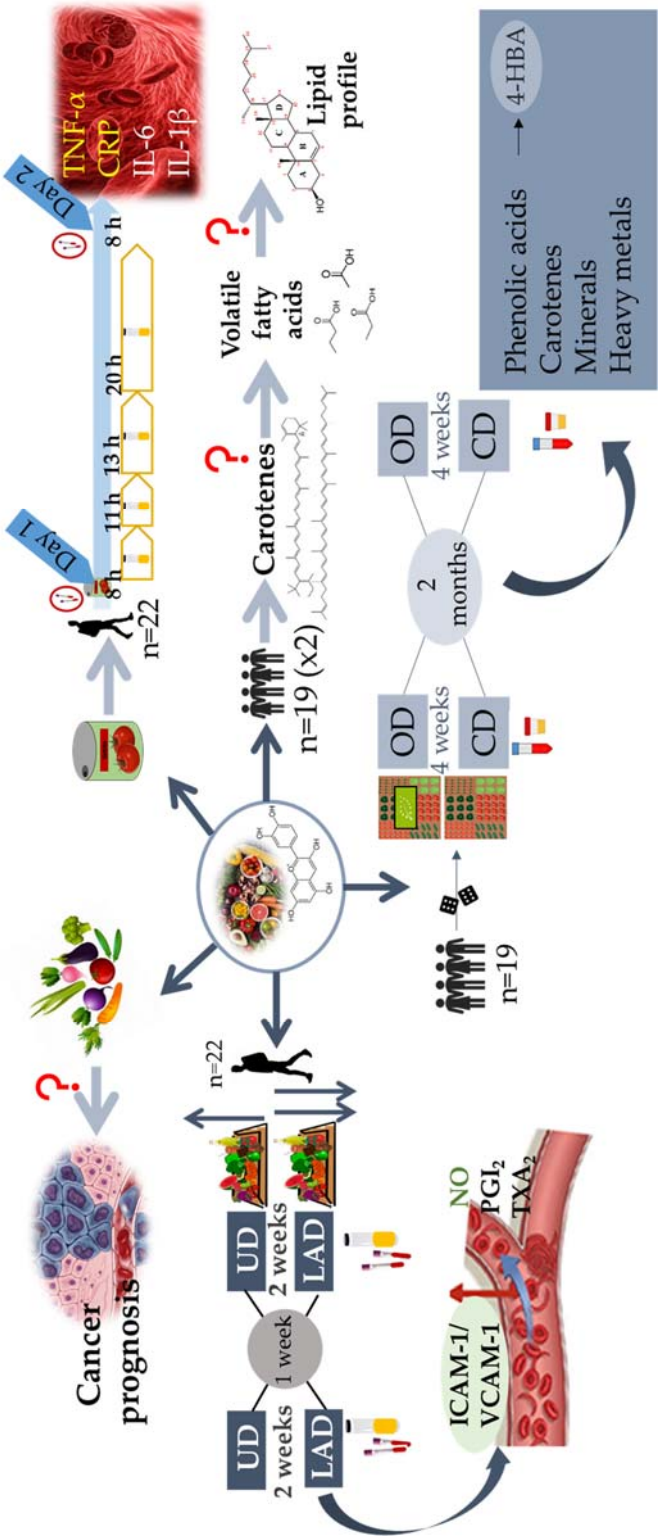
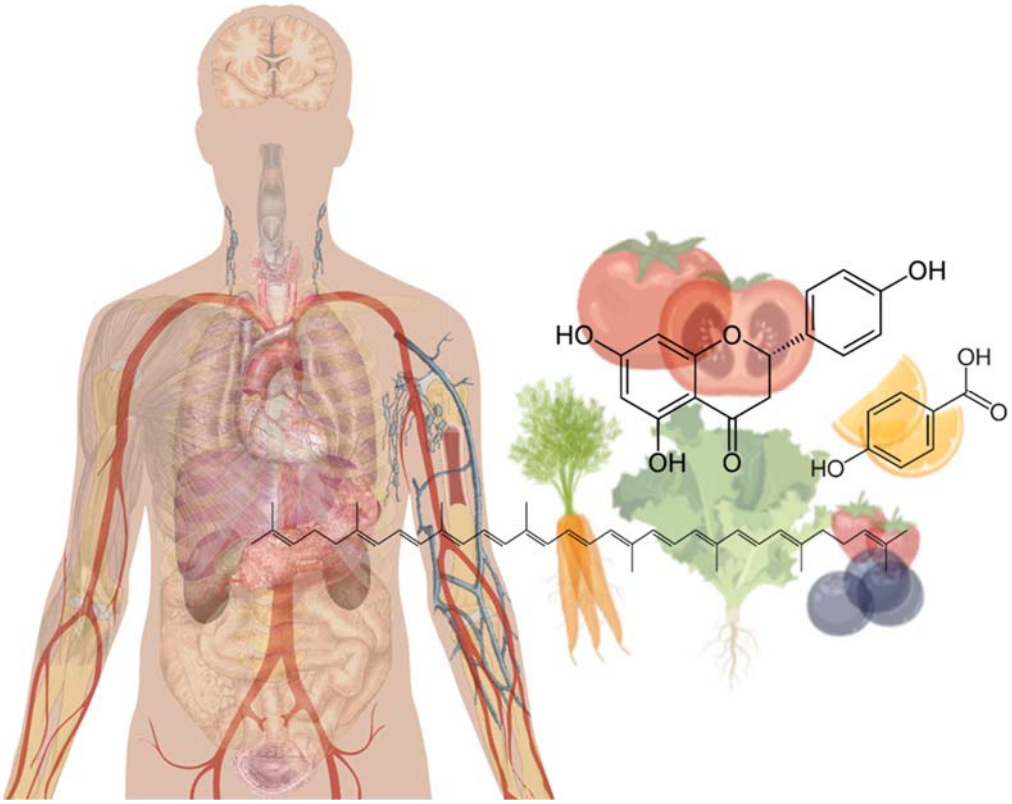


Figure 1. Summary of the studies included in the present thesis.

INTRODUCCIÓN



2. INTRODUCCIÓN

2.1. Epidemiología nutricional

2.1.1. Diseño de estudios epidemiológicos

La Organización Mundial de la Salud (OMS) define la epidemiología como "el estudio de la distribución y los determinantes de estados o eventos (en particular de enfermedades) relacionados con la salud y la aplicación de esos estudios al control de enfermedades y otros problemas de salud" [1]. Los estudios epidemiológicos pueden ser de tipo descriptivo o analítico **(Tabla 1)**.

Tabla 1. Clasificación de los estudios epidemiológicos.

ESTUDIOS EPIDEMIOLÓGICOS	**Analíticos**	**Observacionales**	Cohorte
			Casos y controles
		Experimentales	Ensayos clínicos
	Descriptivos	**Observacionales**	Ensayos comunitarios
			Casos clínicos
			Estudios transversales
			Series de casos
			Estudios ecológicos

Los estudios analíticos se utilizan para analizar los factores determinantes de enfermedades y con ello establecer una relación de causa-efecto (factor de riesgo-enfermedad). Estos últimos pueden ser observacionales o experimentales. Los estudios analíticos observacionales pueden ser transversales (los datos del estudio se analizan en un momento puntual), longitudinales retrospectivos (se analiza en el presente con datos del pasado) y longitudinales prospectivos (se analiza con datos obtenidos posteriormente al comienzo del estudio). A diferencia de los estudios de casos y controles que se muestrean siguiendo el criterio de presencia o ausencia de enfermedad, en los de cohorte se selecciona la muestra según la exposición o no exposición al factor en estudio. Los estudios experimentales, los cuales implican una intervención, se denominan ensayos clínicos. Los ensayos comunitarios se consideran

cuasiexperimentales y evalúan intervenciones en comunidades (o grupos de personas), a diferencia de los ensayos clínicos que lo hacen de forma individual. Los datos experimentales son preferibles a los observaciones en la determinación de causalidad [2]. Los ensayos clínicos pueden ser cruzados, si una misma muestra de individuos realiza todos los tratamientos o intervenciones, o paralelos, si cada grupo de individuos lleva a cabo un tratamiento o intervención diferente. Los estudios cruzados son más robustos que los paralelos, debido a que cada individuo actúa como su propio control, y por lo tanto el tamaño muestras necesario es mucho menor. Sin embargo, el diseño paralelo permite una menor duración del estudio y no tiene riesgo de interacciones entre las intervenciones *(efecto "carry-over")*. Para evitar este posibles efecto residual que puede ocurrir en los ensayos cruzados, es necesario un período de lavado *(wash-out)* suficiente entre las intervenciones [3,4]. Los estudios descriptivos son observacionales y se utilizan para analizar la distribución de enfermedades. Pueden ser casos clínicos, estudios transversales o de prevalencia, series de casos o estudios ecológicos.

A pesar de ser los ensayos clínicos los más adecuados en muchas ocasiones por la posibilidad de manipulación [5], los estudios observacionales son muy utilizados para valorar el riesgo de las enfermedad en relación con uno o varios factores de exposición [6,7]. A priori, el grado de evidencia es mayor cuando los estudios son de intervención, pero dependiendo del control de posibles sesgos y variables de confusión la calidad de los estos puede variar [8]. Además, los estudios epidemiológicos se llevan a cabo en humanos y proporcional un nivel de evidencia mayor que aquellos que se llevan a cabo en animales y los que se realizan *"in vitro"*. Sin embargo, los meta-análisis (**Figura 2**) permiten combinar los resultados de estudios científicos mediante análisis estadístico, alcanzando de esta manera niveles de evidencia más altos [9].

Figura 2. Pirámide de la evidencia de estudios científicos y situación de las publicaciones que forman parte del presente trabajo.

La epidemiología nutricional es una subdisciplina de la epidemiología y proporciona conocimientos específicos sobre la relación entre la dieta y las enfermedades [10]. La epidemiología nutricional es compleja y cuenta con una serie de limitaciones metodológicas. En primer lugar, no se tiene en cuenta las interacciones y sinergias entre los diferentes compuestos de la dieta [11]. Las dietas son variables entre individuos, e incluso en un mismo individuo los hábitos y composición dietética pueden variar. Incluso con dosis controladas, se ha observado una variabilidad interindividual en los marcadores indicadores de consumo dietético que implica una mayor o menor respuesta por parte de los sujetos [12]. Además, la estimación de nutrientes a través del uso de tablas tiene algunos problemas: i) no todos los alimentos están bien caracterizados, ii) no tiene en cuenta todos los factores que afectan al contenido nutricional del alimento (estación, área de procedencia, condiciones de crecimiento, almacenamiento, técnicas de cocinado y procesamiento). También los métodos de evaluación dietética tienen sus inconvenientes (por ejemplo, los participantes pueden no dar los datos exactos de su dieta por no recordar algunos detalles, por falta de colaboración o por infra- o sobre-estimación de las cantidades que

consumen), especialmente, los registros dietéticos (24 horas, una semana…) y los cuestionarios de frecuencia de consumo de alimentos (FFQ). Sin embargo, los FFQ son ampliamente utilizados en la actualidad y se validan mediante la correlación con biomarcadores en muestras biológicas. En cuanto a los estudios, los ensayos clínicos no pueden ser ciegos y el conocimiento de la asignación podría afectar los resultados. Además, la tasa de abandono y el grado de incumplimiento son más altos que en el caso de los estudios farmacológicos [2]. Por lo tanto, en esta disciplina los meta-análisis (análisis estadísticos que combinan los resultados de múltiples estudios científicos que abordan la misma pregunta) pueden ser de gran utilidad para la valoración de la evidencia de los resultados e incluso para establecer recomendaciones [13].

2.1.2. La epidemia de las enfermedades no transmisibles relacionadas con la dieta

Hoy en día, las enfermedades crónicas o enfermedades no transmisibles (ENT) son la principal causa de mortalidad, provocando 41 millones de muertes al año (71% de la mortalidad global), de las cuales 15 millones ocurren de forma prematura (antes de los 70 años) [14]. Las enfermedades cardiovasculares (ECV) y el cáncer son las ENT responsables de un mayor número de muertes [14]. El aumento de las ENT se debe al envejecimiento de la población y a algunos factores que aumentan su riesgo de incidencia y mortalidad, entre los que destacan dietas malsanas, tabaquismo y uso de otros tóxicos, inactividad física y contaminantes (**Figura 3**). Estos factores de riesgo afectan a la homeostasis del ser humano desencadenando la aparición de estas ENT y de otras patologías como la obesidad, diabetes e hipertensión, que se relacionan directamente con el desarrollo de ECV principalmente.

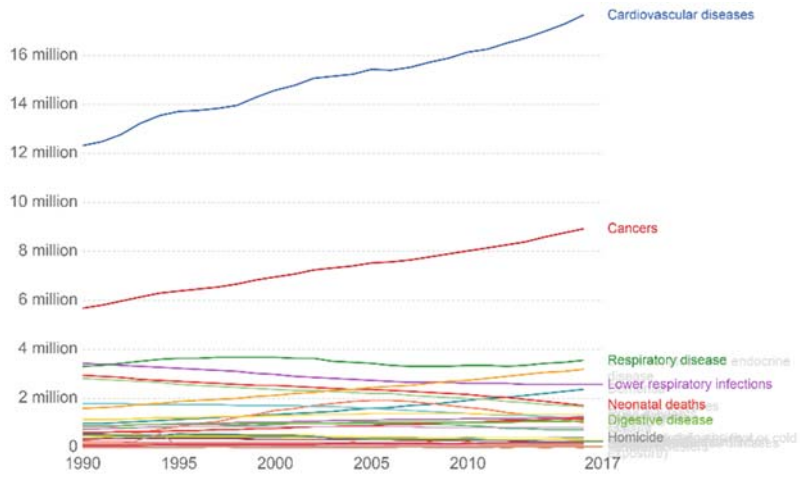


Figura 3. Número de muertes por enfermedades no transmisibles en relación con los factores de riesgo externos (contaminación, dieta y otros factores sobre el estilo de vida) desde el año 1990 al 2017 [15,16].

2.1.2.1. Enfermedades cardiovasculares

Las ECV son la principal causa de mortalidad en el mundo, llegando a producir 17,9 millones de muertes anuales. Casi uno de cada tres fallecimientos ocurre por estos trastornos relacionados con el corazón y los vasos sanguíneos, entre los que se incluyen las enfermedades coronarias, las cerebrovasculares y las reumáticas, siendo las causas principales los ataques al corazón e infarto de miocardio. Sin embargo, es posible la detección precoz y el tratamiento temprano de estas enfermedades debido a su estrecha relación con alteraciones como la hipertensión arterial, la diabetes, la hiperlipidemia y el sobrepeso/obesidad, que a su vez se asocian con factores tales como el consumo de dietas no saludables, el tabaco, el exceso de alcohol y la inactividad física. Otros determinantes considerados en el desarrollo de las ECV son: la edad, la genética, el estrés y el nivel socio-económico. Estas enfermedades afectan especialmente a los países de ingresos bajos o medios, en donde se produce más de un 75% de las muertes por la falta de recursos [17].

A pesar del significante efecto de la edad sobre el desarrollo de estas enfermedades, en las dos últimas décadas ha incrementado considerablemente la incidencia de ECV en adultos de entre 18 y 45 años de edad debido al aumento de los factores de riesgo [18]. En particular, la ingesta de dietas malsanas se relacionaron con un 64% de las ECV en individuos jóvenes (de 25 a 34 años de edad), que resultó casi el doble de la proporción de muertes cardiometabólicas atribuidas a la dieta en individuos ≥75 años [19].

2.1.2.2. Cáncer

El cáncer es la segunda causa de mortalidad en el mundo, ocasionando una de cada seis de muertes al año. En el 2018 se han estimado alrededor de 18 millones de casos de cáncer, de los cuales más de la mitad tuvieron un desenlace fatal. El cáncer engloba un conjunto de enfermedades que pueden afectar a cualquier parte del organismo y que comienza con la transformación de células normales en precancerosas que dan lugar a tumores (o neoplasias) malignos. Debido a la rápida proliferación de estas células cancerosas, el tumor original puede propagarse por otros órganos dando lugar a la metástasis, la principal causa de muerte por cáncer. A diferencia de las ECV, el problema del cáncer es la detección frecuente en una fase avanzada y la falta de diagnóstico y tratamiento. Sin embargo, entre el 30-50% de los cánceres pueden prevenirse evitando los factores de riesgo (tabaquismo, bajo consumo de hortalizas y frutas, consumo de alcohol en exceso, inactividad física, sobrepeso/obesidad, estrés, infecciones microbiológicas, tóxicos ambientales y contaminantes de alimentos y agua). Otros determinantes son el envejecimiento de la población y factores relacionados con la genética y con el estado socio-económico (~70% de las muertes por cáncer se producen en países de ingresos medios y bajos) [20,21].

Se ha especulado que la dieta, además de estar estrechamente relacionada con la aparición de cáncer, puede tener efectos sobre la prognosis. Sin embargo, los estudios realizados hasta la fecha se han centrado especialmente en el caso del cáncer de mama, siendo insuficientes en otros tipos de cáncer [22].

2.2. Nutrición preventiva

Una dieta saludable, rica en compuestos bioactivos, puede disminuir el riesgo de desarrollar ENT debido a su implicación en diversas funciones fisiológicas [23–28]. En los últimos años, se ha descrito una interacción bidireccional entre algunos de estos compuestos, especialmente fenólicos, y la microbiota intestinal [29,30]. Los compuestos bioactivos de la dieta que se relacionan con la microbiota pueden cambiar su composición, y a su vez aquellos que no son absorbidos y llegan al colon pueden dar lugar a productos metabólicos también activos biológicamente. Por lo tanto, una dieta rica en compuestos bioactivos podría tener efectos positivos sobre la microbiota y de forma directa e indirecta disminuir el riesgo de las ENT (**Figura 4**).

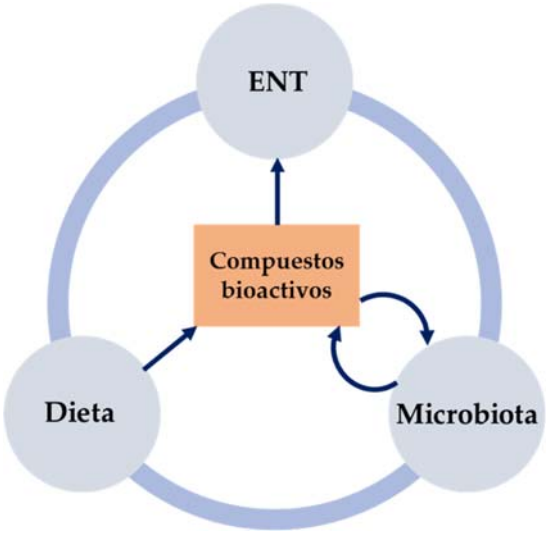


Figura 4. Interacción entre la dieta, los compuestos bioactivos, la microbiota y las enfermedades no transmisibles (ENT).

2.2.1. Dieta mediterránea

La dieta mediterránea (DM) se caracteriza por su riqueza en alimentos de origen vegetal principalmente [28,31]. En 1948, se llevó a cabo el primer estudio en el cual se comparó la dieta que consumían en la isla de Creta con la de Grecia y Estados Unidos y que llevó a una publicación posterior de los resultados en relación con la incidencia de ECV [32]. De forma paralela, el estudio de los Siete Países, liderado por Ancel Keys, puso de manifiesto los beneficios de la dieta que se consumía en los países mediterráneos [33,34]. Desde entonces, numerosas publicaciones científicas han corroborado el efecto protector de la DM sobre las ENT [23–28]. Además, un informe reciente de la OMS sugiere la DM como un patrón dietético idóneo en la prevención de la malnutrición y ENT como la diabetes, las cardiopatías, los accidentes cerebrovasculares y el cáncer [23]. También un estudio publicado en la revista US News and World Report sitúo a la DM en el primer puesto entre las 41 dietas populares identificadas, particularmente por el alto contenido en productos vegetales y la capacidad de prevenir ENT tales como ECV y diabetes, considerándola también la de más fácil seguimiento [35].

En la base de la DM se encuentran las hortalizas y las frutas, esenciales por su composición rica en micronutrientes (vitaminas C, E y folato, provitamina A y minerales), compuestos fitoquímicos (polifenoles, carotenoides, glucosinolatos, esteroles, etc) [36–38] y fibra [39].

El consumo de hortalizas y frutas se ha relacionado con una disminución del riesgo de ENT [40–43]. Una relación inversa con el riesgo de ECV y cáncer ha sido observada entre los grupos estudiados con una mayor ingesta, sin embargo el nivel de evidencia es superior en el caso de las ECV [40,41]. Según el informe publicado por el World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR), el riesgo del cáncer aerodigestivo y algunos otros como el de esófago, pulmón y vejiga podrían disminuir con una dieta que contenga al menos 3 raciones de hortalizas al día y 2 de frutas (considerando una ración = 80 g). Por el contrario, consumir una cantidad menor a la recomendada podría aumentar el riesgo de padecer cáncer colorrectal o gástrico [44].

Además de su efecto beneficioso en la prevención de enfermedades, una ingesta adecuada de hortalizas y frutas también podría disminuir la tasa de mortalidad global, así como el número de muertes por ECV y cáncer [41–43,45]. En el 2017 hubo 3,88 millones de muertes que se atribuyeron a un bajo consumo de hortalizas y frutas (**Figura 5**).

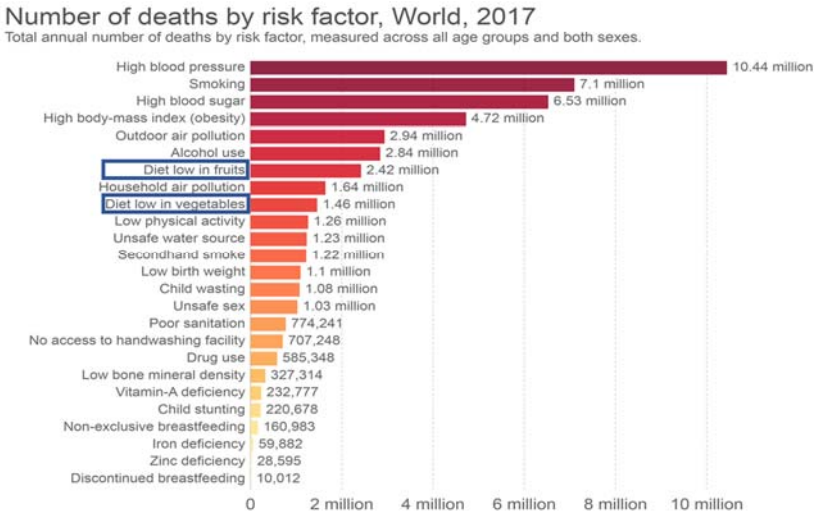


Figura 5. Número de muertes por enfermedades no transmisibles atribuidas a los factores de riesgo en el año 2017 [15,46].

A pesar de que las OMS aconseja un consumo mínimo de 400 gramos de hortalizas y frutas al día (equivalente a 5 raciones diarias) [47], un estudio reciente sugirió incrementarlo a 800 gramos/día para reducir el riesgo de algunas ENT [42]. Sin embargo, según el *European Health Interview Survey* (EHIS) solo un 14,1% de los adultos de la UE alcanza la ingesta mínima propuesta de 5 raciones de hortalizas y frutas al día [48]. Además, se ha observado que algunos factores como el nivel socio-económico

afecta al consumo de estos alimentos, siendo este muy inferior en los países poco desarrollados [49].

Como ya se comentó en la sección *2.1.1*, la dieta se evalúa principalmente a través de FFQ. Además, existen métodos validados que indican la adherencia a la DM a través de cuestiones sobre el patrón de consumo dietético. Un consumo elevado de alimentos de origen vegetal (hortalizas, frutas, legumbres, *sofrito*, frutos secos, y aceite de oliva virgen), moderado de vino, pescado y de carne blanca (pollo, pavo o conejo) y bajo de carnes rojas y procesados, bebidas carbonatadas y bollería industrial son propios de una DM [50,51].

2.2.2. Sofrito: un producto típico de la dieta mediterránea

El cuestionario de 14-items sobre la adherencia a la DM termina con el siguiente ítem: *"¿Cuantas veces a la semana consumes los vegetales cocinados, la pasta, arroz u otros platos aderezados con salsa de tomate, ajo, cebolla o puerro elaborada a fuego lento con aceite de oliva (sofrito)?"* [50,51]. El *sofrito* es un elemento de la DM producido mediante el procesado del tomate junto con aceite de oliva y otros ingredientes como la cebolla, ajo y/o puerro. Se trata de una salsa vegetal rica en compuestos bioactivos, siendo el licopeno el más predominante, seguido del β-caroteno. Entre los compuestos fenólicos identificados, los mayoritarios fueron la rutina, la quercetina y la naringenina [52]. Diversos autores han descrito un aumento en la concentración y absorción de estos fitoquímicos en salsas de tomates que contienen aceite, con respecto al tomate crudo o la preparación sin aceite [53–57]. Además, recientemente se ha observado que la técnica de cocinado utilizada en la preparación del *sofrito* y la concentración de cebolla también afectan positivamente a la liberación de estos compuestos bioactivos [58]. Estos factores favorecen la isomerización del licopeno a su forma *cis*, que es más biodisponible [59].

En cuanto a las consecuencias a nivel de salud que conlleva incluir en la dieta este alimento, las investigaciones sugieren un efecto protector en relación con las ECV y cáncer de próstata [60–64]. El consumo habitual de *sofrito* parece favorecer la regulación de procesos metabólicos, inflamatorios y hormonales [60–63].

2.2.3. Alimentos orgánicos y el impacto sobre la salud (Publicación 1)

Artículo titulado "Organic food and the impact on human health" Publicado en la revista científica "*Critical Reviews in Food Science and Nutrition*" (Índice de impacto: 6.704 (2018); Q1, D1).

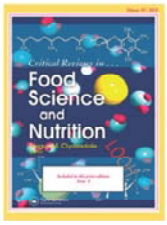
Debido a la divulgación científica y social sobre los efectos beneficiosos de llevar a cabo una dieta sana y sostenible con el medioambiente, en los últimos años ha incrementado notablemente el consumo de alimentos de origen ecológico. En primer lugar, la

agricultura ecológica tiene una limitación más estricta en el uso de pesticidas, fertilizantes sintéticos y antibióticos. Se ha sugerido un valor nutricional mayor en los alimentos de origen ecológico en cuanto al contenido de compuestos bioactivos (polifenoles, carotenoides y vitamina C) y ácidos grasos poliinsaturados, además de una concentración más baja de cadmio.

Sin embargo, aunque se han encontrado efectos beneficiosos en relación con el riesgo de desarrollar enfermedades metabólicas, no hay evidencias claras hasta la fecha que asocien el consumo de alimentos ecológicos con un mejor estado de salud. Algunos factores como el estilo de vida deberían tenerse en cuenta en los estudios observacionales, debido a que los consumidores de estos productos podrían tener hábitos más saludables.

La escasez de estudios y la corta duración de estos, así como la falta de control de todos los factores que pueden afectar (a nivel individual y ambiental) no han permitido hasta la fecha conocer el impacto del seguimiento de una dieta ecológica.

Los detalles sobre la evidencia científica conocida hasta la fecha pueden encontrarse en la publicación titulada *"Organic food and the impact on human health"*, presentada a continuación **(Publicación 1).**



Taylor & Francis

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Organic food and the impact on human health

Sara Hurtado-Barroso, Anna Tresserra-Rimbau, Anna Vallverdú-Queralt & Rosa María Lamuela-Raventós

To cite this article: Sara Hurtado-Barroso, Anna Tresserra-Rimbau, Anna Vallverdú-Queralt & Rosa María Lamuela-Raventós (2019) Organic food and the impact on human health, Critical Reviews in Food Science and Nutrition, 59:4, 704-714, DOI: 10.1080/10408398.2017.1394815

To link to this article: https://doi.org/10.1080/10408398.2017.1394815

Published online: 30 Nov 2017.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
2019, VOL. 59, NO. 4, 704–714
https://doi.org/10.1080/10408398.2017.1394815

Organic food and the impact on human health

Sara Hurtado-Barroso[a,b,c], Anna Tresserra-Rimbau[a,b,c], Anna Vallverdú-Queralt[a,b], and Rosa María Lamuela-Raventós[a,b,c]

[a]Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain; [b]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Spain; [c]INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, Barcelona, Spain.

ABSTRACT

In the last decade, the production and consumption of organic food have increased steadily worldwide, despite the lower productivity of organic crops. Indeed, the population attributes healthier properties to organic food. Although scientific evidence is still scarce, organic agriculture seems to contribute to maintaining an optimal health status and decreases the risk of developing chronic diseases. This may be due to the higher content of bioactive compounds and lower content of unhealthy substances such as cadmium and synthetic fertilizers and pesticides in organic foods of plant origin compared to conventional agricultural products. Thus, large long-term intervention studies are needed to determine whether an organic diet is healthier than a diet including conventionally grown food products. This review provides an update of the present knowledge of the impact of an organic versus a conventional food diet on health.

KEYWORDS

Polyphenols; pesticides residues; allergies; fertility; cardiovascular; gut microbiota

Introduction

"Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, considering that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system" (FAO/WHO Codex Alimentarius Commission, 1999). Although the practice of organic agriculture has traditionally existed, the modern organic movement began in Europe around the 1920s, at the same time as industrialized agriculture. Nonetheless, this movement became highly regarded in the 1970s due to greater knowledge of the adverse effects of fertilizers and pesticides employed in conventional practices. The use of pesticides became popular during the 1960s, mainly dichloro-diphenyl-trichloroethane (DDT), which was first introduced in the 1940s (Durham, 1963). Nonetheless, since the Haughley experiment performed by Balfour in 1939 (Balfour, 1943), which aimed to compare organic and conventional food systems and to study the relationship between health and the use of organic and conventional chemical-based farming, other studies have been performed with the same objective (Brantsæter *et al.*, 2017). In addition, the growing interest in organic agriculture has promoted the foundation of several institutions such as the International Federation of Organic Agriculture Movements (IFOAM).

Since the beginning of the organic farming system, the production and markets for organic products have grown worldwide. In 2015, the global organic market reached 81.6 billion US dollars (76.8 billion euros), with the United States having the largest market for organic food (FiBL). This amount is between 4.5-fold higher than 15 years ago (FiBL). From 1999 to 2015, the number of countries using an organic agriculture system rose from 77 to 179, and more than 84% of the producers are in Asia, Africa and Latin America, being India, Ethiopia and Mexico the main producers (FiBL). However, the largest area of organic agriculture land is Australia, followed by Argentina, the USA and Spain (FiBL). Wild organic collection areas are concentrated in Europe, Africa, Asia and Latin America (FiBL). Certified organic acreage and livestock have also been expanding, mainly for fruit, vegetable, dairy, and poultry production, accounting for over 4% of the total food sales in the US (USDA ERS).

Despite the higher cost and lower productivity of organic compared to traditional agriculture, the trend to consuming these products is rising every year. Indeed, consumers are prepared to pay a higher price for organic products because they believe organic food is healthier and more sustainable with the environment. Organic foods are generally more natural products and involve less processing than conventional food. They are grown with limited synthetic pesticides or fertilizers or routine use of antibiotics or growth hormones (IFOAM). However, the limited use of pesticides and the use of animal feces may be the main cause of microbiological contamination in organic raw vegetables. Although the majority of studies have reported differences between organic compared to conventional food, there is still no common agreement on this subject. A review on the content of secondary metabolites in organic products stated that in terms of nutritional composition it is still not possible to conclude that an organic production system is better than a conventional system (Barański *et al.*, 2017). The aim of the present review was to evaluate the scientific evidence about the benefits of organic compared with conventionally produced food.

CONTACT Rosa María Lamuela-Raventós lamuela@ub.edu

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

Results

Lifestyle of organic versus conventional consumers

To compare organic with conventional foods and their effects on health, the characteristics of the subjects who consume the two kinds of food should be taken into account. In order to achieve this goal, a research group from France designed the NutriNet-Santé study (Hercberg *et al.*, 2010), a prospective observational cohort including 500,000 participants greater than or equal to 18 years of age followed over a 10-year period. The NutriNet-Santé study (Baudry *et al.*, 2015; Eisinger-Watzl *et al.*, 2015) showed that organic food is typically more consumed by women compared to men and by people with a high level of education, who are physically active, have a lower body mass index (BMI) and who follow a vegetarian or vegan diet (Eisinger-Watzl *et al.*, 2015). Baudry *et al.* also observed that pregnant women living in rural areas consume more organic food than those living in urban areas (Baudry *et al.*, 2015), contradicting previous research (Torjusen *et al.*, 2010). The relationship between organic consumption and the use of alcohol and smoking is not clear (Baudry *et al.*, 2015; Eisinger-Watzl *et al.*, 2015; Torjusen *et al.*, 2010).

The main reasons why people choose organic food are health and environmental concerns, absence of contaminants in food and taste (Baudry *et al.*, 2017). Organic food is associated with freshness and naturalness, while conventional food is sometimes related to processed food (Baudry *et al.*, 2017). In addition, it has been reported that products of plant origin are more frequently used in this kind of diets compared to products of animal origin (eggs being an exception) (Baudry *et al.*, 2015). Therefore, in general, an organic-based dietary pattern is associated with a healthier lifestyle. However, this association between organic diet and healthier lifestyle should be considered when evaluating the health outcomes of people consuming organic food.

Is organic food better in bioactive compounds?

The differences between the quality of organic versus non-organic foods remain unclear, although a meta-analysis concluded that organic foods were richer in some healthy components such as polyphenols and had lower or null pesticide and cadmium (Cd) concentrations (Barański *et al.*, 2014) (see Figure 1). Polyphenols are secreted by plants in response to stress stimuli. Plants such as organically produced foods grown with fewer fertilizers are in a more stressful environment and therefore have higher concentrations of these compounds. Conversely, fertilizer may also reduce the stress of the plants. In addition, long-term use of phosphorus fertilizers can increase the level of Cd in conventional crops (Kratz *et al.*, 2016). Vallverdu-Queralt and Lamuela-Raventós reviewed the outcomes of nutrient content in organic and conventional food by foodomic technologies and concluded that organic crops had less nitrogen and higher levels of polyphenols and carotenoids (Vallverdú-Queralt and Lamuela-Raventós, 2016). However, differences in the protein content of the two crop systems is still not clear (Magkos *et al.*, 2003; Palupi *et al.*, 2012; Vallverdú-Queralt and Lamuela-Raventós, 2016) since the higher protein content in conventional food described in several studies may be, in part, due to the nitrogen content of the fertilizers used (Magkos *et al.*, 2003).

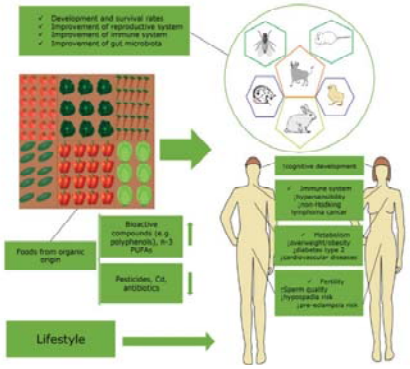


Figure 1. Effects of organic food on animal and human organisms and the possible influence of lifestyle in humans (preliminary results).

However, the quality of protein in organic food is better compared to that of conventionally produced food (Magkos *et al.*, 2003).

Furthemore, differences in fatty acid profiles have been observed. Particularly, organic animal products such as meat and milk have shown a higher content of n-3 PUFA and total PUFA and higher α-linolenic acid, conjugated linoleic acid, vaccenic acid, eicosapentanoic acid and docosapentanoic acid (Palupi *et al.*, 2012; Średnicka-Tober *et al.*, 2016a; Średnicka-Tober *et al.*, 2016b). This is due to forage feeding of organically raised animals. Nonetheless, these differences in n-3 PUFA content and the level of this fatty acid in non-organic meat and milk continue to be too low to have any effect on human health. There also seems to be a trend to higher vitamin C and E and certain mineral concentrations in some organic products (Brantsæter *et al.*, 2017).

Several studies have evaluated the differences between the polyphenol content of organic and conventional grown potatoes, white cabbage, carrots, wheat, pepper, tomatoes, spinach, garlic, bell pepper, maize kernels, red grapes, lettuce, apples, and cauliflowers, as well as tomato-derived foods such as tomato juices and ketchups (Koh *et al.*, 2012; Ren *et al.*, 2001; Vallverdú-Queralt *et al.*, 2012a; Vallverdú-Queralt *et al.*, 2014; Vallverdú-Queralt *et al.*, 2011; Vallverdú-Queralt *et al.*, 2012b; Mitchell *et al.*, *2007*;Borguini, 2006; Győre-Kis et al., 2012; Stracke *et al.*, 2009a; Roussos and Gasparatos, 2009; Wang *et al.*, 2008; Rosati *et al.*, 2014; Lo Scalzo *et al.*, 2013; Raigón *et al.*, 2010; Rossetto *et al.*, 2009; Carbonaro *et al.*, 2002; Asami *et al.*, 2003 and Lima *et al.*, 2008). Koh *et al.* compared the levels of flavonoids in 27 spinach varieties grown in organic and conventional cropping systems (Koh *et al.*, 2012). The levels of flavonoids were significantly higher in organically grown spinach (40.48 ± 6.16 and 2.83 ± 0.03 mg/kg) compared to the conventionally grown product (25.75 ± 6.12 and 2.27 ± 0.02 mg/kg). Ren *et al.* determined the polyphenol content of five vegetables grown in organic and conventional cultivation systems commonly consumed in Japan (collard greens, Chinese cabbage, spinach, garlic and green bell pepper) and found the quercetin and caffeic acid content in organically grown plants to be 1.3–10.4 times higher than in conventional plants, suggesting the influence of the different cultivation practices (Ren *et al.*, 2001). Other studies carried out in tomatoes, tomato juices, and ketchups have also reported

differences between organic and conventional products (Vallverdú-Queralt *et al.*, 2012a; Vallverdú-Queralt *et al.*, 2014; Vallverdú-Queralt *et al.*, 2011; Vallverdú-Queralt *et al.*, 2012b). One study using a metabolomic approach to identify metabolites with the greatest impact on the overall metabolomic profile of organic versus conventional ketchups (Vallverdú-Queralt *et al.*, 2011) showed that the tomato production system may increase the content of phenols and other metabolites in ketchups. Moreover, the same authors also compared the carotenoid content of organic and conventional tomato-based products (tomato juices and ketchups) (Vallverdú-Queralt *et al.*, 2014) and found statistically higher levels of carotenoid compounds in the organic products. Similarly, Mitchell *et al.* carried out a targeted approach to analyze quercetin and kaempferol levels in tomatoes (Mitchell *et al.*, 2007). The levels of quercetin and kaempferol in organic tomatoes were 79 and 97% higher, respectively, than in conventional tomatoes. Another targeted approach found higher levels of phenolic compounds and ascorbic acid in organic tomatoes (Borguini, 2006). Similarly, Györe-Kis *et al.* recorded a higher content of phenolic compounds in nine cultivars of organic tomatoes in three consecutive years (2008, 2009 and 2010) (Györe-Kis et al., 2012).

Stracke *et al.* evaluated the polyphenol profile of apples (Golden Delicious) grown under defined organic and conventional conditions and described that organically produced apples showed significantly higher concentrations of chlorogenic acid, flavonols, flavanols and dihydrochalcones (Stracke *et al.*, 2009a). However, Roussos and Gasparatos assessed the total phenol, total *o*-diphenol, total flavanol and total flavonoid content in apples cultivated according to organic and conventional procedures and found higher contents in the conventional products (Roussos and Gasparatos, 2009).

In another study, Wang *et al.* evaluated the effects of organic or conventional culture systems on sugar, organic acid, anthocyanin, and phenolic content, as well as antioxidant activity in blueberries and concluded that organically produced fruit had a higher content of phytochemicals (Wang *et al.*, 2008).

Another approach was applied to determine whether organic versus conventional practices affect olive fruit and oil composition in two cultivars (Rosati *et al.*, 2014). The characteristics of the fruit (i.e. fresh and dry weight of pulp and pit, and oil content) did not differ and neither did the oil chemical traits except for an increased content of polyphenols in the organic products. A targeted approach was applied to green Italian cauliflowers (HF1 Emeraude and a local variety, Velox) grown in organic and conventional management systems (Lo Scalzo *et al.*, 2013). Compared to the conventional cauliflowers, organic Velox showed a 21 and 13% higher content of polyphenols and carotenoids, respectively.

Other studies have also reported higher concentrations of total phenolic compounds in organic eggplants (Raigón *et al.*, 2010), beets (Rossetto *et al.*, 2009), peaches and pears (Carbonaro *et al.*, 2002), marionberries (Asami *et al.*, 2003) and miscellaneous plants (Lima *et al.*, 2008).

Organic food and human health

Some studies comparing organic with conventional food have described lower pesticide levels and a higher nutritional value in organic crops, but few clinical and epidemiological studies have been carried out so far. However, dietary intervention and epidemiological studies have observed higher amounts of some bioactive compounds in organic crops, which could be linked to a decrease of the risk of chronic diseases, including cardiovascular and neurodegenerative diseases and certain cancers. This suggests that the consumption of organic food may be beneficial (Johansson *et al.*, 2014).

Nutritional markers in humans

Despite the differences in bioactive compounds described in organic compared to conventional food, the nutritional intervention studies performed so far have not shown a clear association between antioxidant levels in humans and the organic or conventional cropping system (Table 1). Three non-cross-over (Caris-Veyrat *et al.*, 2004; Stracke *et al.*, 2010; Stracke *et al.*, 2009b) and three cross-over trials (Akçay *et al.*, 2004; Briviba *et al.*, 2007; Søltoft *et al.*, 2011) did not observe significant differences in the antioxidant capacity or bioavailability of bioactive compounds such as carotenoids, vitamin C, and polyphenols, while two nutritional cross-over intervention studies observed several significant differences (Grinder-Pedersen *et al.*, 2003; Di Renzo *et al.*, 2007). Grinder-Pedersen *et al.* evaluated the intake and excretion of five selected flavonoids in 16 healthy individuals and the effects on biomarkers of oxidative defense (Grinder-Pedersen *et al.*, 2003). They found the urinary excretion of quercetin and kaempferol to be higher after 22 days of organic food intake. It seems that the conditions of food growth may affect the content of the major flavonoids, quercetin and kaempferol, not only in foods, but also in the excretion of urinary flavonoids and biomarkers of oxidation in humans. In the cross-over study carried out by Di Rienzo *et al.* comparing the consumption of organically and conventionally produced apples in 10 healthy men antioxidant levels in plasma were higher after the intake of organic apples (Di Renzo *et al.*, 2007).

Differences between the two cultivation systems in terms of minerals are not clear. A cross-over intervention trial reported no differences in the intake and bioavailability of copper and zinc in men after consuming organic versus conventional products (Mark *et al.*, 2013). Factors such as the kind of soil could be more significant in mineral concentrations than the cultivation system.

Finally, the cross-sectional KOALA study showed higher levels of vaccenic acid and conjugated linoleic acid in breast milk of lactating women who consumed an organic diet (Rist *et al.*, 2007).

Cropping system and diseases

The first studies to evaluate the effects of organic and conventional practices on health were carried out in animals in the 1930s. Improvement in the growth and survival rates, higher fertility/reproductive function, immune stimulation, lower weight gain and more stress resistance have been the main outcomes described to date in rats, mice, rabbits, chicken and bulls fed by organic systems (Velimirov *et al.*, 2010) (see Figure 1), with these results having been corroborated in more recent studies. Roselli *et al.* showed an enhancement in the immune systems of mice fed with organic carrots compared to those fed with conventional carrots (Roselli *et al.*, 2012). A nutrigenomic approach found differences in the jejunal gene expression involved in cholesterol biosynthesis and immunological process in chickens fed an organic diet (De Greeff *et al.*, 2010). Longer

Table 1. Nutritional markers associated with the organic diet intake in humans: Scientific evidences.

End point	Type of Study	Subjects	Intervention	Outcomes	Reference
Antioxidant status	Non-crossover study	36 healthy men, aged 19–54 years	Consumption of 200 g /day blanched carrots from organic or conventional agricultural systems for 2 weeks with a main meal and with a minimum of 10 g fat.	No effect on the biodisponibility of carotenoids.	Stracke et al., 2009b
	Cross-over study	6 healthy men, aged 23–32 years	Consumption of 1000 g of either organically and conventionally produced apples.	No differences between organic and conventional apples on antioxidant capacity of LDL and DNA damage.	Briviba et al., 2007
	Cross-over study	16 healthy volunteer subjects, aged 21–35 years	Consumption for 22 days of the organic and conventional diet.	Higher urinary excretion of flavonoids, particularly quercetin and kaempferol.	Grinder-Pedersen et al., 2003
	Cross-over study	18 adult and healthy men	Conventional or organic using animal manure or organic using cover crops diet for 12 days.	No differences between the agricultural in plasma status of carotenoids.	Soltoft et al., 2011
	Non-crossover study	20 healthy women, aged 21–39 years	Consumption of 96 g/day of organic or conventional tomato puree for 3 weeks.	No significant difference on plasma levels of vitamin C and carotenoids.	Caris-Veyrat et al., 2004
	Cross-over study	10 healthy men, aged 30–65 years	Consumption of a conventional diet first for first 14 days and then an organic diet for other 14 days.	Higher antioxidant capacity (ORAC) after intake of an organic diet.	Di Renzo et al., 2007
	Non-crossover study	43 healthy men, aged 22–40 years	Consumption of organically or conventionally produced apples (500 g/day; 4 weeks) or no apples.	No difference in the bioavailability of apple polyphenols.	Stracke et al., 2010
	Cross-over	8 healthy volunteer subjects, aged 24–45 years	Consumption of organic red wine (200 mL with alcohol content 24 g in men and 100 mL with alcohol content: 12 g in women) and then consumption of non-organic red wine (200 mL with alcohol content 22.4 g in men and 100 mL with alcohol content 11.2 g in women).	No significant differences between two types of wines with respect to LDL-TBARS blood levels.	Akçay et al., 2004
Intake and absortion of minerals	Two cross-over studies	17 and 16 healthy men, aged 18–40 years	Conventional and organic diet and administration of stable enriched isotopes (12 days).	No difference in the intake and absorption of copper and zinc.	Mark et al., 2013
Conjugated linoleic acids in breast milk	Cross-sectional within the study KOALA Birth Cohort Study	312 breastfeeding mothers, 1 month after partum.	Does not apply	Higher rumenic acid and trans-vaccenic acid in human breast milk in the mother's following a diet that contained organic dairy and meat products, in comparison with mother's consuming a conventional diet.	Rist et al., 2007

longevity and fertility were observed in a *Drosophyla melanogaster* fly model incubated in controlled conditions of humidity, temperature and light cycle and fed with homogenized organic food (bananas, potatoes, raisins, soy beans) compared with a conventional diet (Chhabra *et al.*, 2013). Recently, Parelho *et al.* associated higher hepatic lead loads and testicular damage in mice with conventional farming (Parelho *et al.*, 2016). However, Jensen *et al.* observed lower prostaglandin E2 concentrations in rat offspring of mothers fed with the organic plant-based diet compared to those fed with a conventional plant-based diet (Jensen *et al.*, 2013).

In humans, several observational studies have described beneficial effects of organic food on allergic, atopic, eczema and asthma symptoms, as well as other hypersensitivity diseases with the use of immunoglobulin E (Ig E) measurements and questionnaires in children (Alfvén *et al.*, 2006; Alm *et al.*, 1999; Kummeling *et al.*, 2008; Stenius *et al.*, 2011) and adults (Smit *et al.*, 2007) (Table 2). The effects of an organic diet on allergies and atopic diseases were observed mainly in childhood in the PARSIFAL, KOALA and ALADDIN studies. The PARSIFAL (Alfvén *et al.*, 2006) (cross-sectional study) and ALADDIN (Stenius *et al.*, 2011) (prospective study) studies were carried out in children belonging to European families following an anthroposophic or non-anthroposophic lifestyle.

In relation to other diseases, a cross-over study showed a lower risk of developing cardiovascular diseases in both healthy men and patients with chronic kidney diseases after an organic diet (De Lorenzo *et al.*, 2010), while a prospective study did not observe any significant differences in the presentation of cancer in women who ate organic food compared with those who consumed conventional food (Bradbury *et al.*, 2014) (Table 2).

Organic diet on fertility and pregnancy

Table 3 shows the studies related to the effects of organic food intake on fertility and pregnancy in humans. Three cross-sectional studies reported better sperm quality (Abell *et al.*, 1994; Jensen *et al.*, 1996; Juhler *et al.*, 1999) and one described a lower prevalence of previous genital disorders (Jensen *et al.*, 1996) in organic farmers compared to traditional farmers or other workers consuming conventional food. Larsen *et al.* found no significant differences in sperm quality, although organic farmers showed slightly higher inhibin B concentrations and a higher testosterone/sex hormone binding globulin ratio compared to conventional farmers (Larsen *et al.*, 1999). In the two prospective studies within the MoBa study which included a cohort of children and mothers and was aimed at comparing the effects of organic versus conventional food consumption, the risk of pre-eclampsia in pregnant women was found to be lower (Torjusen *et al.*, 2014) as was the risk of presenting

Table 2. Overview of scientifics evidences about the organic food intake on the risk of diseases in humans.

End point	Type of Study	Subjects	Intervention	Outcomes	Reference
Hypersensitivity diseases	Cross-sectional study	675 children, aged 5–13 years, at anthroposophic and neighbouring schools	Does not apply	Lower prevalence of atopy in children from anthroposophic families than in children from other families	Alm et al., 1999
	Cross-sectional study within the PARSIFAL study (Prevention of Allergy Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle)	14893 children, aged 5–13 years, from families with an anthroposophic lifestyle compared with children from appropriate reference groups	Does not apply	Lower prevalence of allergic symptoms and sensitization in children with anthroposophic lifestyle	Alfvén et al., 2006
	Cross-sectional study	1798 male adults, conventional and organic farmers	Does not apply	Lower wheezing with shortness of breath in organic farmers than in conventional farmers, suggesting a lower risk of asthma-like symptoms in organic farmers	Smit et al., 2007
	Prospective study within the ALADDIN study (Assessment of Lifestyle and Allergic Disease During Infancy)	330 children from families with an anthroposophic, partly anthroposophic, or nonanthroposophic lifestyle	Does not apply	Reduced risk of IgE sensitization already in infancy is associated with anthroposophic lifestyle	Stenius et al., 2011
	Cross-sectional study within the KOALA Birth Cohort Study in the Netherlands	2764 children, aged 2 years	Does not apply	Lower eczema risk was associated with consumption of organic dairy products	Kummeling et al., 2008
Cancer	Prospective study	623 080 middle-aged women	Does not apply	Slight or no decrease in the incidence of cancer associated with consumption of organic food, except possibly for non-Hodgkin lymphoma	Bradbury et al., 2014
Cardiovascular diseases	Cross-over study	100 healthy male individuals, aged 30–65 years and 50 male patients with Chronic Kidney Disease but stable renal function, aged 42–54 years	Consumption of mediterranean diet with conventional and organic products for 14 days each intervention	The Italian Mediterranean Organic Diet reduces total homocysteine, phosphorus, microalbuminuria levels and CVD risk in healthy individuals and in CDK patients	De Lorenzo et al., 2010

Table 3. Overview of scientifics evidences about the organic food intake on fertility and pregnancy in humans.

End point	Type of Study	Subjects	Intervention	Outcomes	Reference
Sperm quality and reproductive hormones	Cross-sectional study	30 male organic farmers (aged 28–44 years) and 73 blue-collar workers (aged 20–50 years)	Does not apply	Higher sperm quality in organic farmers	Abell et al., 1994
	Cross-sectional study	55 male organic farmers (aged 20–45 years) and 141 healthy men and working in an airline company (aged 23–43 years)	Does not apply	Higher sperm concentration and lower prevalence of previous genital disorders in men eating organically produced food.	Jensen et al., 1996
	Cross-sectional study	256 male farmers (171 traditional farmers and 85 organic farmers), aged ≤50 years	Does not apply	Lower proportion of morphologically normal spermatozoa in the group of men without organic food intake	Juhler et al., 1999
	Cross-sectional study	256 male farmers (171 traditional farmers and 85 organic farmers), aged ≤50 years	Does not apply	Slight differences in concentrations of reproductive hormones, but no significant differences in semen quality between organic and traditional farmers.	Larsen et al., 1999
Pre-eclampsia	Prospective cohort study within the Norwegian Mother and Child Cohort Study (MoBa)	28 192 pregnant women (nulliparous)	Does not apply	Lower risk of pre-eclampsia who reported frequent consumption of organically produced vegetables in pregnant women.	Torjusen et al., 2014
	Case-control study	306 mothers of boys operated for hypospadias and 306 mothers of healthy boys	Does not apply	Higher prevalence of hypospadias was associated with the consumption of high fat products and nonorganic milk and eggs diary	Christensen et al., 2013
Hypospadias and cryptorchidism	Prospective cohort study within the Norwegian Mother and Child Cohort Study (MoBa)	35107 mothers of singleton maleinfants	Does not apply	Lower prevalence of hypospadias was associated with the organic consumption, particularly organic vegetables and milk and dairy products, but it was not associated with the prevalence of cryptorchidism.	Brantsæter et al., 2016

hypospadias in male infants of mothers eating organically produced food (Brantsæter *et al.*, 2016). Likewise, another case-control study associated non-organic animal products such as milk and eggs with a higher prevalence of hypospadias (Christensen *et al.*, 2013) (Table 3).

Furthermore, it has been demonstrated that pesticides induce adverse health outcomes, such as neurotoxic effects and lower intellectual development (Ross *et al.*, 2013). Thus, in order to decrease the risk of developing certain diseases, dietary exposure to pesticide residues should be reduced, especially among pregnant women and children.

Pesticides: Are they really dangerous?

It is known that the widespread distribution of pesticides in food production could have possible long-term effects, triggering the development of diseases. A controlled study carried out by 85 traditional farmers using pesticides and 36 organic farmers not using pesticides living in the same geographical area showed that the use of pesticides induces genotoxic effects, immunological alterations and genetic damage (Costa *et al.*, 2014). In the Children's Pesticide Exposure Study (CPES), Lu *et al.* studied in 23 children aged 3–11 years who consumed only a conventional diet (Lu *et al.*, 2006; Lu *et al.*, 2008; Lu *et al.*, 2010). The children were fed an organic diet for 5 consecutive days after which several urinary metabolites of organophosphorus pesticides commonly used throughout the world were measured. The children were found to have lower pesticide levels in urine following the organic diet intervention. Likewise, an organic diet was associated with lower urinary pesticides in 4,466 participants aged 45–84 years included in the Multi-Ethnic Study of Atherosclerosis (MESA) (Curl *et al.*, 2015). Additionally, in a prospective, randomized, cross-over study in 30 healthy adults following a diet including at least 80% organic or conventional food during a period of one week, a reduction in organophosphorus metabolite concentrations was observed in the urine of the subjects consuming the mainly organic diet (Oates *et al.*, 2014). A recent report to the European Parliament declared that the risk to human health due to pesticide exposure is far lower for organic than for conventional food (EPRS, European Parliamentary Research Service and STOA, Scientific Foresight Unit).

Use of antibiotics in food-producing animals from organic and conventional farming

At present, the use of antibiotics in farming is controlled and a withdrawal time has been defined for each drug used in food-producing animals. Nevertheless, several studies have reported a lower efficiency of antibiotic treatment in human medical care because of the use of antibiotics in livestock (Martinez, 2009; Laxminarayan *et al.*, 2013; STAG-AMR, WHO), while other studies found no association between the use of antibiotics in farm animals and drug resistance in humans (Mather

et al., 2012; Mather *et al.*, 2013). According to European Legislation, both organic and conventional farming can use antibiotic treatments in animals with diseases (EC, The Council Of the European Union, 2007). However, unlike conventional practices, the use of antibiotics as subtherapeutic prophylactic treatment is forbidden in organic farming (EC, The Council Of the European Union, 2007).

The use of antibiotics is strongly associated with housing in intensive livestock production such as in conventional farming practices. In order to increase production levels, resources such as space and feed are restricted, leading to animal stress and a higher risk of developing diseases requiring antibiotic treatment (Colson *et al.*, 2006; EFSA, 2007).

Risk of microbiological contaminations: Are there differences between organic and conventional food?

This issue is controversial especially taking into account what happened in Europe in 2011 when 3,950 people were affected by *Escherichia coli O104:H4* bacteria and 53 died. This outbreak of foodborne disease was caused by an enteroaggregative *E. coli* (EAEC) strain that had acquired genes producing Shiga toxins present in organic fenugreek sprouts (King *et al.*, 2012).

Contamination by microorganisms such as *E. coli O157:H7* and mycotoxins is more frequent in organic food (Ceuppens *et al.*, 2014; Gomes *et al.*, 2012; Maffei *et al.*, 2013; Oliveira *et al.*, 2010; Kuzdraliński *et al.*, 2013; Piqué *et al.*, 2013). Studies from different areas of the world have shown a greater prevalence of *E. coli* in organic lettuce samples compared to lettuce from conventional agriculture (Ceuppens *et al.*, 2014; Gomes *et al.*, 2012; Maffei *et al.*, 2013; Oliveira *et al.*, 2010). However, a study performed in Canada comparing the levels of *E.coli* in organic and conventional fresh produce samples found no differences between the two groups (Bohaychuck *et al.*, 2009). Khalil and Gomaa detected a higher amount of *E. coli* in leafy greens from Egypt when they were produced by a conventional versus an organic system (Khalil and Gomaa, 2014). Other authors have reported that the microbiological quality and safety of fresh vegetables are not affected by organic or conventional farming practices (Tango *et al.*, 2014; Marine *et al.*, 2015; Kuan *et al.*, 2017). Ürkek *et al.* described significantly lower total aerobic mesophilic bacteria, coliform and yeast and mold counts in raw milk of organic origin in Turkey; however, somatic cell and *Staphylococcus aureus* counts were higher in organic milk (Ürkek *et al.*, 2017).

Mycotoxin levels in organic and conventional food differ. Kuzdraliński *et al.* studied the mycotoxin content of organic and conventional oats from Poland and observed that the number of mycotoxin-positive samples was higher in organic compared to conventional farming (Kuzdraliński *et al.*, 2013). However, the concentration of diacetoxyscirpenol, a mycotoxin from Fusarium, was four times lower in organic oats (Kuzdraliński *et al.*, 2013). A study carried out by Piqué *et al.* showed a higher incidence and concentration of another type of mycotoxin called patulin in organic apple purees compared to conventional purees (Piqué *et al.*, 2013).

Despite evidence indicating the higher risk of microbiological contamination in organic products, the wide use of antibiotics in conventional practices has been associated with the presence of antibiotic-resistant microorganisms in animal products (Saptoka *et al.*, 2014).

Does organic food benefit the gut microbiota?

Several ongoing studies are currently investigating the relationship between diet and the gut microbiota. There is strong evidence regarding the role of gut microbiota in health and its implication in immune-related disorders such as type 2 diabetes and obesity, as well as the risk of developing cardiovascular disease (Tai *et al.*, 2015; Miele *et al.*, 2015). Although the direct effects of an organic diet on the gut microbiota are not yet known, some studies have described a beneficial influence (Figure 2).

The diversity of microbials in soil varies in organic compared to conventional farming (Ottesen *et al.*, 2009; Hartmann *et al.*, 2014). Torjusen *et al.* hypothesized that an organic diet may be associated with a more diverse microbiota due to the intake of probiotic substances from fresh produce such as vegetables (Torjusen *et al.*, 2014). Pesticides and cooking practices have also been associated with the gut microbiota. When food is fermented, pesticide concentrations may be decreased, but may, at the same time, slow the fermentation process by yeast (Regueiro *et al.*, 2013). Polyphenols have been positively related to gut microbiota by the reciprocal relationship that they may contribute to host health benefits (Ozdal *et al.*, 2016; Conlon and Topping, 2016; Xue *et al.*, 2016). The higher polyphenol content of organic compared with conventional food could be an advantage to the development of healthy microbiota.

Hartmann *et al.* (Hartmann *et al.*, 2014) demonstrated that the use of fertilizers and particularly the application and quality of organic fertilizers, is the major determinant of the microbial diversity of soil. A review by Jin *et al.* reported that exposure to environmental pollutants can alter the composition of the gut microbiome, leading to disorders of energy metabolism, nutrient absorption and immune system function or other toxic symptoms (Jin *et al.*, 2017). Conventional cropping practices

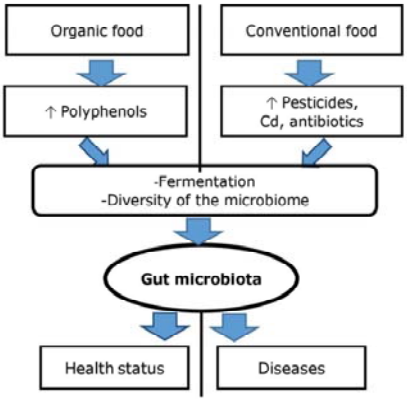


Figure 2. Mechanisms through which organic and conventional food could have effects on the gut microbiota contributing to health and unhealth status respectively (preliminary results).

are related to the use of environmental pollutants such as pesticides (mainly in vegetables and fruits), antibiotics (in meat and milk after administration to animals) and probably a larger amount of some heavy metals (e.g. Cd). Moreover, conventional food is often more processed and is more likely to contain food additives. Several studies in animals showed the potential of pesticides to change the gut microbiota and induce symptoms such as neurotoxicity, hepatotoxicity, immunotoxicity, reproductive toxicity and endocrine disruption because of the antimicrobial activity of some of these pesticides (Jin *et al.*, 2017). In the early 1950s, antibiotics were used by farmers to promote livestock growth. Nowadays, it is known that antibiotics can influence energy metabolism by affecting the gut microbiome (Jin *et al.*, 2017). In addition, the effects of antibiotics on human gut microbiota can persist over longer periods of time (Jakobsson *et al.*, 2010; Fouhy *et al.*, 2012). Exposure to heavy metals and food additives (e.g. non caloric artificial sweeteners and emulsifiers) can also lead to gut microbiota dysbiosis and induce immune-related disorders (Jin *et al.*, 2017).

Thus, it seems that conventional food has indirect negative effects on the gut microbiota because of the toxic components used in cropping. In addition, more diverse microflora has been observed in children from anthroposophic families, as well as a lower incidence of hypersensitivity diseases (Penders *et al.*, 2007). Furthemore, Torjusen *et al.* hypothesized that the incidence of pre-eclampsia could also be related to an altered microbiota (Torjusen et al., 2014). All these aspects should be explored in specific studies aimed at elucidating the role of organic food on human gut microbiota.

Conclusion: Is organic food really healthier?

Organic food seems to be healthier compared to conventional food due to its higher content of bioactive compounds (e.g. polyphenols, vitamin C and carotenoids) and n-3 PUFA, which could be implicated in the incidence of metabolic diseases. Moreover, organic food has a lower Cd content and other unhealthy substances such as pesticides that are related to gut microbiota dysbiosis and immune-related disorders and toxicity in humans.

However, the health outcomes reported by some studies could also be closely linked to the lifestyle of organic food consumers. A lower incidence of metabolic diseases such as cardiovascular diseases, diabetes mellitus type 2 and overweight or obesity can be the result of following a healthy dietary pattern. The diet of consumers of organic foods is richer in fruits, vegetables, legumes and wholegrains and lower in meat intake. Moreover, the greater content of dietetic fiber in organic food may have a positive effect on the gut microbiota and health, reducing the risk of diseases.

Although several studies have attempted to associate health-related biomarkers (such as antioxidant activity and status, LDL oxidation, semen quality, homocysteine, among others) with organic diet intake, the results remain unclear. Thus, at present, pesticide concentrations are the most commonly used biomarkers of conventional food intake.

Few studies have analyzed the effects of organic cropping on health and most of these studies have some limitations such as the short study duration or that they did not take into account crop variety, soil type, weather, climatic conditions and other factors which might affect crop composition. Therefore, long-term, randomized, controlled dietary intervention trials comparing organic and conventional food of the same variety and similar growing conditions are needed to determine the possible beneficial effects of organic diet on human health.

Funding

This work was supported in part by Ministerio de Economia y Competitividad (MCOC) under grant AGL2016-75329-R, Generalitat de Catalunya – Departament d'Agricultura, Ramaderia, Pesca i Alimentació – Direcció General d'Agricultura i Ramaderia under grant 53 05012 2016 and Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN) from the Ministerio de Economía, Industria y Competitividad (MINECO) and Fondo Europeo de desarrolo regional/Fondo social europeo, Unión Europea (FEDER/FSE, UE). The CIBEROBN is an initiative from the Instituto de Salud Carlos III (ISCIII). A.V.-Q. thanks the Ministry of Science, Innovation and Universities for the Ramon y Cajal contract.

References

Abell, A., E. Ernst, and J. P. Bonde. 1994. High sperm density among members of organic farmers' association. *Lancet.* 343:1498.

Akçay, Y. D., H. K. Yıldırım, U. Güvenç, and E. Y. Sözmen. 2004. The effects of consumption of organic and nonorganic red wine on low-density lipoprotein oxidation and antioxidant capacity in humans. *Nutr. Res.* 24:541–554.

Alfvén, T., C. Braun-Fahrländer, B. Brunekreef, E. Von Mutius, J. Riedler, a. Scheynius, M. Van Hage, M. Wickman, M. R. Benz, J. Budde, K. B. Michels, D. Schram, E. Ublagger, M. Waser, and G. Pershagen. 2006. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – The PARSIFAL study. *Allergy Eur. J. Allergy Clin. Immunol.* 61:414–421.

Alm, J. S., J. Swartz, G. Lilja, A. Scheynius, and G. Pershagen. 1999. Atopy in children of families with an anthroposophic lifestyle. *Lancet.* 353:1485–1488.

Asami, D. K., Y.-J. Hong, D. M. Barrett, and A. E. Mitchell. 2003. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. *J. Agric. Food Chem.* 51:1237–1241.

Balfour, L. E. 1943. *The Living Soil (First).* London: Faber and Faber.

Barański, M., D. Średnicka-Tober, N. Volakakis, C. Seal, R. Sanderson, G. B. Stewart, C. Benbrook, B. Biavati, E. Markellou, C. Giotis, J. Gromadzka-Ostrowska, E. Rembialkowska, K. Skwarlo-Sońta, R. Tahvonen, D. Janovská, U. Niggli, P. Nicot, and C. Leifert. 2014. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: A systematic literature review and meta-analyses. *Br. J. Nutr.* 112:794–811.

Barański, M., L. Rempelos, P. Iversen, and C. Leifert. 2017. Effects of organic food consumption on human health; the jury is still out! *Food Nutr. Res.* 61:1287333.

Baudry, J., C. Méjean, B. Allès, S. Péneau, M. Touvier, S. Hercberg, D. Lairon, P. Galan, and E. Kesse-Guyot. 2015. Contribution of organic food to the diet in a large sample of French adults (The NutriNet-Santé cohort study). *Nutrients.* 7:8615–8632.

Baudry, J., S. Péneau, B. Allès, M. Touvier, S. Hercberg, P. Galan, M.-J. Amiot, D. Lairon, C. Méjean, and E. Kesse-Guyot. 2017. Food Choice Motives When Purchasing in Organic and Conventional Consumer Clusters: Focus on Sustainable Concerns (The NutriNet-Santé Cohort Study). *Nutrients.* 9:88.

Bohaychuk, V. M., R. W. Bradbury, R. Dimock, M. Fehr, G. E. Gensler, R. K. King, R. Rieve, and P. Romero Barrios. 2009. A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. *J Food Prot.* 72:415–20.

Borguini, R. G. 2006. *Avaliação do potencial antioxidante e de algumas características físico-químicas do tomate (Lycopersicon esculentum) orgânico em comparação ao convencional.* Biblioteca Digital de Teses e

Dissertações da Universidade de São Paulo, São Paulo. https://doi.org/10.11606/T.6.2006.tde-14082006-153722

Bradbury, K. E., a. Balkwill, E. a. Spencer, a. W. Roddam, G. K. Reeves, J. Green, T. J. Key, V. Beral, and K. Pirie. 2014. Organic food consumption and the incidence of cancer in a large prospective study of women in the United Kingdom. *Br. J. Cancer.* 110:2321–6.

Brantsæter, A. L., H. Torjusen, H. M. Meltzer, E. Papadopoulou, J. a. Hoppin, J. Alexander, G. Lieblein, G. Roos, J. M. Holten, J. Swartz, and M. Haugen. 2016. Organic food consumption during pregnancy and hypospadias and cryptorchidism at birth: The Norwegian mother and child cohort study (MoBa). *Environ. Health Perspect.* 124:357–364.

Brantsæter, A. L., Ta. Ydersbond, Ja. Hoppin, M. Haugen, and H. M. Meltzer. 2017. Organic Food in the Diet: Exposure and Health Implications. *Annu. Rev. Public Health.* 38:annurev-publhealth-031816-044437.

Briviba, K., Ba. Stracke, C. E. Rüfer, B. Watzl, F. P. Weibel, and A. Bub. 2007. Effect of consumption of organically and conventionally produced apples on antioxidant activity and DNA damage in humans. *J. Agric. Food Chem.* 55:7716–7721.

Carbonaro, M., M. Mattera, S. Nicoli, P. Bergamo, and M. Cappelloni. 2002. Modulation of antioxidant compounds in organic vs conventional fruit (peach, Prunus persica L., and pear, Pyrus communis L.). *J. Agric. Food Chem.* 50:5458–62.

Caris-Veyrat, C., M. J. Amiot, V. Tyssandier, D. Grasselly, M. Buret, M. Mikolajczak, J. C. Guilland, C. Bouteloup-Demange, and P. Borel. 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J. Agric. Food Chem.* 52:6503–6509.

Ceuppens, S., C. T. Hessel, R. Q. Rodrigues, S. Bartz, E. C. Tondo, and M. Uyttendaelea. 2014. Microbiological quality and safety assessment of lettuce production in Brazil. *Int J Food Microbiol.* 181:67:76.

Chhabra, R., S. Kolli, and J. H. Bauer. 2013. Organically Grown Food Provides Health Benefits to Drosophila melanogaster. *PLoS One.* 8:1–8.

Christensen, J. S., C. Asklund, N. E. Skakkebæk, N. Jørgensen, H. R. Andersen, T. M. Jørgensen, L. H. Olsen, A. P. Høyer, J. Moesgaard, J. Thorup, and T. K. Jensen. 2013. Association between organic dietary choice during pregnancy and hypospadias in offspring: A study of mothers of 306 boys operated on for hypospadias. *J. Urol.* 189:1077–1082.

Colson, V., P. Orgeur, A. Foury, and P. Mormède. 2006. Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. *Appl. Anim. Behav. Sci.* 98:70–88.

Conlon, M. A., and D. L. Topping. 2016. Dietary polysaccharides and polyphenols can promote health by influencing gut microbiota populations. *Food Funct.* 7:1730–1730.

Costa, C., J. García-lestón, S. Costa, P. Coelho, S. Silva, V. Valdiglesias, F. Mattei, V. D. Armi, S. Bonassi, J. Snawder, and J. P. Teixeira. 2014. Is organic farming safer to farmers' health? A comparison between organic and traditional farming. *Toxicol Lett.* 230:166–176.

Curl, C. L., Saa. Beresford, Ra. Fenske, A. L. Fitzpatrick, C. Lu, Ja. Nettleton, and J. D. Kaufman. 2015. Estimating pesticide exposure from dietary intake and organic food choices: The Multi-Ethnic Study of Atherosclerosis (MESA). *Environ. Health Perspect.* 123:475–83.

De Greeff, A., M. Huber, L. van de Vijver, W. Swinkels, H. Parmentier, and J. Rebel. 2010. Effect of organically and conventionally produced diets on jejunal gene expression in chickens. *Br. J. Nutr.* 103:696–702.

De Lorenzo, A., A. Noce, M. Bigioni, V. Calabrese, D. G. Della Rocca, N. Di Daniele, C. Tozzo, and L. Di Renzo. 2010. The effects of Italian Mediterranean organic diet (IMOD) on health status. *Curr. Pharm. Des.* 16:814–824.

Di Renzo, L., D. Di Pierro, M. Bigioni, V. Sodi, F. Galvano, R. Cianci, L. La Fauci, and A. De Lorenzo. 2007. Is antioxidant plasma status in humans a consequence of the antioxidant food content influence? *Eur. Rev. Med. Pharmacol. Sci.* 11:185–192.

Durham, W. F. 1963. Pesticide residues in foods in relation to human health. In: Residue Reviews / Rückstands-Berichte, 33–81. New York, NY: Springer New York.

Eisinger-Watzl, M., F. Wittig, T. Heuer, I. Hoffmann, H. Verhagen, and S. Scientific Advisor. 2015. Customers Purchasing Organic Food -Do They Live Healthier? Results of the German National Nutrition Survey II. *Eur. J. Nutr. Food Saf.* 5.

EUR-Lex Access to European Union law- Document 32007R0834. Croatian, 2007. The Council Of the European Union. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Official Journal of the European Union.* http://eur-lex.europa.eu/legal-content/EN/TXT/?uri = celex:32007R0834, last access 14 July 2017.

European Parliamentary Research Servive.EPRS and Scientific Foresight Unit, STOA. Brussels, 2016. Human health implications of organic food and organic agriculture. doi:978-92-846-0395-4.

FAO/WHO Codex Alimentarius Commission. 1999.

FiBL -Media release. Nürnberg, February 2017. *The World of Organic Agriculture 2017.* http://www.fibl.org/en/media/media-archive/media-release/article/bio-waechst-weltweit-weiter-509-millionen-hektar-bio flaeche-biomarkt-ueber-80-milliarden-us-dollar.html, last access 14 July 2017.

Fouhy, F., C. M. Guinane, S. Hussey, R. Wall, C. A. Ryan, E. M. Dempsey, B. Murphy, R. P. Ross, G. F. Fitzgerald, C. Stanton, and P. D. Cotter. 2012. High-Throughput Sequencing Reveals the Incomplete, Short-Term Recovery of Infant Gut Microbiota following Parenteral Antibiotic Treatment with Ampicillin and Gentamicin. *Antimicrob. Agents Chemother.* 56:5811–5820.

Gomes Neto, N. J., R. M. L. Pessoa, I. M. B. N. Queiroga, M. Magnani, F. I. de Sousa Freitas, and E. L. de Souza. 2012. Bacterial counts and the occurrence of parasites in lettuce (Lactuca sativa) from different cropping systems in Brazil. *Food Control.* 28:47–51.

Grinder-Pedersen, L., S. E. Rasmussen, S. Bügel, L. V. Jørgensen, L. O. Dragsted, V. Gundersen, and B. Sandström. 2003. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *J. Agric. Food Chem.* 51:5671–5676.

Györe-Kis, G., K. Deák, A. Lugasi, A. Csúr-Vargaa, and L. Helyes. 2012. Comparison of conventional and organic tomato yield from a three-year-term experiment. *Acta Aliment.* 41:486–493.

Hartmann, M., B. Frey, J. Mayer, P. Mäder, and F. Widmer. 2014. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* 9:1177–1194.

Hercberg, S., K. Castetbon, S. Czernichow, A. Malon, C. Mejean, E. Kesse, M. Touvier, and P. Galan. 2010. The Nutrinet-Santé Study: A web-based prospective study on the relationship between nutrition and health and determinants of dietary patterns and nutritional status. *BMC Public Health.* 10:242.

Jakobsson, H. E., C. Jernberg, A. F. Andersson, M. Sjölund-Karlsson, J. K. Jansson, and L. Engstrand. 2010. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One.* 5:e9836.

Jensen, M. M., U. Halekoh, C. R. Stokes, and C. Lauridsen. 2013. Effect of maternal intake of organically or conventionally produced feed on oral tolerance development in offspring rats. *J. Agric. Food Chem.* 61:4831–4838.

Jensen, T. K., A. Giwercman, E. Carlsen, T. Scheike, and N. E. Skakkebaek. 1996. Semen quality among members of organic food associations in Zealand, Denmark. *Lancet.* 347:1844.

Jin, Y., S. Wu, Z. Zeng, and Z. Fu. 2017. Effects of environmental pollutants on gut microbiota. *Environ. Pollut.* 222:1–9.

Johansson, E., A. Hussain, R. Kuktaite, S. C. Andersson, and M. E. Olsson. 2014. Contribution of Organically Grown Crops to Human Health. *Int. J. Environ. Res. Public Health.* 11:3870–3893.

Juhler, R. K., S. B. Larsen, O. Meyer, N. D. Jensen, M. Spano, A. Giwercman, and J. P. Bonde. 1999. Human semen quality in relation to dietary pesticide exposure and organic diet. *Arch Env. Contam Toxicol.* 37:415–423.

Khalil, R., and M. Gomaa. 2014. Evaluation of the microbiological quality of conventional and organic leafy greens at the time of purchase from retail markets in Alexandria, Egypt. *Pol J Microbiol.* 63:237–43.

King, L. A., F. Nogareda, F. X. Weill, P. Mariani-Kurkdjian, E. Loukiadis, G. Gault, N. Jourdan-DaSilva, E. Bingen, M. Macé, D. Thevenot, N. Ong, C. Castor, H. Noël, D. Van Cauteren, M. Charron, V. Vaillant, B. Aldabe, V. Goulet, G. Delmas, E. Couturier, Y. Le Strat, C. Combe, Y. Delmas, F. Terrier, B. Vendrely, P. Rolland, and H. de Valk. 2012. Outbreak of Shiga toxin-producing Escherichia coli O104:H4 associated with organic fenugreek sprouts, France, June 2011. *Clin Infect Dis.* 54:1588–94.

Koh, E., S. Charoenprasert, and A. E. Mitchell. 2012. Effect of organic and conventional cropping systems on ascorbic acid, vitamin C, flavonoids, nitrate, and oxalate in 27 varieties of spinach (Spinacia oleracea L.). *J. Agric. Food Chem.* 60:3144–50.

Kratz, S., J. Schick, and E. Schnug. 2016. Trace elements in rock phosphates and P containing mineral and organo-mineral fertilizers sold in Germany. *Sci. Total Environ.* 542:1013–9.

Kuan, C. H., Y. Rukayadi, S. H. Ahmad, C. W. J. Wan Mohamed Radzi, T. Y. Thung, J. M. K. J.K. Premarathne, W. S. Chang, Y. Y. Loo, C. W. Tan, O. B. Ramzi, S. N. Mohd Fadzil, C. S. Kuan, S. K. Yeo, M. Nishibuchi, and S. Radu. 2017. Comparison of the Microbiological Quality and Safety between Conventional and Organic Vegetables Sold in Malaysia. *Front Microbiol.* 8:1433.

Kummeling, I., C. Thijs, M. Huber, L. P.LvaDe. Vijver, B. E. P. Snijders, J. Pender, F. Stelma, Ran. Ree, P. A.Den. Brandt, and P. C. Dagnelie. 2008. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. *Br. J. Nutr.* 99:598–605.

Kuzdraliński, A., E. Solarska, and J. Mazurkiewicz. 2013. Mycotoxin content of organic and conventional oats from southeastern Poland. *Food Control.* 33:68–72.

Larsen, S. B., M. Spanò, A. Giwercman, and J. P. Bonde. 1999. Semen quality and sex hormones among organic and traditional Danish farmers. ASCLEPIOS Study Group. *Occup. Environ. Med.* 56:139–144.

Laxminarayan, R., A. Duse, C. Wattal, A. K. M. Zaidi, H. F. L. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir, S. Kariuki, Z. A. Bhutta, A. Coates, R. Bergstrom, G. D. Wright, E. D. Brown, and O. Cars. 2013. Antibiotic resistance-the need for global solutions. *Lancet. Infect. Dis.* 13:1057–98.

Lima, G. P. P., S. A. Da Rocha, M. Takaki, P. R. R. Ramos, and E. O. Ono. 2008. Comparison of polyamine, phenol and flavonoid contents in plants grown under conventional and organic methods. *Int. J. Food Sci. Technol.* 43:1838–1843.

Lo Scalzo, R., V. Picchi, C. A. Migliori, G. Campanelli, F. Leteo, V. Ferrari, and L. F. Di Cesare. 2013. Variations in the phytochemical contents and antioxidant capacity of organically and conventionally grown Italian cauliflower (Brassica oleracea L. subsp. botrytis): Results from a three-year field study. *J. Agric. Food Chem.* 61:10335–44.

Lu, C., D. B. Barr, Ma. Pearson, and La. Waller. 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. *Environ. Health Perspect.* 116:537–542.

Lu, C., F. J. Schenck, M. A. Pearson, and J. W. Wong. 2010. Assessing children's dietary pesticide exposure: Direct measurement of pesticide residues in 24-hr duplicate food samples. *Environ. Health Perspect.* 118:1625–1630.

Lu, C., K. Toepel, R. Irish, Ra. Fenske, D. B. Barr, and R. Bravo. 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environ. Health Perspect.* 114:260–263.

Maffei, D. F., N. F. A. Silveira, and M. P. L. M. Catanozi. 2013. Microbiological quality of organic and conventional vegetables sold in Brazil. *Food Control.* 29:226–230.

Magkos, F., F. Arvaniti, and A. Zampelas. 2003. Organic food: Nutritious food or food for thought? A review of the evidence. *Int. J. Food Sci. Nutr.* 54:357–371.

Marine, S. C., S. Pagadala, F. Wang, D. M. Pahl, M. V. Melendez, W. L. Kline, R. A. Oni, C. S. Walsh, K. L. Everts, R. L. Buchanan, and S. A. Micallef. 2015. The growing season, but not the farming system, is a food safety risk determinant for leafy greens in the mid-Atlantic region of the United States. *Appl Environ Microbiol.* 81:2395–407.

Mark, A. B., E. Kápolna, K. H. Laursen, U. Halekoh, S. K. Rasmussen, S. Husted, E. H. Larsen, and S. Bügel. 2013. Consumption of organic diets does not affect intake and absorption of zinc and copper in men – evidence from two cross-over trials. *Food Funct.* 4:409–19.

Martínez, J. L. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut.* 157:2893–2902.

Mather, A. E., L. Matthews, D. J. Mellor, R. Reeve, M. J. Denwood, P. Boerlin, R. J. Reid-Smith, D. J. Brown, J. E. Coia, L. M. Browning, D. T. Haydon, and S. W. J. Reid. 2012. An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations. *Proceedings. Biol. Sci.* 279:1630–9.

Mather, A. E., S. W. J. Reid, D. J. Maskell, J. Parkhill, M. C. Fookes, S. R. Harris, D. J. Brown, J. E. Coia, M. R. Mulvey, M. W. Gilmour, I. Petrovska, E. De Pinna, M. Kuroda, M. Akiba, H. Izumiya, T. R. Connor, M. A. Suchard, P. Lemey, D. J. Mellor, D. T. Haydon, and N. R. Thomson. 2013. Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. *Science.* 341:1514–7.

Miele, L., V. Giorgio, M. A. Alberelli, E. De Candia, A. Gasbarrini, and A. Grieco. 2015. Impact of Gut Microbiota on Obesity, Diabetes, and Cardiovascular Disease Risk. *Curr.Cardiol.Rep.* 17:120.

Mitchell, A. E., Y.-J. Hong, E. Koh, D. M. Barrett, D. E. Bryant, R. F. Denison, and S. Kaffka. 2007. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J. Agric. Food Chem.* 55:6154–9.

Oates, L., M. Cohen, L. Braun, A. Schembri, and R. Taskova. 2014. Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. *Environ. Res.* 132:105–111.

Oliveira, M., J. Usall, I. Viñas, M. Anguera, F. Gatius, and M. Abadias. 2010. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiol.* 27:679–84.

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to animal health and welfare in fattening pigs in relation to housing and husbandry. 2007. *EFSA J.* 5:564.

Organic Basics | IFOAM. https://www.ifoam.bio/en/our-library/organic-basics, last access 14 July 2017.

Ottesen, A. R., J. R. White, D. N. Skaltsas, M. J. Newell, and C. S. Walsh. 2009. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *J. Food Prot.* 72:2321–5.

Ozdal, T., D. A. Sela, J. Xiao, D. Boyacioglu, F. Chen, and E. Capanoglu. 2016. The Reciprocal Interactions between Polyphenols and Gut Microbiota and Effects on Bioaccessibility. *Nutrients.* 8:78.

Palupi, E., A. Jayanegara, A. Ploeger, and J. Kahl. 2012. Comparison of nutritional quality between conventional and organic dairy products: A meta-analysis. *J. Sci. Food Agric.* 92:2774–2781.

Parelho, C., F. Bernardo, R. Camarinho, A. S. Rodrigues, and P. Garcia. 2016. Testicular damage and farming environments – An integrative ecotoxicological link. *Chemosphere.* 155:135–141.

Penders, J., C. Thijs, P. A. van den Brandt, I. Kummeling, B. Snijders, F. Stelma, H. Adams, R. van Ree, and E. E. Stobberingh. 2007. Gut microbiota composition and development of atopic manifestations in infancy: The KOALA Birth Cohort Study. *Gut.* 56:661–7.

Piqué, E., L. Vargas-Murga, J. Gómez-Catalán, J. de Lapuente, and J. M. Llobet. 2013. Occurrence of patulin in organic and conventional apple-based food marketed in Catalonia and exposure assessment. *Food Chem Toxicol.* 60:199–204.

Raigón, M. D., A. Rodríguez-Burruezo, and J. Prohens. 2010. Effects of organic and conventional cultivation methods on composition of eggplant fruits. *J. Agric. Food Chem.* 58:6833–40.

Regueiro, J., O. López-Fernández, R. Rial-Otero, B. Cancho-Grande, and J. Simal-Gándara. 2013. A Review on the Fermentation of Foods and the Residues of Pesticides — Biotransformation of Pesticides and Effects on Fermentation and Food Quality. *Crit. Rev. Food Sci. Nutr.* 55:839–863.

Ren, H., H. Endo, and T. Hayashi. 2001. Antioxidative and antimutagenic activities and polyphenol content of pesticide-free and organically cultivated green vegetables using water-soluble chitosan as a soil modifier and leaf surface spray. *J. Sci. Food Agric.* 81:1426–1432.

Rist, L., A. Mueller, C. Barthel, B. Snijders, M. Jansen, aP. Simões-Wüst, M. Huber, I. Kummeling, U. von Mandach, H. Steinhart, and C. Thijs. 2007. Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. *Br. J. Nutr.* 97:735–743.

Rosati, A., C. Cafiero, A. Paoletti, B. Alfei, S. Caporali, L. Casciani, and M. Valentini. 2014. Effect of agronomical practices on carpology, fruit and oil composition, and oil sensory properties, in olive (Olea europaea L.). *Food Chem.* 159:236–43.

Roselli, M., A. Finamore, E. Brasili, G. Capuani, H. L. Kristensen, C. Micheloni, and E. Mengheri. 2012. Impact of organic and conventional carrots on intestinal and peripheral immunity. *J. Sci. Food Agric.* 92:2913–2922.

Ross, S. M., I. C. McManus, V. Harrison, and O. Mason. 2013. Neurobehavioral problems following low-level exposure to organophosphate

pesticides: A systematic and meta-analytic review. *Crit. Rev. Toxicol.* 43:21–44.

Rossetto, M. R. M., F. Vianello, S.Ada. Rocha, and G. P. P. Lima. 2009. Antioxidant substances and pesticide in parts of beet organic and conventional manure. *African J. Plant Sci.* 3:245–253.

Roussos, P. A., and D. Gasparatos. 2009. Apple tree growth and overall fruit quality under organic and conventional orchard management. *Sci. Hortic. (Amsterdam).* 123:247–252.

Sapkota, A. R., E. L. Kinney, A. George, R. M. Hulet, R. Cruz-Cano, K. J. Schwab, G. Zhang, and S. W. Joseph. 2014. Lower prevalence of antibiotic-resistant Salmonella on large-scale U.S. conventional poultry farms that transitioned to organic practices. *Sci Total Environ.* 476–477:387–92.

Smit, L. A., M. Zuurbier, G. Doekes, I. M. Wouters, D. Heederik, and J. Douwes. 2007. Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. *Occup. Environ. Med.* 64:101–7.

Søltoft, M., A. Bysted, K. H. Madsen, A. B. Mark, S. G. Bügel, J. Nielsen, and P. Knuthsen. 2011. Effects of organic and conventional growth systems on the content of carotenoids in carrot roots, and on intake and plasma status of carotenoids in humans. *J. Sci. Food Agric.* 91:767–775.

Średnicka-Tober, D., M. Barański, C. J. Seal, R. Sanderson, C. Benbrook, H. Steinshamn, J. Gromadzka-Ostrowska, E. Rembiałkowska, K. Skwarło-Sońta, M. Eyre, G. Cozzi, M. K. Larsen, T. Jordon, U. Niggli, T. Sakowski, P. C. Calder, G. C. Burdge, S. Sotiraki, A. Stefanakis, S. Stergiadis, H. Yolcu, E. Chatzidimitriou, G. Butler, G. Stewart, and C. Leifert. 2016a. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: A systematic literature review and meta-and redundancy analyses. *Br. J. Nutr.* 115:1043–1060.

Średnicka-Tober, D., M. Barański, C. Seal, R. Sanderson, C. Benbrook, H. Steinshamn, J. Gromadzka-Ostrowska, E. Rembiałkowska, K. Skwarło-Sońta, M. Eyre, G. Cozzi, M. Krogh Larsen, T. Jordon, U. Niggli, T. Sakowski, P. C. Calder, G. C. Burdge, S. Sotiraki, A. Stefanakis, H. Yolcu, S. Stergiadis, E. Chatzidimitriou, G. Butler, G. Stewart, and C. Leifert. 2016b. Composition differences between organic and conventional meat: A systematic literature review and meta-analysis. *Br. J. Nutr.* 23:1–18.

Stenius, F., J. Swartz, G. Lilja, M. Borres, M. Bottai, G. Pershagen, a. Scheynius, and J. Alm. 2011. Lifestyle factors and sensitization in children – The ALADDIN birth cohort. *Allergy Eur. J. Allergy Clin. Immunol.* 66:1330–1338.

Stracke, B. A., C. E. Rüfer, A. Bub, S. Seifert, F. P. Weibel, C. Kunz, and B. Watzl. 2010. No effect of the farming system (organic/conventional) on the bioavailability of apple (Malus domestica Bork., cultivar Golden Delicious) polyphenols in healthy men: A comparative study. *Eur. J. Nutr.* 49:301–310.

Stracke, B. A., C. E. Rüfer, F. P. Weibel, A. Bub, and B. Watzl. 2009a. Three-year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (Malus domestica Bork. Cultivar "Golden Delicious"). *J. Agric. Food Chem.* 57:4598–605.

Stracke, Ba., C. E. Rüfer, A. Bub, K. Briviba, S. Seifert, C. Kunz, and B. Watzl. 2009b. Bioavailability and nutritional effects of carotenoids from organically and conventionally produced carrots in healthy men. *Br. J. Nutr.* 101:1664–72.

Tai, N., F. S. Wong, and L. Wen. 2015. The role of gut microbiota in the development of type 1, type 2 diabetes mellitus and obesity. *Rev. Endocr.Metab.Disord.* 16:55–65.

Tango, C. N., N. J. Choi, M. S. Chung, and D. H. Oh. 2014. Bacteriological quality of vegetables from organic and conventional production in different areas of Korea. *J Food Prot.* 77:1411–7.

Torjusen, H., A. L. Brantsæter, M. Haugen, J. Alexander, L. S. Bakketeig, G. Lieblein, H. Stigum, T. Næs, J. Swartz, G. Holmboe-Ottesen, G. Roos, and H. M. Meltzer. 2014. Reduced risk of pre-eclampsia with organic vegetable consumption: Results from the prospective Norwegian Mother and Child Cohort Study. *BMJ Open.* 4:e006143.

Torjusen, H., A. L. Brantsæter, M. Haugen, G. Lieblein, H. Stigum, G. Roos, G. Holmboe-Ottesen, and H. M. Meltzer. 2010. Characteristics associated with organic food consumption during pregnancy; data from a large cohort of pregnant women in Norway. *BMC Public Health.* 10:775.

Ürkek, B., M. Şengül, T. Erkaya, and V. Aksakal. 2017. Prevalence and Comparing of Some Microbiological Properties, Somatic Cell Count and Antibiotic Residue of Organic and Conventional Raw Milk Produced in Turkey. *Korean J Food Sci Anim Resour.* 37:264–273.

USDA ERS â€" Organic Agriculture. https://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture.aspx, last access 14 July 2017.

Vallverdú-Queralt, A., O. Jáuregui, A. Medina-Remón, and R. M. Lamuela-Raventós. 2012a. Evaluation of a method to characterize the phenolic profile of organic and conventional tomatoes. *J. Agric. Food Chem.* 60:3373–80.

Vallverdú-Queralt, A., and R. M. Lamuela-Raventós. 2016. Foodomics: A new tool to differentiate between organic and conventional foods. *Electrophoresis.* 37:1784–1794.

Vallverdú-Queralt, A., M. Martínez-Huélamo, I. Casals-Ribes, and R. M. Lamuela-Raventós. 2014. Differences in the carotenoid profile of commercially available organic and conventional tomato-based products. *J. Berry Res.* 4:69–77.

Vallverdú-Queralt, A., A. Medina-Remón, I. Casals-Ribes, M. Amat, and R. M. Lamuela-Raventós. 2011. A metabolomic approach differentiates between conventional and organic ketchups. *J. Agric. Food Chem.* 59:11703–10.

Vallverdú-Queralt, A., A. Medina-Remón, I. Casals-Ribes, and R. M. Lamuela-Raventos. 2012b. Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chem.* 130:222–227.

Velimirov, A., M. Huber, C. Lauridsen, E. Rembiałkowska, K. Seidel, and S. Bügel. 2010. Feeding trials in organic food quality and health research. *J. Sci. Food Agric.* 90:175–182.

Wang, S. Y., C.-T. Chen, W. Sciarappa, C. Y. Wang, and M. J. Camp. 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *J. Agric. Food Chem.* 56:5788–94.

WHO Headquarters. Strategic and Technical Advisory Group on antimicrobial resistance (STAG-AMR) Background. (Geneva, 2014.

Xue, B., J. Xie, J. Huang, L. Chen, L. Gao, S. Ou, Y. Wang, and X. Peng. 2016. Plant polyphenols alter a pathway of energy metabolism by inhibiting fecal Bacteroidetes and Firmicutes in vitro. *Food Funct.* 7:1501–7.

2.3. Compuestos bioactivos de la dieta y sus propiedades beneficiosas en la salud

Los compuestos bioactivos forman parte de la cadena alimentaria y pueden ejercer efectos beneficiosos en la salud humana [65,66]. Por lo general, estos compuestos son fitoquímicos formados durante el metabolismo secundario de las plantas, aunque también pueden proceder de otros organismos como las algas, bacterias y hongos. Como ya se ha comentado en la sección anterior, el consumo dietético de estos compuestos está estrechamente relacionado con la prevención de enfermedades crónicas y degenerativas, como las ECV [67,68], el cáncer [68,69], la diabetes de tipo 2 [70,71], la obesidad [72,73], el síndrome metabólico [74,75], la osteoporosis [68,76] y las enfermedades neurológicas [68,71], encontrándose también asociaciones inversas y significativas con la mortalidad [77,78]. La **Figura 6** representa las principales propiedades otorgadas a estos componentes dietéticos.

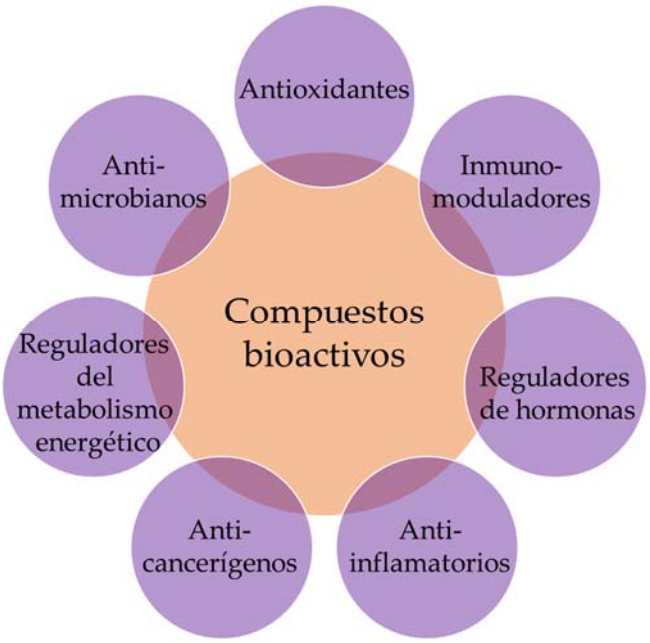


Figura 6. Funciones biológicas de los compuestos bioactivos.

Los compuestos bioactivos pueden disminuir el riesgo de ENT a través de distintas vías de señalización que implican un gran número de proteínas (principalmente de tipo quinasas). Algunos de ellos como los flavonoides y los curcuminoides son capaces de reparar daños en el DNA y modular las reacciones metabólicas de las fases I y II (por ejemplo, a través de la regulación de la citocromo P450 (CYP450), activando o inhibiendo a las isozimas CYP) [79,80]. Muchos de estos compuestos biológicamente

activos son conocidos comúnmente como antioxidantes debido a que suprimen las especies reactivas del oxígeno (ROS) formadas endógena o exógenamente. Los fitoesteroles, organosulfurados y otros fitoquímicos pueden inhibir la actividad del factor nuclear potenciador de las cadenas ligeras kappa de las células B activadas (NF-κB), implicado en la expresión de las citoquinas pro-inflamatorias y el factor de necrosis tumoral (TNF-α). En la presente tesis nos centramos especialmente en la prevención de la inflamación sistémica de bajo grado que precede el desarrollo de las ENT [81,82]. Algunos polifenoles y terpenoides pueden actuar disminuyendo la concentración de citoquinas y otras moléculas proinflamatorias (como las interleuquinas 6 y 1β (IL-6 y IL-1β) y TNF-α) que estimulan la síntesis de la proteína C reactiva (CRP) de fase aguda en el hígado [83]. Además, a través de la inhibición NF-κB, implicada en la producción de enzimas como la óxido nítrico sintasa inducible (iNOS) y la ciclooxigenasa-2 (COX-2), pueden regular la función endotelial favoreciendo la liberación de moléculas vasodilatadoras (NO, prostaciclina (PGI_2), etc) sobre las vasoconstrictoras como el tromboxano A_2 (TXA_2) [84,85]. El NO es un gas de señalización que tiene un papel crítico en la prevención de ECV y otras ENT gracias a su potente capacidad vasodilatadora que disminuye la adhesión/agregación plaquetaria y la formación de trombina, evitando así los daños vasculares. El NO también puede inhibir la expresión de células vasculares y de moléculas de adhesión intercelular y vascular (ICAM-1 y VCAM-1) [86,87]. Los fitoesteroles y organosulfurados también regulan el colesterol, en parte inhibiendo la oxidación de la lipoproteína de baja densidad (LDL), y suprimen la expresión de moléculas de adhesión y otras implicadas en los procesos inflamatorios [85]. Se han observado propiedades anticancerígenas sobre algunos tipos de cáncer entre algunos de estos compuestos bioactivos (glucosinolatos, flavonoides, estilbenos, etc), debido a que pueden inducir la apoptosis, anti-proliferación, anti-invasión y/o autofagia a través de mecanismos como la activación de caspasas, la detención del ciclo celular y la inhibición de neovascularización [88–90]. La vía de señalización más estudiada en la prevención de ENT como las ECV y el cáncer es la del factor nuclear eritroide 2 relacionado con el factor 2 (Nrf2)- elemento de respuesta antioxidante (ARE) [91–93], pero se ha observado que una hiperactivación de Nrf2 puede favorecer la carcinogénesis [94].

Entre los compuestos bioactivos más conocidos y consumidos en mayor concentración se encuentran las vitaminas, los minerales y la fibra dietética, siendo las hortalizas y frutas la fuente principal de su consumo. Además, se conoce un gran número de estructuras químicas procedentes principalmente del metabolismo de las plantas que, a pesar de encontrarse en concentraciones muy bajas, están involucradas en las funciones biológicas del ser humano. En la **Tabla 2** se resumen algunos de fitoquímicos más destacados y sus fuentes dietéticas principales.

Tabla 2. Resumen de los principales fitoquímicos obtenidos de la dieta.

Fitoquímicos		Fuentes dietéticas
Fenoles	Ácidos fenólicos, flavonoides y otros	Frutos rojos, cítricos, hojas verdes, café, cacao, té, vino, aceite de oliva virgen, etc
Carotenoides	Carotenos y xantofilas	Tomate, zanahoria, calabaza, papaya, hojas verdes, etc
Compuestos organosulfurados	Isotiocianatos, glucosinolatos, indoles, sulfurados	Brócoli, ajos, cebollas, etc
Fitoesteroles	Campesterol y sitosterol entre otros	Cereales integrales, legumbres, frutos secos, semillas, etc

A continuación, se explican los compuestos en los que se centra este trabajo: los polifenoles y los carotenoides.

2.3.1. Polifenoles

2.3.1.1. Origen y clasificación

Los compuestos fenólicos son metabolitos secundarios procedentes de las plantas que se sintetizan principalmente a partir de las vías pentosa fosfato-shikimato-fenilpropanoide [70,95,96]. Se trata de un grupo muy amplio de compuestos, pero todos tienen en común un anillo benzoico en su estructura con uno o más radicales hidroxilos [95]. Están presentes en los alimentos de origen vegetal, tales como hortalizas, frutas, frutos secos, aceite de oliva y cacao y en bebidas como el vino, el té y el café [97,98].

Los polifenoles se clasifican en dos grupos: flavonoides y no flavonoides (**Figura 7**). Los flavonoides tienen una estructura de tipo C_6-C_3-C_6, basada en la unión de dos anillos aromáticos por una cadena alifática de tres carbonos [95]. Son los más abundantes y se dividen a su vez en subgrupo, entre los cuales destacan las flavanonas, los flavanoles, las antocianinas, los flavonoles, las flavonas y las isoflavonas. Las flavanonas son características de las frutas cítricas, y entre ellas se encuentran algunos compuestos como la naringenina y la hesperidina. Los flavanoles como la (-)-epicatequina y la (+)-catequina son típicos en el cacao. Dentro de las antocianidinas se encuentran la cianidina, la pelargonidina y la petunidina, carcterísticas de los frutos rojos y las uvas. La cebolla es rica en flavonoles como la quercetina y la isorhamnetina. Entre las flavonas, la apigenina y luteolina están presentes en hortalizas como las alcachofas. La soja se caracteriza por su contenido en isoflavonas, entre las cuales cabe destacar la genisteína, la daizceina y la gliceteina. Por otro lado, los ácidos fenólicos son el grupo más abundante y estudiado dentro de los no flavonoides. Entre los ácidos fenólicos se encuentran los ácidos benzoicos y cinámicos y están presentes en una gran variedad de alimentos vegetales. Otros compuestos no flavonoides son los estilbenos,

como el resveratrol (en alimentos como el vino, las uvas y los cacahuetes), los lignanos, como las enterolactonas (presentes en las semillas de lino y sésamo), y otros fenoles como el tirosol y el hidroxitirosol, típicos del aceite de oliva. A pesar de que algunos polifenoles son característicos de ciertos alimentos concretos (como por ejemplo, las flavanonas de frutas cítricas y las isoflavonas de la soja), la mayoría de los compuestos fenólicos están presente en una variedad de productos vegetales, y a su vez los alimentos que ingerimos contienen un complejo de estos compuestos [99–101].

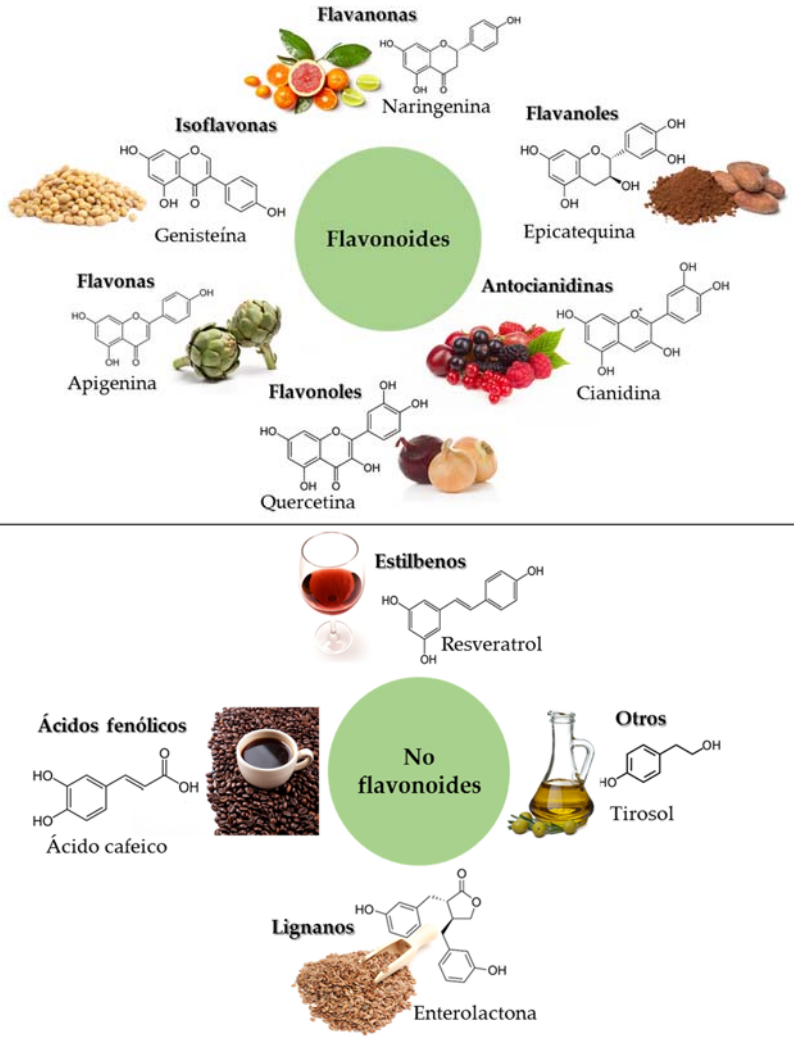


Figura 7. Clasificación de los polifenoles de la dieta.

2.3.1.2. Bioaccesibilidad y biodisponibilidad

El término "bioaccesibilidad" hace referencia a la fracción del compuesto liberada desde la matriz del alimento y que está disponible durante la absorción intestinal, mientras que la "biodisponibilidad" indica la fracción que es digerida, absorbida y utilizada en el metabolismo fisiológico [102–104].

Los polifenoles son ingeridos en la dieta principalmente en forma de glicósidos y al llegar al intestino pueden convertirse en agliconas para ser absorbidos. Este proceso puede llevarse a cabo por dos mecanismos: (i) mediante la lactasa-floricina hidrolasa (LPH), localizada en el borde en cepillo de los enterocitos apicales de las microvellosidades intestinales, y (ii) mediante la β-glucosidasa citosólica (CBG), que se encuentran dentro de las propias células epiteliales. La LPH se une de forma más específica a los flavonoides-*O*-D-glucósidos, y debido al incremento de la hidrofobicidad, los fenoles atraviesan la membrana plasmática del enterocito mediante difusión pasiva. En cambio, para que se lleve a cabo la hidrólisis a través de la CBG, los polifenoles más polares tienen que atravesar la membrana del enterocito. Se ha sugerido que el transportador de glucosa activo dependiente de sodio 1 (SGLT1) está involucrado en este proceso. Antes de pasar a la circulación sanguínea, las agliconas son modificadas estructuralmente mediante las enzimas sulfotransferasas (SULTs), uridina-5'-difosfato glucuronosiltransferasas (UGT) y catecol-*O*-metiltransferasas (COMTs) dando lugar a metabolitos de la fase II. Algunos de los metabolitos que no atraviesan la barrera basolateral vuelven al lumen del intestino delgado a través de la familia de transportadores dependientes de adenosín-trifosfato (ABC). Los compuestos conjugados que pasan al torrente sanguíneo pueden distribuirse a los tejidos diana o llegar al hígado a través de la vena porta hepática, donde tienen lugar más reacciones metabólicas de fase II. Mediante la recirculación enterohepática, los compuestos metabolizados pueden volver al intestino delgado a través de la bilis [95,105]. Tras la circulación enterohepática, los polifenoles son excretados a través de la orina y de la bilis [106].

Por otro lado, los polifenoles que no son absorbidos en el intestino delgado pasan al colon, donde la microbiota puede hidrolizar los glicósidos en agliconas y degradarlos produciendo los ácidos fenólicos. De esta forma, estos metabolitos de menor peso molecular pueden absorberse desde el colon, viajar al hígado y conjugarse o directamente ser excretados [106–108]. Bang *et al*., observaron que los flavonoides unidos a ramnosa alcanzan el colon y son hidrolizados por enzimas como las α-ramnosidasas, secretadas por bacterias de la microbiota (como *Bifidobacterium dentium*), para su posterior absorción [109]. Dentro del grupo de los no flavonoides, se han encontrado algunos polímeros de ácidos hidroxicinámicos, resistentes a la acción de la LPH y de la CBG, que no pueden ser absorbidos en el intestino delgado y se

metabolizan en el colon produciendo ácidos hidroxifenilacéticos e hidroxifenilpropiónicos [110]. Los compuestos fenólicos desconjugados (no glicosilados) pueden ser metabolizados por la microbiota, mediante la fisión de anillos y diversas reacciones (deshidroxilación, desmetilación y descarboxilació), y además pueden producir metabolitos conjugados como sulfatos a partir de la actividad de enzimas arilsulfotransferasas (ATSs) generadas por las bacterias [111–113].

La concentración de agliconas en el plasma después de la ingesta es muy baja debido a que, a excepción de algunos compuestos como la (+)-catequina y (-)-epicatequina que no se encuentran glicosilados en los alimentos y son absorbidos fácilmente en el intestino delgado, los polifenoles de los alimentos sufren diversas modificaciones para su posterior absorción [107,108]. La tasa de absorción de los polifenoles, por lo tanto, es muy baja y variable según la estructura química del compuesto [101,108]. Se excretan a través de la orina, bilis o heces, siendo la orina la principal vía de excreción incluso para los metabolitos formados por la microbiota [114]. Además, se ha observado una correlación entre el consumo dietético de polifenoles y su excreción urinaria [115].

La bioaccesibilidad y biodisponibilidad de los polifenoles dependen de diversos factores que se resumen a continuación:

Factores relacionados con el ambiente. Factores externos como los estímulos estresores pueden favorecer la síntesis de estos compuestos debido a que, aunque muchos de los polifenoles son compuestos del metabolismo secundario de las plantas, también se producen como mecanismos de defensa. Factores como la variedad, el grado de madurez, el área geográfica, el clima, la estación, el tipo de suelo y el tipo de cultivo influyen en la composición de polifenoles presentes finalmente en el alimento. Por ejemplo, la concentración de ácidos fenólicos disminuye generalmente durante el proceso de madurez, mientras que la de antocianinas aumenta [101]. Algunos autores han observado un mayor contenido de polifenoles en alimentos originados mediante agricultura ecológica respecto a los métodos convencionales [116–121], posiblemente debido al limitado uso de fertilizantes y pesticidas que impulsa a las plantas a producir mecanismos para liberarse del estrés. Por lo tanto, la concentración final de polifenoles en los alimentos está muy influenciada por los factores ambientales que afectan a su síntesis.

Factores relacionados con el alimento. La matriz alimentaria es otro factor crucial, debido a que los polifenoles pueden interactuar con los nutrientes (hidratos de carbono, proteínas, lípidos y fibras) y con el alcohol [99]. Por ejemplo, algunos autores han sugerido que la fibra puede retrasar la biodisponibilidad de los polifenoles [103,122]. Sin embargo, el trabajo llevado a cabo por Tamura *et al*. demostró que la pectina mejoraba la biodisponibilidad de la quercetina, formada a partir de la

hidrólisis de la rutina a través de cambios en la microbiota [123]. El efecto positivo de la presencia de grasa sobre la biodisponibilidad de los polifenoles también ha sido descrito en diversas investigaciones [53,124].

Por otro lado, el procesado (tratamiento térmico, homogenización, liofilización y cocinado) y almacenamiento pueden tener efectos, principalmente negativos, sobre la biodisponibilidad de los polifenoles. Crozier *et al*., observaron que las cebollas y los tomates perdían entre un 75 y un 80% de quercetina al ser hervidos durante 15 minutos, el 65% después de cocinarse en un microondas y el 30% tras freírse [125]. Sin embargo, mayores concentraciones de naringenina y ácido clorogénico se cuantificaron en plasma después de consumir tomates cocinados respecto a los frescos [126].

Factores relacionados con el propio compuesto. La velocidad y el grado de absorción de los polifenoles dependen de la concentración del compuesto, así como de su estructura química y la del azúcar al que está unido [99,108].

Interacciones. Además de las interacciones descritas con otros nutrientes, los polifenoles pueden interaccionar con proteínas como la albúmina y con otros polifenoles [99,101]. En un estudio llevado a cabo por Tagashira *et al*. se observó que las catequinas unidas a galato o pirogalol favorecían la acumulación y absorción de catequinas [127].

Factores relacionados con el huésped. También están implicados aquellos factores relacionados con el intestino, como la actividad enzimática y la composición microbiana, y otros como el sexo, la edad, la genética y el estado fisiológico. Por lo tanto, la producción de metabolitos activos varía mucho entre los individuos [99].

2.3.2. Carotenoides

2.3.2.1. Origen y clasificación

Los carotenoides son pigmentos liposolubles procedentes del metabolismo de las plantas u otros organismos no fotosintéticos (algas, hongos y bacterias). La condensación "cabeza-cola" de tres moléculas de isopentenil difosfato (IPP) y una de dimetilalil difosfato (DMAPP) da lugar al geranilgeranil pirofosfato (GGPP), un terpeno de 20 átomos de carbonos. La unión de dos terpenos GGPP, catalizada por la GGPP sintetasa, da lugar al fitoeno (C_{40}), a partir del cual se sintetizan los carotenoides mediante la enzima fitoeno sintetasa (PSY) [128].

Según su estructura, los carotenoides se clasifican en dos grupos: carotenos y xantofilas. Los carotenos son hidrocarburos (solo contienen átomos de carbono e hidrógeno), y las xantofilas, derivadas de los carotenos, también contienen uno o más

átomos de oxígeno. En la **Figura 8** se presentan los principales carotenos (β-caroteno, α-caroteno y licopeno) y xantofilas (luteína, zeaxantina y β-criptoxantina) consumidos en la dieta. Los alimentos de coloración amarilla, naranja o roja son ricos en carotenoides, por lo general, y deben su coloración a estos compuestos (zanahorias, calabaza, papaya, tomate…). Además, una fuente rica en carotenos y xantofilas son los vegetales de hoja verde, como las espinacas y la col kale [128,129]. Aunque predominan en los alimentos vegetales, algunos alimentos de origen animal como la leche, la yema del huevo y el salmón también contienen carotenoides [130].

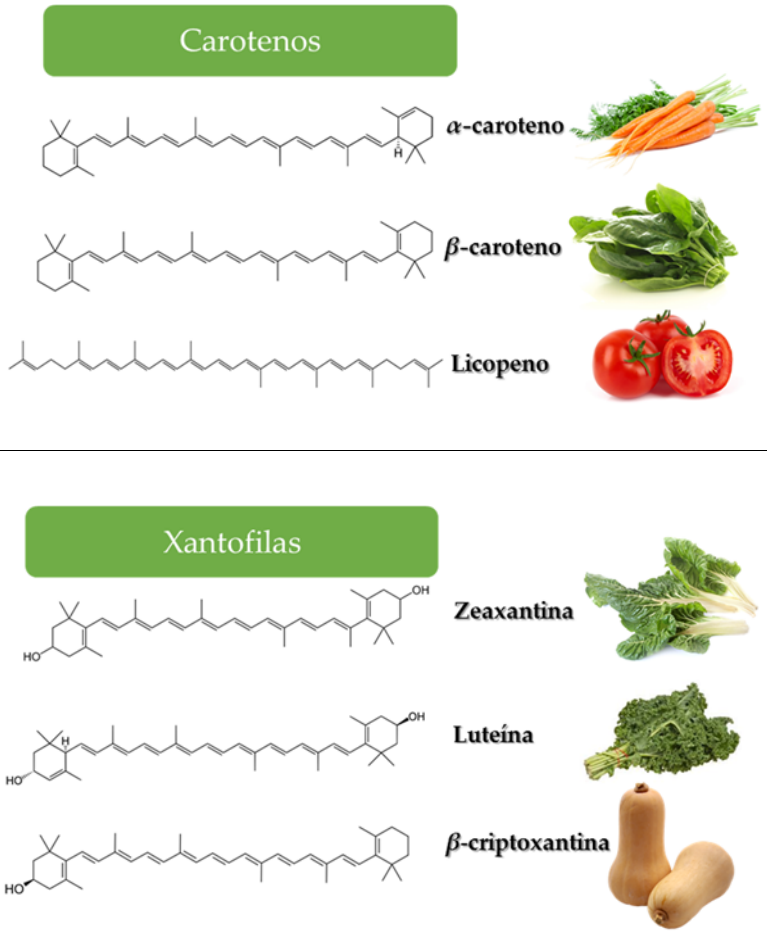


Figura 8. Clasificación de los carotenoides de la dieta.

En la naturaleza, los carotenoides se encuentran principalmente en su conformación all-*trans* (isoforma *E*), que es la más estable. Sin embargo, debido a la presencia de

dobles enlaces en su estructura, se oxidan e isomerizan a la conformación *cis* (isoforma *Z*), especialmente durante el procesamiento térmico de los alimentos [131].

2.3.2.2. Bioaccesibilidad y biodisponibilidad

Los carotenoides son liberados desde la matriz alimentaria y solubilizados en pequeñas gotas lipídicas para su posterior incorporación en las micelas a través de enzimas lipasas. Estas micelas están formadas por ácidos biliares unidos a componentes de la digestión de los triacilglicéridos, fosfolípidos y esteres de colesterol que generan los ácidos grasos libres, los mono- y di-acilglicéridos, los lisofosfolípidos y el colesterol libre; y son necesarias para el transporte y absorción de los carotenoides en el enterocito. La absorción puede llevarse a cabo por difusión pasiva o a través de la unión a receptores de membranas como el de clase B tipo I (SR-B1), el determinante de grupo 36 (CD36) y la proteína Niemann-Pick C1-Like 1 (NPC1L1) [132,133].

En el interior del enterocito, los carotenoides pro-vitamina A, como el β-caroteno, se transforman en retinal, a través de la enzima β-caroteno oxigenesa 1 (BCO1). Posteriormente, el retinal puede convertirse en retinol, mediante la enzima retinol deshidrogenasa (RDH) y producir los esteres de retinilo a partir de la lecitin-retinol-aciltransferasa (LRAT). De forma paralela, algunos carotenoides forman apo-carotenoides a partir de su degradación mediante enzimas como la β-caroteno oxigenesa 2 (BCO2). Los carotenoides y sus derivados son incorporados en quilomicrones y llegan a la circulación sanguínea, en donde los quilomicrones son hidrolizados por lipoproteínas lipasas para poder entrar dentro de los hepatocitos. Los carotenoides se liberan de los quilomicrones remanentes y se incorporan en las lipoproteínas de muy baja densidad (VLDL). A través de las partículas VLDL se transportan triacilglicéridos y carotenoides hacia los tejidos adiposos y periféricos. Las partículas VLDL se transforman en otras de menor tamaño, las LDL, que contiene menor carga de triacilglicéridos y liberan carotenoide a los tejidos periféricos. A partir de las VLDL, las xantofilas también pueden ser transferidas a las lipoproteínas de alta densidad (HDL) para su posterior distribución hacia los tejidos, pero no se ha observado que este mecanismo ocurre con los carotenos [132,133]. Los carotenoides pueden almacenarse principalmente en el tejido adiposo o ser utilizados por los diferentes tejidos, y finalmente se excretan a través de las heces [128].

A continuación, se resumen los factores que afectan a la bioaccesibilidad y biodisponibilidad de los carotenoides:

Factores relacionados con el ambiente. Factores como la variedad, el grado de madurez, el área geográfica, el clima, la estación, el tipo de suelo y el tipo de cultivo influyen de manera significativa en el contenido y la conformación del alimento [134,135]. Por ejemplo, algunos autores han encontrado concentraciones superiores de

estos compuestos en alimentos cultivados de forma ecológica en comparación con aquellos obtenidos a través de métodos convencionales, aunque otros trabajos no han observado los mismos resultados [121,136,137].

Factores relacionados con el alimento. El procesado del alimento permite su liberación desde el cloroplasto o cromoplasto, favoreciendo así su bioaccesibilidad. Además, como ya se ha descrito anteriormente, el tratamiento térmico de los alimentos conlleva reacciones de oxidación produciéndose así los denominados isómeros *cis* [138].

La matriz del alimento también afecta a la bioaccesibilidad y biodisponibilidad de los carotenoides. Como cabe esperar por su composición liposoluble, los lípidos aumentan la liberación de estos compuestos desde el alimento y su absorción en el organismo [53,54,134,139]. Se especula que un consumo de 3-5 g. de grasa en cada comida puede favorecer la absorción de los carotenoides ingeridos [139], siendo importante el tipo de grasa utilizada. Se han sugerido mejores resultados sobre la bioaccesibilidad de los carotenoides cuando se añade aceite de oliva en comparación con otras matrices lipídicas [134]. Sin embargo otros componentes de la dieta, como las fibras (principalmente las pectinas), disminuyen la bioaccesibilidad y biodisponibilidad de los carotenoides [103,140].

Factores relacionados con el propio compuesto. La concentración y la estructura química de los carotenoides van a determinar su forma y grado de liberación y absorción. Los compuestos más polares (xantofilas) son más biodisponibles que los más apolares (carotenos), debido a que alcanzan más fácilmente las micelas y consecuentemente son más absorbidos por los enterocitos.

Otra cuestión importante es su conformación. Algunos autores han demostrado que la isomerización del licopeno da lugar a compuestos más biodisponibles y bioactivos [141]. Sin embargo, los isómeros *cis* de β-caroteno parecen ser menos biodisponibles que el all-*trans*-β-caroteno [142].

Interacciones. Además de los efectos ya descritos sobre la grasa y la fibra en los alimentos, las interacciones con otros fitoquímicos pueden aumentar o favorecer la biodisponibilidad de los carotenoides. En general, se ha especulado que las interacciones entre carotenoides no son favorables [139,143]. De forma indirecta, otros compuestos bioactivos como los fitoesteroles también pueden disminuir la absorción de los carotenoides a través de la reducción del colesterol plasmático [144].

Factores relacionados con el huésped. La biodisponibilidad de los carotenoides también depende de algunos factores como el sexo, la edad, la genética y los relacionados con el sistema digestivo (como por ejemplo el pH y la concentración de ácidos biliares) [145–148].

2.3.3. Microbiota y compuestos bioactivos: post-bióticos

En los últimos años, el número de estudios que abordan el papel de la dieta sobre la microbiota intestinal y el impacto sobre la salud ha aumentado considerablemente [149–155]. Más allá de los conocidos prebióticos (ingredientes fermentados selectivamente que dan lugar a cambios específicos en la composición y/o la actividad de la flora gastrointestinal, confiriendo así beneficios a la salud del huésped) y probióticos (microorganismos vivos que, cuando son administrados en cantidad adecuada, ejercen un efecto beneficioso sobre la salud del huésped), recientemente ha surgido el término "postbióticos". Los postbióticos, también conocidos como metabióticos, son factores solubles que pueden ser secretados por bacterias vivas o liberarse tras la lisis bacteriana [156–158]. Entre los más estudiados se encuentran los ácidos grasos volátiles (VFA), los ácidos orgánicos (como por ejemplo los ácidos fenólicos y los indoles), la metilalamina, las vitaminas del grupo B y K, los aminoácidos de cadena ramificada, péptidos (como la lactocepina), el ácido biliar, los polisacáridos, los ácidos teicoicos y lipoteicoicos, los muropéptidos derivados de peptidoglucanos y los plasmalógenos [155,156,159]. Las investigaciones en este campo han demostrado el beneficioso papel de estos compuestos en la salud (**Figura 9**), debido principalmente a sus propiedades antimicrobianas, antioxidantes e inmunomodulatorias [156,160–162]. El uso de postbióticos sintetizados artificialmente como alternativa a los probióticos ha sido sugerido por varios autores en la prevención y tratamiento de enfermedades relacionadas con la disbiosis (alteración de la microbiota intestinal) [156,158,163–165].

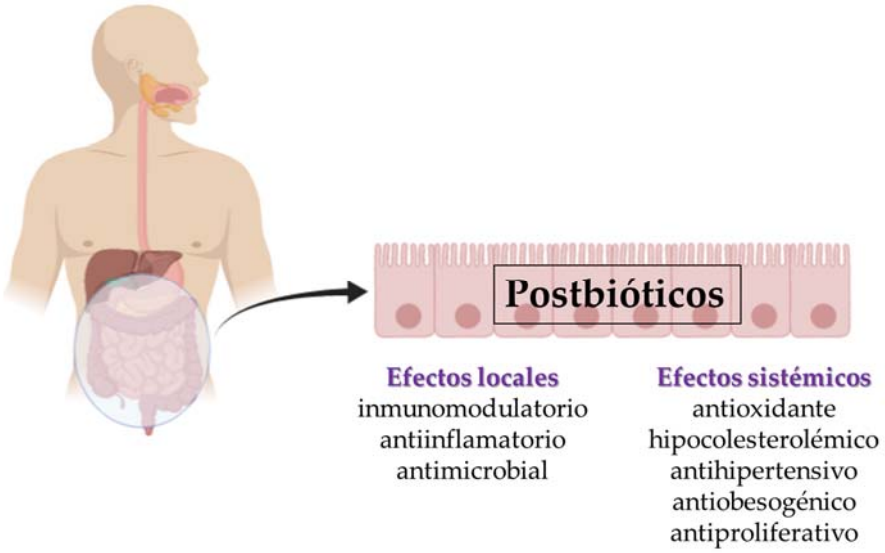


Figura 9. Efectos beneficiosos de los postbióticos en la salud (adapatada de Aguilar-Toalá *et al.* [156]).

En los subapartados siguientes (*2.2.3.1. y 2.2.3.2.*) se explicarán más detalles sobre los ácidos grasos de cadena y los ácidos fenólicos, que forman parte del contenido de la tesis.

2.3.3.1. Ácidos grasos volátiles

Los VFA son producidos por bacterias intestinales anaeróbicas en el colon a través de la fermentación de fibra dietética principalmente [166,167]. Los más abundantes y estudiados son el acetato (C2), propionato (C3) y butirato (C4) (**Figura 10**); y a pesar de que el acetato representa aproximadamente el 50% de los VFA en el colon, la proporción puede variar dependiendo de factores como la composición de la microbiota, la dieta y los procesos de fermentación [168–170]. Otros VFA minoritarios en el colon son el valerato (C5), el caproato (C6) y el heptanoato (C7) [171]. Por lo general, el acetato y el propionato pueden elongarse a través de la microbiota produciendo butirato y valerato, respectivamente, y además a partir del butirato se puede sintetizar el caproato y a partir del valerato el heptanoato [172,173]. Adicionalmente, la microbiota intestinal también puede utilizar proteínas como sustrato para producir los ácidos grasos de cadena ramificada (BCFA, isobutirato, isovalerato e isocaproato) a partir de los aminoácidos de cadena ramificada (leucina, isoleucina y valina) [174]. El consumo dietético de BCFA proviene principalmente de los productos lácteos y la mayoría de estudios al respecto se enfocan en la presencia de estos ácidos grasos en la microbiota neonatal [175–177].

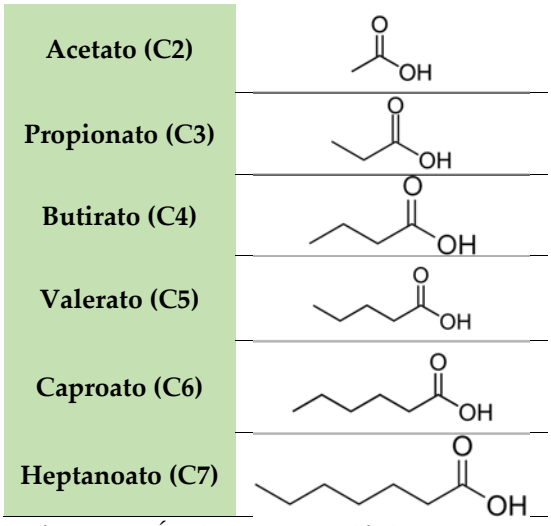


Figura 10. Ácidos grasos volátiles (C2-C7).

La señalización de VFA está mediada por los receptores acoplados a proteínas G (GPRs), GPR41 (FFAR3), GPR43 (FFAR2) y GPR109A (HCAR2), expresados

predominantemente en el tejido adiposo, el intestino y las células inmunes. GPR41 y GPR43 tienen mayor afinidad por el acetato y el propionato, mientras que el butirato inhibe la histona desacetilasa (HDAC) a través de GPR109A [149,155,178,179]. El papel de los VFA, incluyendo los BCFA, en la regulación del metabolismo energético ha sido descrito en diversos estudios [170,179–181]. Además, se ha observado que el butirato es el sustrato energético preferido por los colonocitos, por lo que podría estar implicado en la prevención y tratamiento del cáncer colorrectal [182–184]. El butirato también está implicado en la síntesis del colesterol y su función también parece relevante en la disminución del riesgo de enfermedades cardiovasculares [185].

2.3.3.2. Ácidos fenólicos

Los ácidos fenólicos pueden proceder directamente del consumo dietético o producirse a partir del metabolismo microbiano de otros polifenoles, principalmente ácidos hidroxicinámicos y flavonoides. Debido a la baja absorción en el intestino delgado, más del 90% de los polifenoles llegan al intestino grueso, donde son metabolizados por la microbiota del colon, y finalmente se absorben como ácidos fenólicos de bajo pesos molecular [106,155,186]. Los ácidos fenólicos, según su estructura, puedes ser: fenilacéticos, fenilpropiónicos, cinámicos y sus derivados y benzoicos y sus derivados (**Figura 11**). Los flavonoides que alcanzan el colon se someten a una fisión en anillo dando lugar a los ácidos fenólicos, que a su vez pueden sufrir más reacciones convirtiéndose en otros productos metabólicos [111]. Estos polifenoles pueden tener efectos beneficiosos sobre la salud debido a que interacciona con la microbiota y pueden cambiar su composición y posiblemente favorecer la producción de VFA [29,187–189]. En la sección *2.3.1.* sobre los polifenoles se pueden encontrar más detalles de estos compuestos.

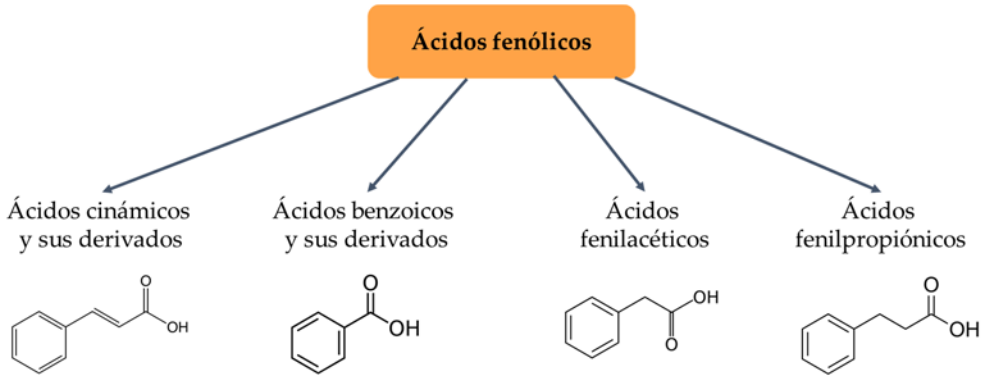


Figura 11. Clasificación de ácidos fenólicos producidos por la microbiota.

Puntos claves:

- Una dieta rica en hortalizas y frutas, como la mediterránea, puede prevenir el riesgo de la incidencia y mortalidad de enfermedades crónicas como las cardiovasculares y el cáncer.

- Se han propuesto recomendaciones dietéticas para la prevención de los diferentes cánceres y estos consejos son también dirigidas a los pacientes de cáncer (excepto en el caso del cáncer de mama, en donde existen guías específicas). Sin embargo, las necesidades dietéticas para los supervivientes de cáncer podrían diferir de aquellas que se plantean en la prevención primaria.

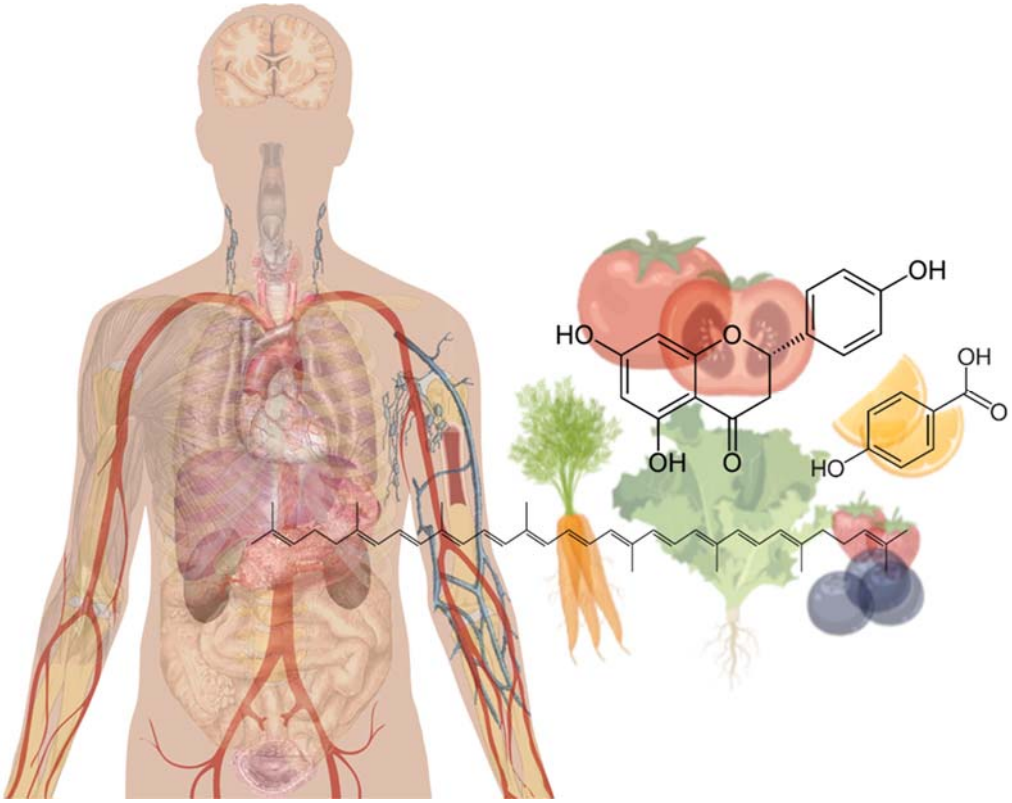
- Se ha demonstrado que las hortalizas y frutas contienen compuestos bioactivos beneficiosos para la salud cardiovascular a través del mantenimiento de la homeostasis vascular e inflamatoria, en cambio, no hay estudios suficientes que sugieran la necesidad de su consumo especialmente cuando no existen otros factores de riesgo aparentes (tabaquismo, uso de drogas, metabólicos, etc).

- La bioaccesibilidad y biodisponibilidad de estos compuestos biológicamente activos depende de muchos factores, entre los cuales destacan el sistema de cultivo empleado, el procesado de los alimentos, la matriz alimentaria y las interacciones entre los ingredientes y compuestos. A pesar de la evidencia descrita hasta la fecha en este campo, son necesarias más investigaciones que aborden el impacto de estos factores sobre la biodisponibilidad y consecuentemente sobre la actividad biologica de estos compuestos.

- Además de proceder directamente de la dieta, algunos compuestos fenólicos también se producen a través del metabolismo microbiano de los polifenoles. Se ha descrito previamente que estos metabolitos de la microbiota estan implicados en mantenimiento de la salud.

- Los acidos grasos volatiles son los principales metabolitos formados por la microbiota intestinal y están directamente implicados en el mantenimiento de la homeostasis energética en el ser humano. En la actualidad, surge la nesesidad de estudiar las interacciones bidireccionales entre la actividad metabolica microbiana y el consumo dietetico de compuestos bioactivos, lo cual tiene potentes efectos sobre la salud humana.

HIPÓTESIS Y OBJETIVOS



3. HIPÓTESIS Y OBJETIVOS

Hipótesis

La hipótesis general de la tesis es que **la dieta mediterránea, rica en hortalizas y frutas, tiene efectos positivos sobre los procesos vasculares y pro-inflamatorios relacionados con la salud cardiovascular y puede asociarse a un mejor pronóstico en supervivientes de cáncer debido a su riqueza en compuestos bioactivos.**

Objetivos

El objetivo general de la tesis fue **evaluar el efecto de consumir una dieta rica en hortalizas y frutas, de tipo mediterránea, sobre la homeostasis endotelial e inflamatoria en sujetos sanos y sobre el pronóstico de pacientes con cáncer.**

Para llevar a cabo el objetivo general, se plantearon los siguientes objetivos específicos:

1. Evaluar cómo afecta el consumo a corto plazo de una dieta baja en alimentos ricos en polifenoles sobre la función endotelial en sujetos sanos. Por ello se han propuesto:
   1.1. Monitorizar la excreción de los polifenoles totales en orina como marcador de cumplimiento de la intervención dietética.
   1.2. Evaluar el impacto de la dieta baja en polifenoles sobre principales biomarcadores implicados en los procesos vasculares en muestras biológicas de plasma y orina.

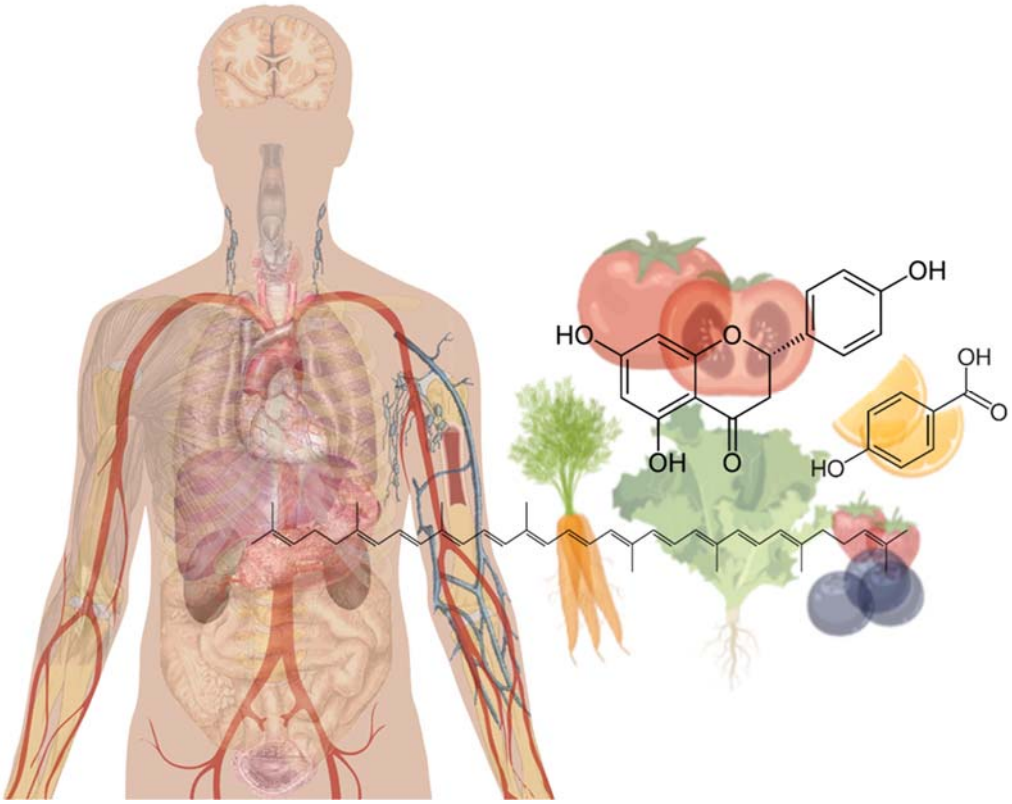
2. Evaluar cómo afecta el consumo de una dosis única de *sofrito* (240 g/ 70 kg de peso corporal) al estado inflamatorio en voluntarios sanos. Para alcanzar este objetivo se han definido los siguientes pasos intermedios:
   2.1. Valorar los cambios de la excreción de polifenoles totales en orina y la concentración de carotenoides en plasma de los voluntarios tras la ingesta del *sofrito*.
   2.2. Evaluar el efecto del consumo agudo de *sofrito* sobre algunos de los principales biomarcadores pro-inflamatorios en plasma.
   2.3. Relacionar los cambios en los marcadores proinflamatorios estudiados con los niveles de polifenoles totales excretados y carotenoides biodisponibles tras la intervención.

3. Comparar el efecto de una dieta mediterránea de origen ecológico *versus* otra similar de origen convencional de un mes de duración en las muestras biológicas de voluntarios sanos en relación con los siguientes parámetros:
   -el nivel de compuestos bioactivos, carotenos y ácidos fenólicos en muestras biológicas
   -el estatus antropométrico y los parámetros fisiológicos y bioquímicos básicos de los voluntarios
   -la concentración plasmática de minerales y metales

4. Evaluar la interacción entre los compuestos bioactivos procedentes de la dieta y del metabolismo de la microbiota y sus efectos sobre los marcadores lipídicos en sujetos sanos. Para ello se ha planteado:
   4.1. Analizar la relación de los carotenos en plasma, biomarcadores del consumo de hortalizas y frutas, y la producción de ácidos grasos volátiles en heces de sujetos sanos.
   4.2. Determinar si los niveles de carotenos y ácidos grasos volátiles se asocian con la concentración de colesterol (HDL, LDL y total) y los triglicéridos.

5. Analizar si el consumo de hortalizas y frutas se asocia con un mejor pronóstico de cáncer mediante meta-análisis. Para alcanzar este objetivo se ha evaluado si el consumo de estos grupos de alimentos y sus subgrupos disminuyen:
   - la recurrencia de los diferentes tipos de cáncer (excepto el cáncer de mama)
   - la mortalidad por cáncer y global en los pacientes de cáncer en distintas localizaciones (excepto en el caso del cáncer de mama).

RESULTADOS



4. RESULTADOS

En esta sección, se exponen los resultados obtenidos durante la tesis doctoral. Estos son explicados en las 5 publicaciones que se presentan a continuación tras un previo resumen de su contenido (consultar la **Figura 2** de la *sección 2.1.1.* sobre la clasificación de las publicaciones presentes en la tesis en relación con los diseños de estudios epidemiológicos). Tres de los manuscritos presentados han sido publicados en revistas científicas indexadas del primer cuartil. Las otras dos publicaciones también han sido enviadas a revistas científicas del primer cuartil y se encuentras actualmente en fase de revisión.

4.1. Publicación 2: El cambio a una dieta baja en polifenoles durante dos semanas altera los biomarcadores vasculares en hombres sanos.

Artículo titulado "Changing to a Low-Polyphenol Diet Alters Vascular Biomarkers in Healthy Men after Only Two Weeks". Publicado en la revista científica "*Nutrients*" (Índice de impacto: 4,171 (2018); Q1).

Una dieta rica en compuestos bioactivos tiene efectos beneficiosos sobre la salud cardiovascular. A pesar de que un gran número de investigaciones ha corroborado esta hipótesis, la mayoría de los autores han observado estas consecuencias en poblaciones de riesgo (presión arterial alta, hipercolesterolemia, síndrome metabólico, etc) o en pacientes. Sin embargo, en este trabajo nos planteamos que una dieta pobre en estos compuestos bioactivos podría ser perjudicial a corto plazo y sin ninguna alteración aparente en los marcadores de salud cardiovascular. Para ello, se llevó a cabo un estudio cruzado en el cual se incluyeron 22 hombres sanos de entre 18 y 32 años que llevaron a cabo durante dos semanas una dieta baja en antioxidantes (especialmente en polifenoles) (LAD) y durante otras dos semanas su dieta habitual (UD). En la intervención LAD, los voluntarios evitaron el consumo de alimentos ricos en polifenoles como las hortalizas y frutas (máximo de dos raciones al día), el cacao, el café y el té **(Tabla 3)**. En cambio, la UD fue una dieta similar a la mediterránea (adherencia moderada *versus* Baja adherencia en la LAD). Como marcador de cumplimiento se analizaron los polifenoles totales en orina. La TPE fue significativamente menor después de la LAD en comparación con la UD (79 ± 43 *versus* 123 ± 58 mg equivalentes de ácido gálico (GAE)/g creatinina, $p = 0{,}007$) y se relacionó directamente con la adherencia a la dieta mediterránea ($r = 0{,}59$, $p = 0{,}003$). Los marcadores vasculares que se determinaron después de cada intervención fueron los siguientes: NO, TXA_2, PGI_2, ICAM-1 y VCAM-1. La concentración plasmática de NO disminuyó significativamente después de la LAD (52 ± 28 en LAD *versus* 80 ± 34 μM en UD, $p = 0{,}002$) y esta reducción se correlacionó con la menor adherencia a la dieta mediterránea ($r = 0{,}54$, $p = 0{,}008$). A pesar de no observar cambios significativos en el resto de los biomarcadores, el cociente TXA_2/PGI_2 aumentó significativamente (0,09 ± 0,08 en LAD *versus* 0,16 ± 0,14 en UD, p=0,048). En conclusión, el seguimiento de una dieta baja en antioxidantes a corto plazo puede afectar negativamente a la homeostasis vascular, incluso cuando no hay un riesgo aparente de ECV.

nutrients

MDPI

Article

Changing to a Low-Polyphenol Diet Alters Vascular Biomarkers in Healthy Men after Only Two Weeks

Sara Hurtado-Barroso [1,2,3], Paola Quifer-Rada [1,4], José Fernando Rinaldi de Alvarenga [1,3], Silvia Pérez-Fernández [5,6], Anna Tresserra-Rimbau [2,7] and Rosa M. Lamuela-Raventos [1,2,3,*]

1 Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain; sara.hurtado_17@ub.edu (S.H.-B.); pquifera@gmail.com (P.Q-R.); zehfernando@gmail.com (J.F.R.d.A.)
2 CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, 28029 Madrid, Spain; anna.tresserra@iispv.cat
3 INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08028 Barcelona, Spain
4 Department of Endocrinology & Nutrition, CIBER of Diabetes and Associated Metabolic Diseases, Biomedical Reseacrh Institute Sant Pau, Hospital de la Santa Creu i Sant Pau, 08041 Barcelona, Spain
5 Cardiovascular Epidemiology and Genetics Group, REGICOR, IMIM (Instituto Hospital del Mar de Investigaciones Médicas), 08003 Barcelona, Spain; sperez@imim.es
6 Center for the Biomedical Research Network in Cardiovascular Diseases (CIBERCV), Institute of Health Carlos III, 28029 Madrid, Spain
7 Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Faculty of Medicine and Health Sciences, Pere Virgili Health Research Center, University Rovira i Virgili, 43201 Reus, Spain
* Correspondence: lamuela@ub.edu; Tel.: +34-934-034-843

Received: 26 October 2018; Accepted: 12 November 2018; Published: 14 November 2018

Abstract: Bioactive dietary compounds play a critical role in health maintenance. The relation between bioactive compound intake and cardiovascular health-related biomarkers has been demonstrated in several studies, although mainly with participants who have altered biochemical parameters (high blood pressure, high cholesterol, metabolic syndrome, etc.). The aim of this study was to evaluate if adopting a diet low in polyphenol-rich food for two weeks would affect vascular biomarkers in healthy men. In a crossover study, 22 healthy men were randomly assigned to their usual diet (UD), consuming healthy food rich in polyphenols, or to a low antioxidant diet (LAD), with less than two servings of fruit and vegetables per day and avoiding the intake of cocoa products, coffee and tea. As a marker of compliance, total polyphenols in urine were significantly lower after the LAD than after the UD (79 ± 43 vs. 123 ± 58 mg GAE/g creatinine). Nitric oxide levels were also reduced (52 ± 28 in LAD vs. 80 ± 34 µM in UD), although no significant changes in cellular adhesion molecules and eicosanoids were observed; however, an increasing ratio between thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2) was reached ($p = 0.048$). Thus, a slight dietary modification, reducing the consumption of polyphenol-rich food, may affect vascular biomarkers even in healthy individuals.

Keywords: bioactive compounds; nitric oxide; vascular biomarkers; low antioxidant diet; Mediterranean diet; eicosanoids; adhesion molecules

1. Introduction

It is broadly accepted that bioactive compounds of plant origin play a crucial protective role against non-communicable diseases (NCDs). Polyphenols are plant secondary metabolites with one or

more aromatic rings that bear at least one hydroxyl group. More than 8000 phenolic structures have been described [1] and several hundred are found in edible vegetables [2]. In the last years, phenolic compounds have been widely studied due to their close association with the prevention of NCDs such as cardiovascular diseases (CVDs) [3–5]. Polyphenols are known to have anti-inflammatory properties and improve endothelial function [6–8].

Current research about the protective role of polyphenols against CVD is focused on their action on the endothelium [9–11]. Endothelium-dependent vasodilator factors include molecules like nitric oxide (NO) and prostacyclin (PGI_2), while the endothelium-dependent vasoconstrictor response involves reactive oxygen species (ROS) and thromboxane A_2 (TXA_2) [12]. NO is a gaseous signaling molecule mainly produced from the amino acid L-arginine by nitric oxide synthase [13] with a crucial role in the prevention of CVD and other NCDs. Endothelium-derived NO is a powerful vasodilator that inhibits platelet adhesion and aggregation as well as thrombin formation, thus preventing vascular injuries. Moreover, NO can inhibit the expression of vascular cell and intercellular adhesion molecules (VCAM-1 and ICAM-1) [14,15].

The entry of polyphenols into endothelial cells is apparently facilitated by specific membrane receptors and transport systems [16–18], which increase the formation of NO through different pathways (some involving ROS) [19,20]. The activation of soluble guanylyl cyclase by NO results in the formation of cyclic guanosine monophosphate (cGMP) that initiate the relaxation of endothelium [12]. However, further research is needed to establish the mechanisms involved in the interaction between the molecules, the triggering of signals and the biological responses. These mechanisms have been studied but mainly with supplements or extracts containing polyphenols at a far higher concentration than those consumed in the usual diet. Moreover, many studies have been performed in elderly populations at risk of developing NCDs due to altered biochemical parameters (high blood pressure, high cholesterol, metabolic syndrome, etc.) or even in patients with a diagnosed disease [21].

Thus, in the present study we aimed to study if a short-term dietary intervention involving a reduced consumption of polyphenol-rich food could affect vascular biomarkers in healthy young men at no apparent risk of developing a disease.

2. Materials and Methods

2.1. Study Subjects

Twenty-two healthy men between 18 and 32 years of age took part in the interventional study from November 2015 to April 2016. Only male adults were enrolled in this trial to avoid effects related to hormonal fluctuations during the menstrual cycle [22,23]. Exclusion criteria were body mass index (BMI) over 30 kg/m^2, a history of CVDs, hypertension and dyslipidemia, chronic illness or homeostatic disorder and toxic habits (such as tobacco, alcohol and drug consumption). Volunteers were asked to maintain the same level of physical activity throughout the study. Figure 1 shows the flowchart of subjects in the trial.

All participants provided written informed consent prior to starting the intervention. The study was carried out according to the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Clinical Investigation of the University of Barcelona (Barcelona, Spain). The clinical trial was registered and given the International Standard Randomized Controlled Trial Number (http://www.isrctn.com/) of ISRCTN17867378.

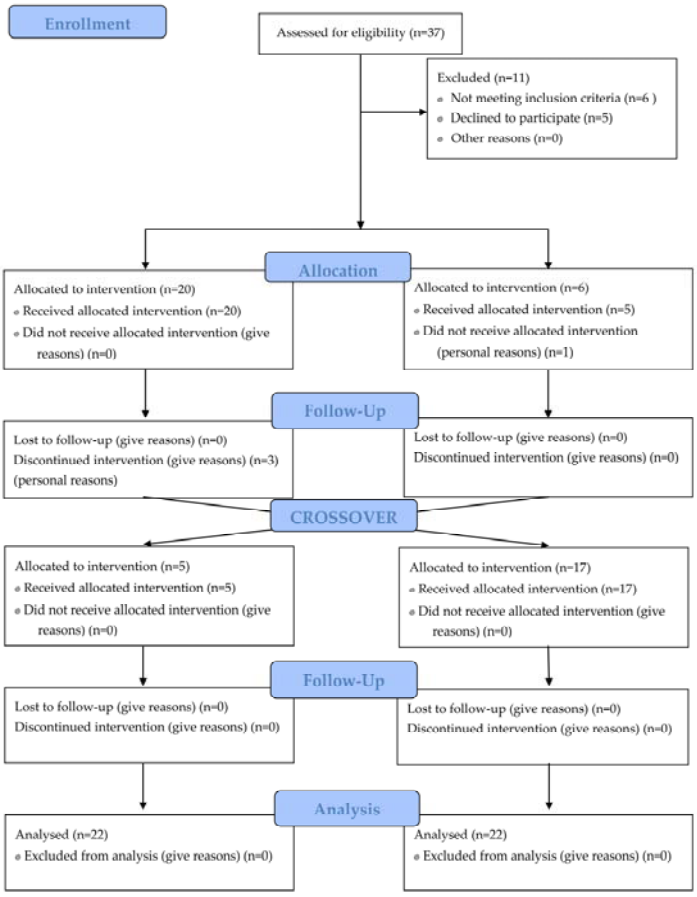


Figure 1. Flow diagram showing the participation of volunteers in each phase of the trial. The usual diet (UD) is shown on the left side of the graph and the low antioxidant diet (LAD) on the right side.

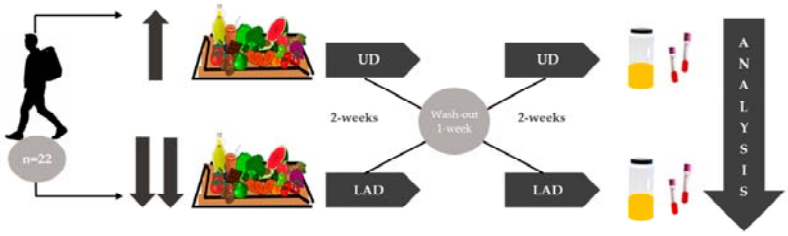


Figure 2. General scheme of the crossover trial with two intervention periods.

2.2. Study Design

The study was open, crossover and controlled (Figure 2), and every participant underwent two 2-week interventions, and ate their usual diet (UD) and a low antioxidant diet (LAD), with a week of washout in between. Advice about changes in diet was given. Participants following the LAD were asked not to consume more than 2 servings of fruits or vegetables per day and their consumption of polyphenol-rich items, such as coffee, tea or chocolate, was restricted. Supplementary Table S1 shows all the recommendations to follow a low antioxidant diet (LAD).

In contrast, in the UD there was no restriction on polyphenol-rich food (fruit, vegetables, extra-virgin olive oil, tea, etc.) consumption. The study was run in the Department of Nutrition, Food Science and Gastronomy of the Food Science Torribera Campus of the University of Barcelona (Spain).

2.3. Dietary and Physical Activity Assessments

After each intervention, subjects filled out a 3-day food recall questionnaire. The dietary registers were analyzed using nutrition analysis software developed at the University of Barcelona, PCN Pro software (Programa de Càlcul Nutricional Professional, Santa Coloma de Gramenet, Barcelona, Spain). In addition, a 14-point Mediterranean Diet Adherence questionnaire was used to compare the two diets [24]. Physical activity was measured using the validated Spanish version of the Minnesota leisure-time physical activity questionnaire [25].

2.4. Blood and Urine Collection

Fasting blood and urine samples were collected after each dietary intervention between 8:00 and 9:00 a.m. Blood was drawn from the arm via venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA), and plasma was separated after centrifugation at 2070× *g* for 15 min at 4 °C. Plasma and urine were aliquoted and stored at −80 °C until the day of the analysis.

2.5. Clinical and Anthropometric Measurements

Diastolic and systolic blood pressure (DBP and SBP) as well as heart rate (HR) were measured in triplicate after each intervention the morning in fasting conditions. Biochemistry parameters in plasma were evaluated at an external laboratory (mdb lab Durán Bellido). C-reactive protein (CRP) was measured by an immunoturbidimetric method. HDL, LDL, total cholesterol and triglycerides were analyzed by an enzymatic method. Urea and uric acid were analyzed by enzymatic and enzymatic/chromogen methods, respectively. Creatinine was determined by reaction kinetics of the Jaffe method (as modified by Larsen). Total proteins and albumin were measured by the endpoint biuret reaction and bromocresol green methods, respectively. Biochemistry measurements were performed once the studied completed in the samples stored at −80 °C.

Body mass index (BMI) was calculated from the weight and height, and the waist-hip ratio (WHR) from the measurements of the waist and hip circumferences taken at the visit after each intervention in the early morning.

2.6. Quantification of Total Polyphenol Excretion (TPE) in Urine Samples

A solid phase extraction (SPE) using a 96-well plate cartridge (Oasis MAX, Waters Co., Milford, MA, USA) was performed in diluted urine samples and total polyphenol excretion (TPE) was measured by a Folin–Ciocalteu reaction according to Medina-Remon et al. [26]. The spectrophotometry analysis was carried out at a wavelength of 765 nm. The results were expressed as mg of gallic acid equivalent (GAE)/g of creatinine.

2.7. Determination of Plasmatic Inflammatory Biomarkers

The cell adhesion molecules, sICAM-1 and sVCAM-1, were measured by a ProcartaPlex Multiplex Immunoassay kit (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). Plasma samples were

diluted 1:200 and assayed in duplicate. The assay was performed through a MAGPIX® instrument (Luminex, Co., Austin, TX, USA) and data were processed with ProcartaPlex Analyst software.

Before the determination of NO, plasma samples were filtered using the Amicon® Ultra 30K (Merck KGaA, Darmstadt, Germany) device over microcentrifuge tubes and centrifuged at 14,000× *g* for 1 h at 4 °C. The NO amount was indirectly determined using a nitrate/nitrite colorimetric assay kit (Cayman Chem. Co., Ann Arbor, MI, USA, ref. 780001). The detection limit of the assay was approximately 1 µM nitrite, considering a 1:2 dilution of plasma samples. The analysis was carried out in triplicate.

2.8. Determination of Eicosanoids in Urine

The PGI_2 and TXA_2 were indirectly quantified by measuring PGIM (Prostaglandin I Metabolite) and 11-dehydro thromboxane B_2, respectively. Both molecules were determined in urine using two competitive enzyme-linked immunosorbent assay (ELISA) kits acquired from Cayman Chem. Co. (Ann Arbor, MI, USA, ref. 501100 and 519510). The PGIM assay has a range from 39 to 5000 pg/mL and a sensitivity (80% B/B0) of approximately 120 pg/mL. The 11-dehydro thromboxane B_2 assay has a range from 15.6 to 2000 pg/mL and a sensitivity (80% B/B0) of approximately 34 pg/mL. The analysis was carried out in triplicate. The TXA_2:PGI_2 ratio was calculated.

2.9. Statistical Analysis

Normality was checked by a Shapiro–Wilk test. Non-normal variables were log-transformed. The non-normal variables with values close to zero were transformed by inverse hyperbolic sine (IHS) function. Linear regression models were assayed both to calculate the raw *p*-value and *p*-value of the differences adjusted by mean-centered age (years) and physical activity (METS/day). A *p*-value of <0.05 was considered statistically significant.

Correlations between the MedDiet score and the analyzed biomarkers were determined using a correlation matrix that considers repeated measures [27]. The Pearson coefficient (*r*) was calculated. Significant correlations ($p < 0.05$) are shown in the results.

All the analysis was performed using the software R, version 3.4.2. (R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria [28]).

3. Results

3.1. Characteristics of Participants

Twenty-two men completed the study. Baseline characteristics remained constant throughout the study (age and METS/day). In addition, anthropometric and clinical measurements did not change significantly after the LAD compared to the control (UD) ($p > 0.05$) (Table 1).

Table 1. Characteristics of all participants of the study.

	UD	LAD	*p*	Adjusted *p* [a]
Baseline characteristics				
Age (years)	24 ± 1			
Physical activity (METS/day)	743 ± 75			
Anthropometric measurements				
BMI (kg/m^2)	24.9 ± 0.79	24.8 ± 0.79	0.375	0.33
WHR	0.84 ± 0.01	0.84 ± 0.01	1	0.89
Clinical measurements				
SBP (mmHg)	123 ± 2	122 ± 2	0.864	0.90
DBP (mmHg)	76 ± 2	76 ± 2	0.893	0.995
HR (bpm)	66 ± 2	63 ± 2	0.109	0.198
CRP (mg/dL)	0.10 ± 0.02	0.09 ± 0.01	0.893	0.925
Total cholesterol (mmoles/L)	3.84 ± 0.12	3.80 ± 0.12	0.547	0.551
HDL (mmoles/L)	1.37 ± 0.06	1.36 ± 0.05	0.865	0.832
LDL (mmoles/L)	2.04 ± 0.11	1.97 ± 0.11	0.250	0.306
Triglycerides (mmoles/L)	0.94 ± 0.08	1.04 ± 0.12	0.470	0.525
Urea (mmoles/L)	5.56 ± 0.30	5.94 ± 0.25	0.211	0.365
Creatinine (µmoles/L)	76 ± 1.51	76.3 ± 1.60	0.510	0.752
Uric acid (µmoles/L)	319 ± 10.95	332 ± 10.51	0.138	0.136
Total proteins (g/L)	73.1 ± 0.57	73.9 ± 0.54	0.272	0.324
Albumin (g/L)	46.8 ± 0.50	46.6 ± 0.41	0.655	0.608

Data are mean ± standard error of the mean (SEM). [a] Baseline measures, age and physical activity were used to adjust the *p*-value.

3.2. Dietary Intake

Table 2 shows the differences between the two interventions (UD and LAD) in nutrients and healthy food intake. Although the diets were similar in macronutrients (except for the fat content), the LAD had a lower concentration of vitamins, minerals, and fiber. Levels of magnesium, potassium and iron differed significantly between the diets, as did vitamins A, E, C and folate ($p \leq 0.001$), because the UD involved a higher consumption of fruits and vegetables rich in these compounds ($p < 0.001$), as well as of cocoa ($p < 0.001$), tea ($p < 0.001$), olive oil ($p = 0.01$), and nuts and seeds ($p < 0.001$).

The score categories of adherence to the MedDiet are high (≥10), moderate (6–9) and low (≤5). According to the scores obtained in the 14-item questionnaire, the adherence to the MedDiet in the UD was moderate (8.5 ± 0.4), and low in the LAD (4.7 ± 0.3), the two dietary interventions being significantly different ($p < 0.001$).

Table 2. Changes in the dietary parameters after 2 weeks.

	UD	LAD	*p*	Adjusted *p* [a]
Nutrients				
Energy (kcal/day)	2393 ± 130	2194 ± 127	0.083	0.033 *
Carbohydrates (g/day)	256 ± 15	245 ± 14	0.445	0.498
Fat (g/day)	106 ± 9	83 ± 7	0.005 *	0.001 *
Protein (g/day)	100 ± 6	109 ± 7	0.256	0.473
Cholesterol (mg/day)	301 ± 30	241 ± 21	0.046 *	0.025 *
Fiber (g/day)	30 ± 2	16 ± 1	<0.001 *	<0.001 *
Ca (mg/day)	869±49	936 ± 91	0.452	0.816
P (mg/day)	1599±82	1612 ± 97	0.897	0.711
Mg (mg/day)	436±25	314 ± 30	<0.001 *	<0.001 *
Na (mg/day)	2495±273	2945 ± 280	0.065	0.107
K (mg/day)	4237±180	2950 ± 325	<0.001 *	<0.001 *
Fe (mg/day)	17±0.98	12 ± 0.99	0.001 *	0.002 *
Zn (mg/day)	11±0.72	10 ± 0.60	0.217	0.057
Vit. A (mcg r.e./day)	1460±232	242 ± 45	<0.001 *	<0.001 *
Vit E (mg t.e./day)	15±1.19	8 ± 0.70	<0.001 *	<0.001 *
Vit. C (mg/day)	255±25	39 ± 6	<0.001 *	<0.001 *
Folate (mcg/day)	511±34	243 ± 33	<0.001 *	<0.001 *
Food				
Vegetable (g/day)	402 ± 38	120 ± 25	<0.001 *	<0.001 *
Fruit (g/day)	368 ± 53	48 ± 16	<0.001 *	<0.001 *
Nuts and seeds (g/day)	15 ± 5	0.2 ± 0.2	<0.001 *	<0.001 *
Virgin olive oil (g/day)	33 ± 2	26 ± 2	0.009 *	0.01 *
Pulses (g/day)	19 ± 6	16 ± 6	0.210	0.295
Cereals (g/day)	202 ± 20	275 ± 17	0.001 *	0.001 *
Cocoa (g/day)	13 ± 3	0.7 ± 0.6	<0.001 *	<0.001 *
Coffee (g/day)	63 ± 24	23 ± 9	0.186	0.162
Tea (g/day)	112 ± 29	0	<0.001 *	<0.001 *
White meat (g/day)	70 ± 14	79 ± 14	0.353	0.312
Fish (g/day)	61 ± 12	65 ± 17	0.768	0.885
Wine (g/day)	5 ± 3	0	0.163	0.107
Beer (g/day)	16 ± 8	13 ± 10	0.201	0.215
Mediterranean diet score				
MedDiet score [†]	8.5 ± 0.4	4.7 ± 0.3	<0.001 *	<0.001 *

Data are mean ± SEM. [†] MedDiet score obtained from 14-item Mediterranean Diet Adherence Screener. [a] Baseline measures, age and physical activity were used to adjust the *p*-value. * Significant difference ($p < 0.05$).

3.3. TPE in Urine after Each Dietary Intervention

Figure 3 shows a significant decrease of TPE, expressed in gallic acid equivalents (GAE) and corrected by creatinine, in participants after following the LAD for two weeks. The difference in TPE between the two interventions was around 45 mg GAE/g creatinine ($p = 0.007$).

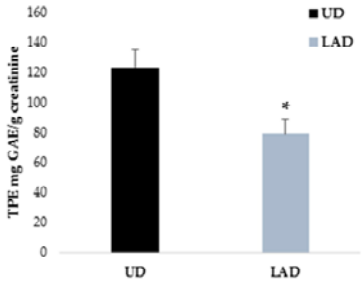


Figure 3. Comparison of total polyphenol excretion (TPE) in urine after each intervention. Data shown are mean ± SEM. * Significant differences with $p < 0.05$.

3.4. Inflammatory Molecules in Plasma and Urine

Significant changes in NO levels ($p = 0.001$ and adjusted $p = 0.002$) were observed. Figure 4A shows the reduction of NO concentration after the short-term diet with food containing lower amounts of bioactive compounds compared to the UD intervention (52 ± 28 µM in LAD vs. 80 ± 34 µM in UD).

Although there were negative changes in the inflammatory parameters (cellular adhesion molecules and eicosanoids) after the LAD, these differences were not significant (Figure 4B–E). The TXA_2:PGI_2 ratio, an indicator of the balance of platelet function, increased considerably from 0.09 ± 0.08 to 0.16 ± 0.14 ($p = 0.051$ and adjusted $p = 0.048$, Figure 4F).

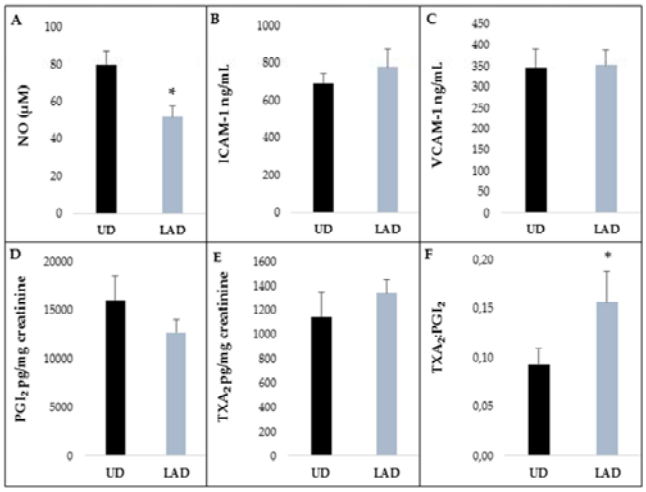


Figure 4. (**A**) NO (**B**) ICAM-1 (**C**) VCAM-1 (**D**) PGI_2 (**E**) TXA_2 and (**F**) ratio TXA_2/PGI_2 between both interventions. Data shown are mean ± SEM. The UD is in black and the LAD is in gray; * significant differences with $p < 0.05$.

3.5. Correlation between Biomarkers and Mediterranean Diet Adherence

After the short-term intervention with low-antioxidant foods, significant changes were observed in the TPE and NO. Both parameters correlated positively with the MedDiet. Thus, TPE in urine and

NO concentration in plasma had decreased in participants after following the diet poor in bioactive compounds (Figure 5A,B).

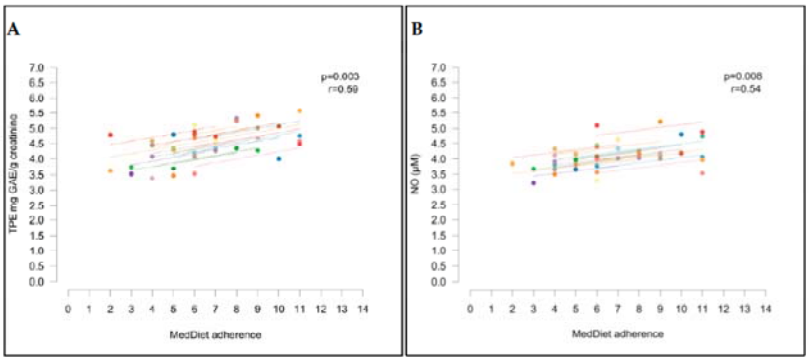


Figure 5. Correlation of Mediterranean diet adherence with TPE (**A**) and NO (**B**).

4. Discussion

In our study, changing to a diet low in polyphenols affected NO expression and modified the anti-inflammatory response in young healthy men, although without reaching significance, except for the ratio TXA_2:PGI_2 probably due to the low number of subjects and the short period of the intervention.

Regarding the results of the health-related biomarkers, our study suggests that following a diet low in bioactive compounds, measured by the validated biomarker of TPE, for a period as short as two weeks, reduced plasmatic NO in healthy volunteers. Several studies have shown the protective role of polyphenols or polyphenol-rich food on endothelium function [10] and that following a Mediterranean diet is beneficial for vascular health. Schroeter et al. reported an acute increase in the concentration of circulating NO species after the consumption of flavanol-rich cocoa in a crossover trial with healthy male subjects [29]. These authors also observed a positive correlation between the chronic ingestion of flavanol-rich cocoa and the urinary excretion of NO metabolites, at least partially due to the flavanol (−)-epicatechin, in a cross-sectional study of the Kuna population [29]. Likewise, in elderly participants at high cardiovascular risk from the PREDIMED trial, a significant increase of total plasma NO was observed after a one-year intervention with an extra intake of extra virgin olive oil or nuts [8]. This increase was associated with a significant change in the blood pressure (BP) and correlated with the TPE in urine [8]. In another PREDIMED substudy, an increase in serum NO (nitrate + nitrite) after a Mediterranean Diet + extra virgin olive oil intervention was negatively correlated with BP in hypertensive women [30]. In addition, in a crossover study where healthy subjects consumed an ice cream based on dark cocoa powder with a hazelnut and green tea extract, an increase in total serum polyphenol and NO levels were found and correlated [31].

Although in our study a significant change in BP and higher NO levels did not correlate with TPE in urine, a positive correlation was obtained between total plasma NO and MedDiet adherence. Thus, NO levels in plasma were lower in participants after following the LAD rather than the UD ($p = 0.008$, $r = 0.54$). Other authors performing crossover trials where healthy subjects consumed polyphenol-rich products or extracts have also found an increase of plasma or urinary NO [32–34]. In a parallel study with elderly pre-hypertensive or stage 1 hypertensive participants without concomitant risk factors, significant changes in BP and NO were observed after an intervention with dark chocolate for 18 weeks [35]. A reduction in BP and a rise of plasma NO, both correlated with each other,

were observed in men at high cardiovascular risk after an intervention with dealcoholized red wine in a crossover trial [36]. In addition, 24 men with metabolic syndrome consuming a grape supplement in a 30-day-crossover trial had higher levels of plasma NO, correlated with a decrease in systolic BP, although the change in NO was not significant [21]. However, in contrast with the aforementioned studies, a recent crossover trial studying the acute effect of coffee in healthy volunteers did not find significant differences in plasma NO concentrations or BP [37]. Likewise, no changes in concentration of plasma NO were observed in subjects at risk of CVDs consuming a beverage based on wild blueberries daily for six weeks [38].

The pro-inflammatory response increased in participants after the two-week LAD, although changes in plasma ICAM-1 nor VCAM-1 were not significant. In accordance with our results, Taubert et al. reported lower levels of ICAM-1 in plasma after the consumption of a grape supplement, and although there was not a significant difference in VCAM-1, the reduction of this biomarker was positively correlated with a decrease in systolic BP [35]. Similar results were obtained in another parallel trial where hypertensive subjects consumed 150 mL/day of pomegranate juice for 2 weeks and a reduction of the VCAM-1 biomarker and BP was observed [39]. Several other authors have observed positive effects on CAMs after consumption of polyphenol extracts or polyphenol-rich products [6,9,40,41]. However, another trial did not find any effect on ICAM-1 or VCAM-1 [38,42,43].

The balance of the vasodilation and vasoconstrictor molecules, PGI_2 and TXA_2, is also crucial for vascular health. Both eicosanoids are synthesized from arachidonic acid by cyclooxygenase isoforms (COX_1 and COX_2). In the present study, a notable increase in the TXA2:PGI2 ratio was observed, being almost double after the LAD intervention compared to the UD ($p = 0.048$), suggesting that polyphenols could contribute to the maintenance of vascular homeostasis. The observed trend was for PGI2 to decrease and TXA2 to increase after the LAD, although the change of both biomarkers did not reach significance. Dietary polyphenols regulate immune cells as well as the expression of several pro- and anti-inflammatory genes and cytokines by different pathways (MAPK, NF-kB and arachidonic acid), contributing to anti-inflammatory, immune-modulatory, and antioxidant activities [44].

The effect of polyphenol-rich products on eicosanoids has been demonstrated. Pignatelli et al. showed that following a Mediterranean diet reduces the level of TXA_2 in atrial fibrillation patients [45]. Also, the intake of extra virgin olive oil, a typical polyphenol-rich product of the Mediterranean diet, reduced the serum TXB_2 concentration in healthy subjects [46]. However, other authors have obtained inconclusive results. In a crossover study, platelet TXA_2 production in smokers, which was higher than in healthy subjects, did not change after consuming 40 g of milk chocolate or of dark chocolate [47]. No effect on TXA2 and PGI2 metabolites in urine, or on the ratio of both molecules, was found in healthy subjects consuming an American diet and another supplemented with cacao/chocolate (containing 466mg of procyanidins) for four weeks [48]. Moreover, after consuming a serving of aronia-citrus juice for 45 days, 16 triathletes had lower urinary excretion of 11-dehydro-TXB_2, but no significant change was observed in the 2,3-dinor-6-keto-PGF1α (PGI_2 metabolite) [49].

A strong point of this study is its focus on healthy young volunteers (most of them university students), which is atypical in this research area. Moreover, the intervention was carried out with a normal diet, reducing the intake of polyphenol-rich food for only a short period. The limitations of the study design are the small size of the sample, limited to a male population, the non-randomized design, and the lack of baseline measures. However, as the UD was the normal diet of the volunteers, it was not considered necessary to take baseline measures into account (data not shown).

5. Conclusions

These results highlight that adopting a diet low in polyphenols for a period as short as two weeks can alter the total plasma NO concentration in healthy young men. An increasing imbalance between TXA_2 and PGI_2 was also observed, although the changes in both eicosanoids did not reach significance. Thus, this study confirms the health protective role of polyphenol-rich food and suggests that avoiding

or reducing the consumption of food rich in these compounds has a negative effect on vascular biomarkers, even after a short period and in healthy subjects.

Supplementary Materials: The following is available online at http://www.mdpi.com/2072-6643/10/11/1766/s1, Table S1: Recommendations to follow a low antioxidant diet (LAD).

Author Contributions: S.H.-B., J.F.R.d.A., P.Q.-R., A.T.-R. and R.M.L.-R. designed the study. S.H.-B. conducted the research. P.Q.-R. collaborated in the processing of the data. S.P.-F. performed the statistical analysis. S.H.-B. wrote the manuscript. R.M.L.-R. was the main responsible of the project and final content. All participants reviewed and approved the paper.

Funding: This research was funded by CYCIT from Ministerio de Ciencia, Innovación y Universidades), grant number AGL2016-79113-R and Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERobn).

Acknowledgments: First: we thank all the participants of the study. S.H.B received support from the Ministerio de Educación, Cultura y deporte (MECD) through predoctoral scholarship FPU (FPU14/01715). P.Q.-R. is thankful for the Sara Borrell postdoctoral program from the Instituto de Salud Carlos III (ISCIII). J.F.R.d.A. is grateful to the Science without Borders program for the predoctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—Brazil (233576/2014-2). A.T.R. thanks the Juan de la Cierva postdoctoral program (FJCI-2016-28694) from the Ministerio de Economía, Industria y Competitividad.

Conflicts of Interest: Lamuela-Raventós reports receiving lecture fees from Cerveceros de España; and receiving lecture fees and travel support from Adventia. Nevertheless, these foundations were not involved in the study design, the collection, analysis and interpretation of data, the writing of the manuscript or the decision to submit the manuscript for publication. The other authors declare no conflict of interest.

References

1. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (Poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892. [CrossRef] [PubMed]
2. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An online comprehensive database on polyphenol contents in foods. *Database* **2010**, *2010*, bap024. [CrossRef] [PubMed]
3. Murillo, A.G.; Fernandez, M.L. The relevance of dietary polyphenols in cardiovascular protection. *Curr. Pharm. Des.* **2017**, *23*, 2444–2452. [CrossRef] [PubMed]
4. Tressera-Rimbau, A.; Arranz, S.; Eder, M.; Vallverdú-Queralt, A. Dietary polyphenols in the prevention of stroke. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 7467962. [CrossRef] [PubMed]
5. Williamson, G. The role of polyphenols in modern nutrition. *Nutr. Bull.* **2017**, *42*, 226–235. [CrossRef] [PubMed]
6. Medina-Remón, A.; Casas, R.; Tressserra-Rimbau, A.; Ros, E.; Martínez-González, M.A.; Fitó, M.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventos, R.M.; Estruch, R.; et al. Polyphenol intake from a Mediterranean diet decreases inflammatory biomarkers related to atherosclerosis: A substudy of the PREDIMED trial. *Br. J. Clin. Pharmacol.* **2017**, *83*, 114–128. [CrossRef] [PubMed]
7. Croft, K.D. Dietary polyphenols: Antioxidants or not? *Arch. Biochem. Biophys.* **2016**, *595*, 120–124. [CrossRef] [PubMed]
8. 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.; et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutr. Metab. Cardiovasc. Dis.* **2015**, *25*, 60–67. [CrossRef] [PubMed]
9. Forte, M.; Conti, V.; Damato, A.; Ambrosio, M.; Puca, A.A.; Sciarretta, S.; Frati, G.; Vecchione, C.; Carrizzo, A. Targeting nitric oxide with natural derived compounds as a therapeutic strategy in vascular diseases. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 7364138. [CrossRef] [PubMed]
10. Oak, M.-H.; Auger, C.; Belcastro, E.; Park, S.-H.; Lee, H.-H.; Schini-Kerth, V.B. Potential mechanisms underlying cardiovascular protection by polyphenols: Role of the endothelium. *Free Radic. Biol. Med.* **2018**. [CrossRef] [PubMed]
11. Yamagata, K.; Tagami, M.; Yamori, Y. Dietary polyphenols regulate endothelial function and prevent cardiovascular disease. *Nutrition* **2015**, *31*, 28–37. [CrossRef] [PubMed]

12. Vanhoutte, P.M.; Shimokawa, H.; Feletou, M.; Tang, E.H.C. Endothelial dysfunction and vascular disease-a 30th anniversary update. *Acta Physiol.* **2017**, *219*, 22–96. [CrossRef] [PubMed]
13. Zhao, Y.; Vanhoutte, P.M.; Leung, S.W.S. Vascular nitric oxide: Beyond eNOS. *J. Pharmacol. Sci.* **2015**, *129*, 83–94. [CrossRef] [PubMed]
14. Vita, J.A.; Keaney, J.F. Endothelial function: A barometer for cardiovascular risk? *Circulation* **2002**, *106*, 640–642. [CrossRef] [PubMed]
15. Herman, A.G.; Moncada, S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. *Eur. Heart J.* **2005**, *26*, 1945–1955. [CrossRef] [PubMed]
16. Tachibana, H.; Koga, K.; Fujimura, Y.; Yamada, K. A receptor for green tea polyphenol EGCG. *Nat. Struct. Mol. Biol.* **2004**, *11*, 380–381. [CrossRef] [PubMed]
17. Schramm, D.D.; Collins, H.E.; German, J.B. Flavonoid transport by mammalian endothelial cells. *J. Nutr. Biochem.* **1999**, *10*, 193–197. [CrossRef]
18. Jin, X.; Yi, L.; Chen, M.; Chen, C.; Chang, H.; Zhang, T.; Wang, L.; Zhu, J.; Zhang, Q.; Mi, M. Correction: Delphinidin-3-Glucoside protects against oxidized low-density lipoprotein-induced mitochondrial dysfunction in vascular endothelial cells via the sodium-dependent glucose transporter SGLT1. *PLoS ONE* **2014**, *9*. [CrossRef]
19. Kim, J.H.; Auger, C.; Kurita, I.; Anselm, E.; Rivoarilala, L.O.; Lee, H.J.; Lee, K.W.; Schini-Kerth, V.B. Aronia melanocarpa juice, a rich source of polyphenols, induces endothelium-dependent relaxations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase. *Nitric Oxide* **2013**, *35*, 54–64. [CrossRef] [PubMed]
20. Kim, J.; Formoso, G.; Li, Y.; Potenza, M.A.; Marasciulo, F.L.; Montagnani, M.; Quon, M.J. Epigallocatechin Gallate, a Green Tea Polyphenol, Mediates NO-dependent Vasodilation Using Signaling Pathways in Vascular Endothelium Requiring Reactive Oxygen Species and Fyn. *J. Biol. Chem.* **2007**, *282*, 13736–13745. [CrossRef] [PubMed]
21. Barona, J.; Aristizabal, J.C.; Blesso, C.N.; Volek, J.S.; Fernandez, M.L. Grape polyphenols reduce blood pressure and increase flow-mediated vasodilation in men with metabolic syndrome. *J. Nutr.* **2012**, *142*, 1626–1632. [CrossRef] [PubMed]
22. Kawano, H.; Motoyama, T.; Kugiyama, K.; Hirashima, O.; Ohgushi, M.; Yoshimura, M.; Ogawa, H.; Okumura, K.; Yasue, H. Menstrual cyclic variation of endothelium-dependent vasodilation of the brachial artery: Possible role of estrogen and nitric oxide. *Proc. Assoc. Am. Physicians* **1996**, *108*, 473–480. [PubMed]
23. Whitcomb, B.W.; Mumford, S.L.; Perkins, N.J.; Wactawski-Wende, J.; Bertone-Johnson, E.R.; Lynch, K.E.; Schisterman, E.F. Urinary cytokine and chemokine profiles across the menstrual cycle in healthy reproductive-aged women. *Fertil. Steril.* **2014**, *101*, 1383–1391. [CrossRef] [PubMed]
24. Schröder, H.; Fitó, M.; Estruch, R.; Martínez-González, M.A.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M.; et al. A short screener is valid for assessing mediterranean diet adherence among older spanish men and women. *J. Nutr.* **2011**, *141*, 1140–1145. [CrossRef] [PubMed]
25. Elosua, R.; Marrugat, J.; Molina, L.; Pons, S.; Pujol, E. Validation of the minnesota leisure time physical activity questionnaire in spanish men. The MARATHOM Investigators. *Am. J. Epidemiol.* **1994**, *139*, 1197–1209. [CrossRef] [PubMed]
26. Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M.-Á.; Diez-Espino, J.; Lamuela-Raventos, R.M. Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal. Chim. Acta* **2009**, *634*, 54–60. [CrossRef] [PubMed]
27. rmcorr: Repeated Measures Correlation. Available online: https://cran.r-project.org/web/packages/rmcorr/index.html (accessed on 13 November 2018).
28. The R Project for Statistical Computing. Available online: https://www.r-project.org/ (accessed on 13 November 2018).
29. Schroeter, H.; Heiss, C.; Balzer, J.; Kleinbongard, P.; Keen, C.L.; Hollenberg, N.K.; Sies, H.; Kwik-Uribe, C.; Schmitz, H.H.; Kelm, M. (−)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 1024–1029. [CrossRef] [PubMed]

30. Storniolo, C.E.; Casillas, R.; Bulló, M.; Castañer, O.; Ros, E.; Sáez, G.T.; Toledo, E.; Estruch, R.; Ruiz-Gutiérrez, V.; Fitó, M.; et al. A Mediterranean diet supplemented with extra virgin olive oil or nuts improves endothelial markers involved in blood pressure control in hypertensive women. *Eur. J. Nutr.* **2017**, *56*, 89–97. [CrossRef] [PubMed]
31. Sanguigni, V.; Manco, M.; Sorge, R.; Gnessi, L.; Francomano, D. Natural antioxidant ice cream acutely reduces oxidative stress and improves vascular function and physical performance in healthy individuals. *Nutrition* **2017**, *33*, 225–233. [CrossRef] [PubMed]
32. Hollands, W.J.; Hart, D.J.; Dainty, J.R.; Hasselwander, O.; Tiihonen, K.; Wood, R.; Kroon, P.A. Bioavailability of epicatechin and effects on nitric oxide metabolites of an apple flavanol-rich extract supplemented beverage compared to a whole apple puree: A randomized, placebo-controlled, crossover trial. *Mol. Nutr. Food Res.* **2013**, *57*, 1209–1217. [CrossRef] [PubMed]
33. Hall, W.L.; Formanuik, N.L.; Harnpanich, D.; Cheung, M.; Talbot, D.; Chowienczyk, P.J.; Sanders, T.A.B. A Meal Enriched with Soy Isoflavones Increases Nitric Oxide-Mediated Vasodilation in Healthy Postmenopausal Women. *J. Nutr.* **2008**, *138*, 1288–1292. [CrossRef] [PubMed]
34. Ochiai, R.; Sugiura, Y.; Otsuka, K.; Katsuragi, Y.; Hashiguchi, T. Coffee bean polyphenols ameliorate postprandial endothelial dysfunction in healthy male adults. *Int. J. Food Sci. Nutr.* **2015**, *66*, 350–354. [CrossRef] [PubMed]
35. Taubert, D.; Roesen, R.; Lehmann, C.; Jung, N.; Schömig, E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. *JAMA* **2007**, *298*, 49. [CrossRef] [PubMed]
36. Chiva-Blanch, G.; Urpi-Sarda, M.; Ros, E.; Arranz, S.; Valderas-Martinez, P.; Casas, R.; Sacanella, E.; Llorach, R.; Lamuela-Raventos, R.M.; Andres-Lacueva, C.; et al. Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide: Short communication. *Circ. Res.* **2012**, *111*, 1065–1068. [CrossRef] [PubMed]
37. Boon, E.A.J.; Croft, K.D.; Shinde, S.; Hodgson, J.M.; Ward, N.C. The acute effect of coffee on endothelial function and glucose metabolism following a glucose load in healthy human volunteers. *Food Funct.* **2017**, *8*, 3366–3373. [CrossRef] [PubMed]
38. Riso, P.; Klimis-Zacas, D.; Del Bo', C.; Martini, D.; Campolo, J.; Vendrame, S.; Møller, P.; Loft, S.; De Maria, R.; Porrini, M. Effect of a wild blueberry (*Vaccinium angustifolium*) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. *Eur. J. Nutr.* **2013**, *52*, 949–961. [CrossRef] [PubMed]
39. Asgary, S.; Sahebkar, A.; Afshani, M.R.; Keshvari, M.; Haghjooyjavanmard, S.; Rafieian-Kopaei, M. Clinical evaluation of blood pressure lowering, endothelial function improving, hypolipidemic and anti-inflammatory effects of pomegranate juice in hypertensive subjects. *Phyther. Res.* **2014**, *28*, 193–199. [CrossRef] [PubMed]
40. Casas, R.; Sacanella, E.; Urpí-Sardà, M.; Chiva-Blanch, G.; Ros, E.; Martínez-González, M.-A.; Covas, M.-I.; Rosa Ma Lamuela-Raventos, R.M.; Salas-Salvadó, J.; Fiol, M.; et al. The effects of the mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial. *PLoS ONE* **2014**, *9*, e100084. [CrossRef] [PubMed]
41. Coimbra, S.R.; Lage, S.H.; Brandizzi, L.; Yoshida, V.; da Luz, P.L. The action of red wine and purple grape juice on vascular reactivity is independent of plasma lipids in hypercholesterolemic patients. *Brazilian J. Med. Biol. Res.* **2005**, *38*, 1339–1347. [CrossRef]
42. Park, E.; Edirisinghe, I.; Choy, Y.Y.; Waterhouse, A.; Burton-Freeman, B. Effects of grape seed extract beverage on blood pressure and metabolic indices in individuals with pre-hypertension: A randomised, double-blinded, two-arm, parallel, placebo-controlled trial. *Br. J. Nutr.* **2016**, *115*, 226–238. [CrossRef] [PubMed]
43. Dohadwala, M.M.; Holbrook, M.; Hamburg, N.M.; Shenouda, S.M.; Chung, W.B.; Titas, M.; Kluge, M.A.; Wang, N.; Palmisano, J.; Milbury, P.E.; et al. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *Am. J. Clin. Nutr.* **2011**, *93*, 934–940. [CrossRef] [PubMed]
44. Yahfoufi, N.; Alsadi, N.; Jambi, M.; Matar, C.; Yahfoufi, N.; Alsadi, N.; Jambi, M.; Matar, C. The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients* **2018**, *10*, 1618. [CrossRef] [PubMed]

45. Pignatelli, P.; Pastori, D.; Farcomeni, A.; Nocella, C.; Bartimoccia, S.; Vicario, T.; Bucci, T.; Carnevale, R.; Violi, F. Mediterranean diet reduces thromboxane A2 production in atrial fibrillation patients. *Clin. Nutr.* **2015**, *34*, 899–903. [CrossRef] [PubMed]
46. Bogani, P.; Galli, C.; Villa, M.; Visioli, F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis* **2007**, *190*, 181–186. [CrossRef] [PubMed]
47. Carnevale, R.; Lofredo, L.; Pignatelli, P.; Nocella, C.; Bartimoccia, S.; Di Santo, S.; Martino, F.; Catasca, E.; Perri, L.; Violi, F. Dark chocolate inhibits platelet isoprostanes via NOX2 down-regulation in smokers. *J. Thromb. Haemost.* **2012**, *10*, 125–132. [CrossRef] [PubMed]
48. Wan, Y.; Vinson, J.A.; Etherton, T.D.; Proch, J.; Lazarus, S.A.; Kris-Etherton, P.M. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr.* **2001**, *74*, 596–602. [CrossRef] [PubMed]
49. García-Flores, L.A.; Medina, S.; Gómez, C.; Wheelock, C.E.; Cejuela, R.; Martínez-Sanz, J.M.; Oger, C.; Galano, J.-M.; Durand, T.; Hernández-Sáez, Á.; et al. Aronia-citrus juice (polyphenol-rich juice) intake and elite triathlon training: A lipidomic approach using representative oxylipins in urine. *Food Funct.* **2018**, *9*, 463–475. [CrossRef] [PubMed]

Suplemento

Tabla 3. Recomendaciones para el seguimiento de una dieta baja en antioxidantes.

Restringidos	Moderados	Permitidos
ALIMENTOS Y ADITIVOS		
Cereales integrales y maíz	Hortalizas: ajo, cebolla, nabo, berenjena con piel, alcachofas, lechuga iceberg	Cereales refinados (arroz blanco, pasta, etc)
Queso curado	Soja y derivados (por ejemplo, el tofu)	Carne
Vegetales de hoja verde (espinacas, acelgas, berros, etc)	Habas	Pescado
Otras hortalizas: zanahorias, tomate, brócoli, col, pimiento, puerro, calabaza, espárragos, judías verdes, boniato	Frutas: piña, aguacate, limón, kiwi, banana	Productos lácteos desnatados
Frutas: naranjas, mandarinas, pomelo, uvas, frutos rojos (fresas, arándanos, moras, frambuesas, etc), granadas, ciruelas, papaya, melón, persimón, melocotón	Aceite de oliva	Clara de huevo
Aceitunas		Hortalizas: berenjena sin piel, apio, calabacín, coliflor, rábano, patata, pepino
Guisantes		Setas
Frutos secos (nueces, almendras, etc)		Frutas: manzana sin piel, peras sin piel, higos, piña el almíbar
Hierbas aromáticas (perejil, cilantro, orégano, etc)		Legumbres: judías blancas, garbanzos, lentejas
Especias (cúrcuma, pimentón, etc)		
Mostaza		
Algas		
Cacao		
BEBIDAS		
Té e infusiones	Café (máximo uno al día)	Agua
Cerveza	Bebidas destiladas	Gaseosa
Vino, cava o sidra		Refrescos
Bebidas de cacao		Caldos de pollo o pescado (sin vegetales)
Zumos de fruta		
Bebidas vegetales (bebida de avena, de almendras, etc)		

El consumo de hortalizas y frutas se restringió a 2 raciones al día (~160 g/día), muy por debajo de las recomendaciones dietéticas que indican un consumo de 5 raciones al día de hortalizas y frutas combinadas (400 g/ día).

4.2. Publicación 3: El efecto agudo de una dosis única de *sofrito* sobre los biomarcadores de inflamación en hombres sanos.

Artículo titulado "Acute Effect of a Single Dose of Tomato *Sofrito* on Plasmatic Inflammatory Biomarkers in Healthy Men". Publicado en la revista científica "*Nutrients*" (Índice de impacto: 4,171 (2018); Q1).

El *sofrito* es una salsa típica de la dieta mediterránea que contiene tomate como ingrediente principal. Se obtiene a través del procesamiento térmico junto con otros componentes: aceite de oliva y cebolla (opcionalmente ajo o puerro). La suma de estos factores (procesamiento térmico, matriz lipídica y sinergias) sugiere que los compuestos bioactivos liberados desde el *sofrito* son más biodisponibles y consecuentemente tienen efectos beneficiosos en la salud. Por lo tanto, en este trabajo nos planteamos que el consumo de *sofrito* podría prevenir las ENT evitando la inflamación sistémica de bajo grado en personas sanas. En el estudio se incluyeron 22 hombre adultos que consumieron una única dosis de *sofrito* de 240 g/ 70 kg después de tres días sin ingerir tomate ni derivados y una dieta baja en alimentos ricos en antioxidantes el día previo y durante el estudio. A las 24 horas de la intervención, se observaron diferencias significativas en la concentración de todos los carotenoides analizados y de algunos marcadores pro-inflamatorios. A pesar de que la TPE fue significativa a las 5 horas de la ingesta, el aumento máximo se encontró en la orina recogida entre las 12 y las 24 horas después de la intervención. En cuanto a los parámetros de inflamación analizados, la CRP y el TNF-α disminuyeron después de la intervención (p = 0,010 y 0,011, respectivamente), sin embargo, no se encontraron diferencias significativas en las citoquinas pro-inflamatorioas (IL-6 e IL-1β). La concentración plasmática de TNF-α se correlacionó inversamente con la TPE y con la concentración de β-caroteno en plasma (r = 0,42, p = 0,048 y r = 0,41, p = 0,050, respectivamente). Estos resultados indican que los efectos beneficiosos en la salud de los productos del tomate no son atribuidos solamente al licopeno, sino también a otros fitoquímicos como los polifenoles y el β-caroteno.

nutrients

MDPI

Article

Acute Effect of a Single Dose of Tomato *Sofrito* on Plasmatic Inflammatory Biomarkers in Healthy Men

Sara Hurtado-Barroso [1,2,3], Miriam Martínez-Huélamo [1,3], Jose Fernando Rinaldi de Alvarenga [1,3], Paola Quifer-Rada [4], Anna Vallverdú-Queralt [1,2,3], Silvia Pérez-Fernández [5,6] and Rosa M. Lamuela-Raventós [1,2,3,*]

1 Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain; sara.hurtado_17@ub.edu (S.H.-B.); mmartinezh8@gmail.com (M.M.-H.); zehfernando@gmail.com (J.F.R.d.A.); avallverdu@ub.edu (A.V.-Q.)
2 CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, 28029 Madrid, Spain
3 INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08921 Santa Coloma de Gramenet, Spain
4 Department of Endocrinology & Nutrition, CIBER of Diabetes and Associated Metabolic Diseases, Biomedical Research Institute Sant Pau, Hospital de la Santa Creu i Sant Pau, 08041 Barcelona, Spain; pquifer@santpau.cat
5 Cardiovascular Epidemiology and Genetics Group, REGICOR, IMIM (Instituto Hospital del Mar de Investigaciones Biomédicas), 08003 Barcelona, Spain; sperez@imim.es
6 Center for the Biomedical Research Network in Cardiovascular Diseases (CIBERCV), Institute of Health Carlos III, 28029 Madrid, Spain
* Correspondence: lamuela@ub.edu; Tel.: +34-934-034-843

Received: 8 March 2019; Accepted: 11 April 2019; Published: 15 April 2019

check for updates

Abstract: *Sofrito* is a Mediterranean tomato-based sauce that typically also contains olive oil, onion, and garlic. The preparation of *sofrito* modifies the bioactive compounds (carotenoids and polyphenols) in the ingredients to more bioavailable forms, promoting *cis*-lycopene formation and polyphenol bioaccessibility. To evaluate the health benefits of this cooking technique, the effect of consuming an acute dose of *sofrito* on the inflammatory status was studied. In a clinical trial, 22 healthy male subjects consumed a single dose of *sofrito* (240 g/70 kg) after three days without ingesting any tomato products and following a low-antioxidant diet the day before the intervention. Plasma carotenoids and total polyphenol excretion (TPE) were evaluated, as well as the inflammatory biomarkers C-reactive protein (CRP), interleukin-6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α). After the *sofrito* intake, a significant decrease in CRP ($p = 0.010$) and TNF-α ($p = 0.011$) was observed, but only TNF-α was inversely correlated with an increase in TPE and plasma β-carotene (not the major carotenoid, lycopene). The positive health effects of this tomato-based product may be attributed not only to lycopene, but to the bioactive compounds of all the ingredients.

Keywords: carotenoids; polyphenols; TNF-α; CRP; lycopene; β-carotene; bioavailability; Mediterranean; onion; extra virgin olive oil

1. Introduction

Sofrito is a sauce commonly used to prepare dishes in Mediterranean cuisine. It is based on tomato but also contains other ingredients, typically olive oil, onion, and garlic. The regular consumption of this sauce is included in a validated 14-item questionnaire to evaluate adherence to the Mediterranean diet [1,2].

Numerous studies have provided evidence for the protective role of tomato-based products and their bioactive compounds against the development of cardiovascular diseases and cancer [3,4], which is partly attributed to positive effects on inflammatory biomarkers [5–7].

The polyphenol and carotenoid profile of *sofrito* varies according to its composition [8], but as tomato is the principle ingredient, the major carotenoids are lycopene and β-carotene [8]. Carotenoids in food are mainly all-*trans* isomers, whereas *cis*-isomers predominate in the human organism [9]. Factors such as cooking practices and the food matrix promote the isomerization of carotenoids and their bioavailability [9–11], the latter being increased by the presence of lipids [12–14]. Our research group recently reported an enhanced formation of *cis*-lycopene in *sofrito* associated with the concentration of onion and the cooking time [15]. The total polyphenol content of tomatoes could be increased by processing [16], and the bioaccessibility of tomato polyphenols is enhanced by processing and oil addition [17–19]. Thus, due to its phytochemical content, *sofrito* seems to be a health-promoting component of the Mediterranean diet.

Although the impact of tomato products on health has been mainly associated with lycopene [4,20,21], the other bioactive compounds present in *sofrito* could also be implicated [22,23]. Previous studies have reported inhibitory effects of dietary phytochemicals on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation in macrophages, resulting in a decrease in pro-inflammatory cytokines and chemokines like interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF-α) [24,25]. As these molecules stimulate the production of C-reactive protein (CRP) in the liver, which activates NF-κB [26], their reduction could diminish the CRP level. In the current work, it was hypothesized that phytochemical compounds present in *sofrito* could improve the baseline inflammation level. Thus, the aim of this study was to evaluate the effect of a single dose of 240 g/70 kg of *sofrito* on the regulation of inflammatory biomarkers in healthy humans and to identify the biomarkers responsible for these changes.

2. Materials and Methods

2.1. Sofrito Samples

Sofrito samples were supplied by Gallina Blanca (GB Foods, Spain) and consisted of a mix of tomato (50%), onion (37%), extra virgin olive oil (12%), and salt. Every sample was packed in a glass jar that contained 350 g of the *sofrito*. The nutritional and phytochemical composition of *sofrito* are provided in Tables S1 and S2.

2.2. Participants

The study was carried out with twenty-two healthy male volunteers aged between 18 and 32. All participants provided written informed consent in advance. Only men were enrolled in the trial to avoid effects related to hormonal fluctuations during the menstrual cycle [27–29]. Exclusion criteria were chronic illness or homeostatic disorder, history of cardiovascular diseases, hypertension or dyslipidemia, toxic habits (such as use of tobacco, alcohol, and drugs), and tomato or onion allergy or intolerance.

2.3. Intervention

In this open, uncontrolled and acute nutritional study, all participants ingested a single portion of *sofrito* (240 g/70 kg body weight) in a state of fasting. Before the intervention, volunteers avoided eating tomatoes and their by-products for three days. One day before the intervention, they also followed a low antioxidant diet, which was continued until the last blood sample was drawn (24 hours after consumption of *sofrito*). The details of the diet are presented in Supplementary Material Table S3.

The study was run in the Department of Nutrition, Food Sciences and Gastronomy of the Food and Nutrition Torribera Campus, University of Barcelona (Spain), according to the principles of the Declaration of Helsinki. The protocol was approved by the Ethics Committee of Clinical

Investigation of the University of Barcelona (Barcelona, Spain). The clinical trial was registered and given the International Standard Randomized Controlled Trial Number (http://www.isrctn.com/) of ISRCTN17867378.

2.4. Dietary and Physical Activity Assessments

In order to complete the dietary register, subjects were asked to fill out a three-day food recall on the day of the intervention. These were analyzed using PCN Pro software (Programa de Càlcul Nutricional Professional, Santa Coloma de Gramenet, Barcelona). Physical activity was measured by the validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire [30].

2.5. Extraction of Biological Samples

Fasting blood and urine samples were drawn before *sofrito* consumption (baseline extraction) and up to 24 hours afterwards (Figure 1). Blood samples were collected via venipuncture in the arm through EDTA tubes, and plasma was separated after centrifugation at 1902 g for 15 min at 4 °C. Plasma and urine were aliquoted and stored at −80 °C until the day of analysis.

Figure 1. Timeline of sample collection before and after intake of *sofrito*. On the left, baseline extraction of blood and urine. On the right, sample drawn after *sofrito* consumption: collection of blood at 24 h and cumulative urine at 0–3, 3–5, 5–12, and 12–24 h.

2.6. Clinical and Biochemical Evaluations

The diastolic and systolic blood pressure (DBP and SBP, respectively) and heart rate (HR) were measured in triplicate by a blood pressure monitor before and after the intake of a single dose of *sofrito*.

Biochemistry parameters in plasma were evaluated in an external laboratory (mdb.lab Durán Bellido, Barcelona). High density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol and triglycerides were analyzed by an enzymatic method. Urea and uric acid were analyzed by enzymatic and enzymatic/chromogen methods, respectively. Creatinine was determined by reaction kinetics of the Jaffe method (as modified by Larsen). Total proteins and albumin were measured by the endpoint biuret reaction and bromocresol green methods, respectively.

2.7. Analysis of Total Polyphenol Excretion in Urine

After a solid phase extraction (SPE) using a 96-well plate cartridge (Oasis MAX), total polyphenol excretion (TPE) analysis was performed in 2 mL of diluted (1:1) and acidified urine samples by the Folin–Ciocalteu method [31]. The spectrophotometry analysis was carried out using a Thermo Scientific Multiskan®Spectrum (Thermo Fisher Scientific, Vantaa, Finland, ref. 15019000) at a wavelength of 765 nm. The results are expressed as mg of gallic acid equivalent/L of urine (GAE/L). A cumulative urinary excretion curve for total polyphenols was calculated from 0 to 24 h.

2.8. Quantitative Analysis of Carotenoids in Plasma

Carotenoids were extracted by liquid–liquid extraction from plasma samples collected at 0 h and 24 h [32]. Chromatographic analysis of carotenoids was performed by HPLC-UV-DAD, using an HP 1100 HPLC system (Hewlett-105 Packard, Waldbronn, DE) containing a quaternary pump coupled to a DAD G1315B. The separation was carried out with Milli-Q water, methanol (MeOH) and methyl-tert-butyl ether (MTBE) (Panreac Quimica S.A., Barcelona, Spain), according to a procedure

previously validated in our group [32]. A Waters RP column YMC Carotenoid S-5 µm (250 mm × 4.6 mm) and a precolumn YMC Guard Cartridge Carotenoid S-5 µm (20 mm × 4.0 mαm) were used.

Zeaxanthin (Extrasynthese, Genay, France), lutein, cryptoxanthin, α-carotene, β-carotene 9- and 13-*cis*-β-carotene (Sigma-Aldrich, St. Louis, MO, USA), lycopene (Fluka, Bucks, Switzerland), and 5-*cis*-lycopene (CaroteNature GmbH, Münsingen, Switzerland) were used as standards. These were pooled and prepared in synthetic human plasma (Sigma-Aldrich, St. Louis, MO, USA).

The sensitivity of each analyte was 0.703 µmol/L (lutein), 0.352 µmol/L (zeaxanthin), 0.362 µmol/L (cryptoxanthin), 0.480 µmol/L (*trans*-β-apo-8′-carotenal), 0.745 µmol/L (13-*cis*-β-carotene), 0.373 µmol/L (9-*cis*-β-carotene and *trans*-β-carotene), and 0.186 µmol/L (*trans* and *cis*-lycopenes) [32].

2.9. Determination of Plasmatic Inflammatory Biomarkers

Plasmatic C-reactive protein (CRP) was measured by an immunoturbidimetric method from external services (mdb.lab Durán Bellido) at baseline and 24 h after consumption of *sofrito*.

The concentrations of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) were assayed in plasma using the Immunoassay Kit (R&D Systems Inc., Minneapolis, USA, refs. HS600B, HSTA00E, HSLB00D). The sensitivity of each analyte was 0.110 pg/mL, 0.049 pg/mL, and 0.063 pg/mL, respectively. Plasma samples were assayed in duplicate.

2.10. Statistical Analysis

Normality of distribution was assessed by a Shapiro–Wilk test. In order to compare baseline and post-intervention values, a linear regression analysis was used for the normal variables (SDP and DBP, HR, HDL, uric acid, albumin and TNF-α). Non-normal variables were assessed by a non-parametric Wilcoxon signed-rank test. The log-transformed TPE variable was analyzed by a Bonferroni post hoc test to compare the excretion of total polyphenols at the different time points. Statistical analyses were performed with SPSS Version 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

The correlation analysis was carried out using a correlation matrix that considers repeated measures [33] with software R, version 3.4.2. The Pearson coefficient (r) was calculated. Significant correlations ($p < 0.05$) are shown in the results.

3. Results

3.1. Characteristics of Participants

Table 1 provides anthropometric measurements of the participants, as well as the degree of adherence to the Mediterranean diet, level of physical activity and nutrient intake at the study baseline.

Table 1. Anthropometric parameters of subjects, physical activity in leisure time according to the Minnesota questionnaire, and the mean nutrient intake from a 3-day food recall.

Characteristics	
Age (years)	23.64 ± 0.86
BMI (kg/m^2)	24.91 ± 0.79
WHR	0.84 ± 0.01
MedDiet adherence (score) [1]	8.5 ± 0.4
Physical activity in leisure time (METs/d)	746 ± 71
Energy (kcal/day)	2393 ± 129
Total fats (g/day)	106 ± 9
Saturated fats (g/day)	32 ± 3
Monounsaturated (g/day)	47 ± 4
Polyunsaturated (g/day)	19 ± 2
Cholesterol (mg/day)	301 ± 30
Carbohydrate (g/day)	256 ± 15
Protein (g/day)	100 ± 6
Fiber (g/day)	30 ± 2

Data are mean ± standard error of the mean (SEM). BMI: body mass index, WHR: waist–hip ratio, MedDiet: Mediterranean diet, METs/d: metabolic equivalent of task per day. [1] The score categories of adherence to the MedDiet are high (≥10), moderate (6–9), and low (≤5).

3.2. Clinical Measures

Clinical measures are shown in Table 2. Systolic blood pressure did not change after consumption of *sofrito* compared to baseline, whereas diastolic blood pressure decreased (p = 0.006). The total cholesterol was lower after the intervention, mainly due to a reduction of HDL (p = 0.005 and 0.015), although significant changes in LDL and triglycerides were not observed. Urea, total proteins, and albumin decreased significantly (p = 0.023, 0.011, and 0.015).

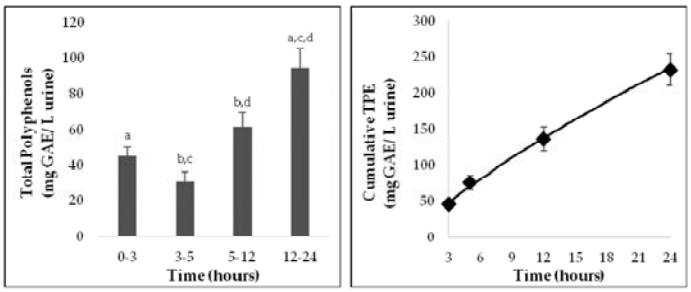
Table 2. Clinical parameters of all participants in the study.

Measures	Baseline	ACS	*P*
DBP (mmHg) #	76 ± 2	71 ± 2	0.006 *
SBP (mmHg) #	123 ± 2	124 ± 2	0.550
HR (bpm) #	66 ± 21	62 ± 2	0.061
Total cholesterol (mmol/L) #	3.83 ± 0.12	3.71 ± 0.12	0.005 *
HDL (mmol/L)	1.37 ± 0.06	1.32 ± 0.06	0.015 *
LDL (mmol/L) #	2.04 ± 0.11	1.98 ± 0.11	0.130
Triglycerides (mmol/L) #	0.94 ± 0.08	0.89 ± 0.06	0.531
Urea (mmol/L)	5.56 ± 0.30	5.08 ± 0.28	0.023 *
Creatinine (µmol/L)	76 ± 2	74 ± 1	0.157
Uric acid (µmol/L) #	319 ± 11	319 ± 10	1.000
Total proteins (g/L) #	73 ± 0.6	71± 0.6	0.011 *
Albumin (g/L) #	47 ± 0.5	46 ± 0.5	0.015 *

Data are mean ± SEM. ACS: after consumption of *sofrito* (at 24 h). DBP: diastolic blood pressure. SBP: systolic blood pressure. HR: heart rate. * p-value < 0.05. # Data analyzed by linear regression. The remaining data were analyzed by the Wilcoxon test.

3.3. Phenolic Excretion in Urine

As a biomarker of polyphenol intake from the *sofrito*, the urinary excretion of total polyphenols from baseline until 24 hours after consumption was analyzed (Figure 2A). The TPE increased from baseline to 5 h after intake, and even more so from 12 to 24 h (p = 0.001), as illustrated by the cumulative curve in Figure 2B.



(**A**) (**B**)

Figure 2. (**A**) Concentration of total polyphenols in the cumulative urine after consumption of a single serving of *sofrito*. (**B**) Cumulative urinary excretion curve of total polyphenols. Urinary total polyphenol excretion is shown: 0–3 (3 h), 0–5 (5 h), 0–12 (12 h), and 0–24 (24 h). The same letters (a–d) refer to statistically significant differences as follows. a: p = 0.001; b: p = 0.003; c: p <0.001, and d: p = 0.048. Data are expressed as mean + SEM.

3.4. Quantification of Carotenoids in Plasma

Levels of carotenoids in plasma were evaluated before and after *sofrito* consumption (Table 3). All target carotenoids increased after the intervention, the predominant ones being 5-*cis*-lycopene, β-carotene and *trans*-lycopene. Total lycopene increased from 43% to 59% of total carotenoids. Xanthophylls, lutein, and cryptoxanthin increased significantly ($p = 0.001$ and 0.012) and zeaxanthin was detected in plasma after *sofrito* consumption but not at baseline. In all participants, a high concentration of carotenes was found at 24 h of *sofrito* intake. Notably, the changes in *trans*-β-carotene were highly significant ($p < 0.001$), and the 9-*cis*-isoform and 13-*cis*-β-carotene were observed after the intervention but not at baseline. Other carotenes that increased were *trans*-lycopene ($p < 0.001$) and 13- and 5-*cis*-lycopene ($p = 0.005$ and <0.001). The concentration of 9-*cis*-lycopene was detected after the consumption of *sofrito* but not at baseline. In addition, the total *cis*-lycopene was significantly higher ($p < 0.001$), as was the total lycopene ($p < 0.001$). In summary, the increase in carotenoid concentration was statistically significant after the consumption of *sofrito*, total carotenoids being three-fold higher compared to the baseline level ($p < 0.001$).

Table 3. Concentration of carotenoids in plasma at baseline and 24 h after consumption of *sofrito*.

Analyte	Baseline	ACS	*p*
Lutein (µmol/L)	1.12 ± 0.02	1.69 ± 0.27	0.001 *
Zeaxanthin (µmol/L)	n.d.	0.65 ± 0.19	-
Cryptoxanthin (µmol/L)	1.08 ± 0.12	1.34 ± 0.15	0.012 *
trans-β-carotene (µmol/L)	3.12 ± 0.58	6.64 ± 0.88	<0.001 *
13-*cis*-β-carotene (µmol/L)	n.d.	0.75 ± 0.15	-
9-*cis*-β-carotene (µmol/L)	n.d.	0.95 ± 0.22	-
trans-lycopene (µmol/L)	2.15 ± 0.30	6.33 ± 1.53	<0.001 *
5-*cis*-lycopene (µmol/L)	1.87 ± 0.28	7.93 ± 2.73	<0.001 *
13-*cis*-lycopene (µmol/L)	0.21 ± 0.11	2.08 ± 0.78	0.005 *
9-*cis*-lycopene (µmol/L)	n.d.	0.90 ± 0.58	-
Total *cis*-β-carotene (µmol/L)	n.d.	1.92 ± 0.33	-
Total β-carotene	3.45 ± 0.67	8.56 ± 1.13	<0.001 *
Total cis-lycopene isomers (µmol/L)	2.09 ± 0.32	10.91 ± 4.00	<0.001 *
Total lycopene (µmol/L)	4.24 ± 0.59	17.23 ± 5.50	<0.001 *
Total carotenoids (µmol/L)	9.97 ± 0.96	29.25 ± 6.45	<0.001 *

Data are mean ± SEM. ACS: after consumption of sofrito (at 24 h). * Significant differences when *p*-value < 0.05. Data were analyzed by the Wilcoxon test. n.d.: not detectable.

3.5. Inflammatory Biomarkers

After the consumption of *sofrito*, all the inflammatory biomarkers studied decreased compared to the baseline. However, the changes were only significant for CRP ($p = 0.010$) and TNF-α ($p = 0.011$) (Figure 3A and 3C), but not IL-6 (Figure 3B). In no case was IL-1β detected (data not shown).

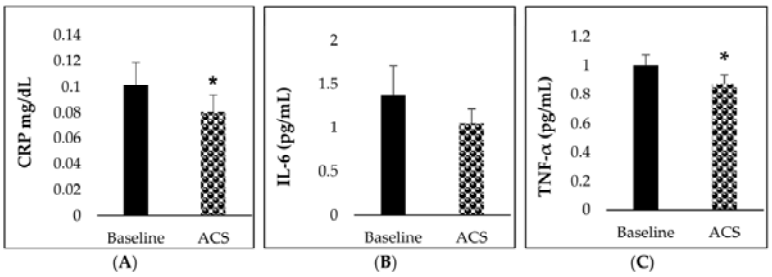


Figure 3. Concentration of inflammatory biomarkers at baseline and after consumption of *sofrito*. (**A**) CRP, (**B**) IL-6 and (**C**) TNF-α. Data are mean ± SEM, * p-value < 0.05. ACS: After consumption of *sofrito* (at 24 h). CRP: C-reactive protein; IL: interleukin; TNF-α: tumor necrosis factor alpha.

3.6. Correlations between Inflammatory Biomarkers and Bioactive Compounds

In order to evaluate the role of TPE in the decrease of inflammatory biomarkers, a correlation study was performed. The reduction of TNF-α, but not IL-6 or CRP, was inversely correlated with the excretion of total polyphenols. Figure 4 shows the negative correlation between TNF-α and urinary TPE ($r = -0.42$, $p = 0.048$).

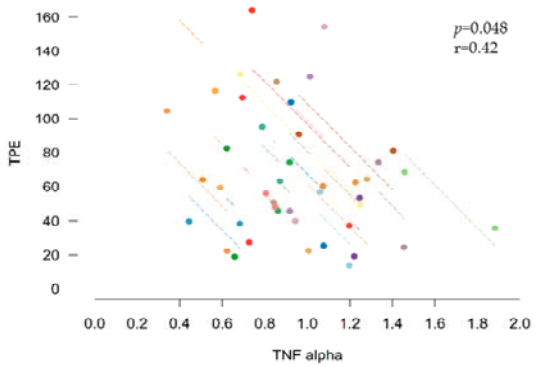


Figure 4. Correlation between tumor necrosis factor alpha (TNF-α) and total polyphenol excretion (TPE).

In addition, an inverse correlation was observed between the concentration of β-carotene in plasma and TNF-α, but not CRP and IL-6 ($r = -0.41$, $p = 0.05$) (Figure 5).

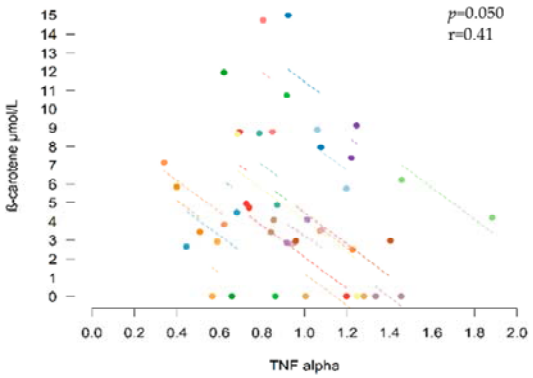


Figure 5. Correlation between TNF-α and β-carotene.

4. Discussion

In this clinical trial, healthy individuals showed higher carotenoid levels in plasma 24 h after the consumption of *sofrito*. Arranz et al. reported higher plasma concentrations of *cis*-isomers of lycopene but not of other *cis*-carotenoids after the ingestion of tomato sauce without onion and garlic [12]. In the current study, both *cis*- and *trans*-isoforms of β-carotene and lycopene increased after *sofrito* intake. Not detected at baseline, 13 and 9-*cis*-isomer of β-carotene and 9-*cis*-lycopene were quantified after the intervention.

TPE levels had increased significantly at 12-24 h after *sofrito* intake compared to the baseline. In a previous study by our group, also in healthy volunteers, changes in polyphenol excretion were noted earlier, at 6-12 h after the consumption of a tomato-based product without onion [19]. Thus, the presence of polyphenols from onion in the *sofrito* could have a delaying effect on the urinary TPE [34].

It was initially hypothesized that phytochemicals in *sofrito* could affect the regulation of systemic pro-inflammatory markers through the inhibition of NF-κB, triggering a decrease in cytokines, chemokines and CRP [24–26]. After the *sofrito* intake, a significant reduction of CRP and TNF-α biomarkers (p = 0.010 and 0.011) in plasma was observed, but not of IL-6. The decrease in TNF-α was inversely correlated with TPE and the level of β-carotene.

In a previous study, both CRP and TNF-α decreased significantly in healthy volunteers after the consumption of tomato juice twice daily for 2 weeks [5]. In obese and overweight participants who consumed tomato juice for 20 days, the concentration of TNF-α decreased, and in obese subjects the level of IL-6 was significantly reduced, but no changes in CRP were observed in either case [35]. In contrast, in a parallel study, a significant decrease in CRP was observed in women (but not men) suffering from heart failure who consumed tomato juice for a month [36]. Gender-related differences in CRP have been previously described [37,38]. In a crossover trial, tomato paste attenuated the increase in IL-6 after a high-fat meal in healthy volunteers [6]. However, Valderas-Martínez et al. reported that healthy subjects showed a significant reduction in IL-6 only after consuming tomato sauce containing olive oil (a single dose), but not raw tomato or tomato sauce without olive oil [7]. A study in schoolchildren found that plasma β-carotene levels were inversely related to IL-6 and CRP, particularly the former, but not to TNF-α [39]. In patients with cardiovascular diseases, β-carotene was significantly correlated with IL-6 [40] and CRP [22]. In contrast, other authors did not find changes in these inflammatory biomarkers after interventions with tomato products or a high-tomato diet [41–46].

In summary, an acute effect on inflammatory biomarkers was observed at 24 h after the administration of a single dose of 240 g/70 kg of *sofrito*. Improvements in inflammatory biomarkers

after the ingestion of tomato products have been attributed mainly to lycopene. However, the results reported here indicate that when the product contains other ingredients, such as onion and virgin olive oil, different bioactive compounds such as polyphenols and carotenoids such as β-carotene may be responsible for the anti-inflammatory effects.

A strong point of this work is that relatively few acute studies have been carried out on the impact of diet on inflammatory biomarkers in healthy humans. The significant reduction in TNF-α and CRP levels after the ingestion of a single dose of tomato-based *sofrito*, rich in bioactive compounds, suggests this Mediterranean sauce may contribute positively to the regulation of the inflammatory status, even in individuals with optimal health.

The main limitation of the study is the lack of controls. Nevertheless, the analyses were carried out 24 h after the intervention in the same conditions to avoid changes due to circadian rhythms, and the volunteers continued a low antioxidant diet after the consumption of *sofrito*.

5. Conclusions

The addition of *sofrito* to dishes and cuisine could contribute to the maintenance of health. An improvement in inflammatory biomarkers, particularly CRP and TNF-α, was observed after a single dose of *sofrito*, even though the subjects were healthy. Beneficial effects of *sofrito* intake could be partly attributed to the presence of bioactive compounds (polyphenols and carotenoids) from the ingredients. Although the health-protecting role of lycopene from tomato has been extensively studied, other carotenoids such as β-carotene (also found in large amounts in tomato by-products) seem to modulate the regulation of the inflammatory status.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/4/851/s1. Table S1: Nutritional value of *sofrito*; Table S2: Comparison of phytochemical composition of tomato and *sofrito* samples; Table S3: Recommendations to follow a low antioxidant diet.

Author Contributions: S.H.-B., M.M.-H., J.F.R.d.A., P.Q.-R., A.V.-Q., and R.M.L.-R. designed the study. S.H.B., M.M.H., J.F.R.d.A., and A.V.Q. contributed in the methodology of analysis. S.H.B., P.Q.-R., and S.P.-F. carried out the data analysis. S.H.B. wrote the manuscript. All participants reviewed and approved the paper. R.M.L.-R. was the main responsible of the project and final content.

Funding: This work was supported in part by AGL2013-49083-C3-1-R AGL2016-75329-R) and the Instituto de Salud Carlos III, ISCIII (CIBEROBN) from the Ministerio de Ciencia, Innovación y Universidades) (AEI/FEDER, UE) and Generalitat de Catalunya (GC) 2017 SGR196.

Acknowledgments: We thank all the volunteers of the study for their participation and GB Foods for providing the *sofrito*. S.H.B. is grateful for the predoctoral scholarship FPU (FPU14/01715) from the Ministerio de Educación. J.F.R.d.A. is thankful for the Science without Borders program for the predoctoral scholarship from Conshelo Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—Brazil (233576/2014-2). P.Q.-R. is grateful for the Sara Borrell postdoctoral program from the Instituto de Salud Carlos III (ISCIII). A.V.Q. is thankful to the Spanish Ministry of Science, Innovation and Universities for the Ramon y Cajal contract.

Conflicts of Interest: Dra. R.M.L.-R. reports receiving lecture fees from Cerveceros de España, and receiving lecture fees and travel support from Adventia. Nevertheless, these foundations were not involved in the study design, the collection, analysis and interpretation of data, the writing of the manuscript, or the decision to submit the manuscript for publication. The other authors declare no conflict of interest.

References

1. Martínez-González, M.A.; García-Arellano, A.; Toledo, E.; Salas-Salvadó, J.; Buil-Cosiales, P.; Corella, D.; Covas, M.I.; Schröder, H.; Arós, F.; Gómez-Gracia, E.; et al. PREDIMED Study Investigators, for the P. S. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: The PREDIMED trial. *PLoS ONE* **2012**, *7*, e43134. [CrossRef] [PubMed]
2. Schröder, H.; Fitó, M.; Estruch, R.; Martínez-González, M.A.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M.; et al. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older Spanish Men and Women. *J. Nutr.* **2011**, *141*, 1140–1145. [CrossRef]
3. Willcox, J.K.; Catignani, G.L.; Lazarus, S. Tomatoes and Cardiovascular Health. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 1–18. [CrossRef] [PubMed]

41. Upritchard, J.E.; Sutherland, W.H.; Mann, J.I. Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* **2000**, *23*, 733–738. [CrossRef]
42. Blum, A.; Monir, M.; Khazim, K.; Peleg, A.; Blum, N. Tomato-rich (Mediterranean) diet does not modify inflammatory markers. *Clin. Investig. Med.* **2007**, *30*, E70–E74. [CrossRef]
43. Thies, F.; Masson, L.F.; Rudd, A.; Vaughan, N.; Tsang, C.; Brittenden, J.; Simpson, W.G.; Duthie, S.; Horgan, G.W.; Duthie, G. Effect of a tomato-rich diet on markers of cardiovascular disease risk in moderately overweight, disease-free, middle-aged adults: A randomized controlled trial. *Am. J. Clin. Nutr.* **2012**, *95*, 1013–1022. [CrossRef]
44. Tsitsimpikou, C.; Tsarouhas, K.; Kioukia-Fougia, N.; Skondra, C.; Fragkiadaki, P.; Papalexis, P.; Stamatopoulos, P.; Kaplanis, I.; Hayes, A.W.; Tsatsakis, A.; et al. Dietary supplementation with tomato-juice in patients with metabolic syndrome: A suggestion to alleviate detrimental clinical factors. *Food Chem. Toxicol.* **2014**, *74*, 9–13. [CrossRef]
45. Gajendragadkar, P.R.; Hubsch, A.; Mäki-Petäjä, K.M.; Serg, M.; Wilkinson, I.B.; Cheriyan, J. Effects of Oral Lycopene Supplementation on Vascular Function in Patients with Cardiovascular Disease and Healthy Volunteers: A Randomised Controlled Trial. *PLoS ONE* **2014**, *9*, e99070. [CrossRef] [PubMed]
46. Colmán-Martínez, M.; Martínez-Huélamo, M.; Valderas-Martínez, P.; Arranz-Martínez, S.; Almanza-Aguilera, E.; Corella, D.; Estruch, R.; Lamuela-Raventós, R.M. trans-Lycopene from tomato juice attenuates inflammatory biomarkers in human plasma samples: An intervention trial. *Mol. Nutr. Food Res.* **2017**, *61*, 1600993. [CrossRef] [PubMed]

4.3. Publicación 4: El compuesto fenólico 4-hidroxibenzoico aumenta después de la intervención con una dieta orgánica.

Artículo titulado "Increase of 4-Hydroxybenzoic, a Bioactive Phenolic Compound, after an Organic Intervention Diet" Publicado en la revista científica "*Antioxidants*" (Índice de impacto: 4,520 (2018); Q1, D1).

El consumo de alimentos ecológicos ha aumentado en los últimos años debido, en gran parte, a que se piensa que son más nutritivos y saludables. Sin embargo, hay pocos estudios hasta la fecha que avalen esta premisa. A pesar de que se ha sugerido que los alimentos cultivados mediante métodos ecológicos tienen mayores concentraciones de fitoquímicos, los ensayos en humanos sobre los efectos de estos alimentos y sus compuestos bioactivos son muy escasos y no concluyentes. Con el objetivo de evaluar las diferencias nutricionales en el organismo después de consumir una dieta ecológica, se ha llevado a cabo la investigación resumida en la publicación presente. Diecinueve sujetos (10 mujeres y 9 hombres) sanos de entre 18 y 40 años fueron incluido en un estudio piloto cruzado, aleatorizado y controlado. Cada individuo realizó dos intervenciones de un mes de duración: una dieta saludable con alimentos ecológicos (OD) y una dieta similar con alimentos cultivados mediante métodos convencionales (CD). Al inicio y al final de cada intervención se recogieron las muestras biológicas de los participantes en la que se evaluaron los parámetros biológicos, la concentración de minerales y metales pesados y los niveles de algunos compuestos bioactivos (ácidos fenólicos y carotenos). Un aumento significativo en la excreción del metabolito 4-hidroxibenzoico (4-HBA) fue observado después de la dieta ecológica con respecto a la convencional (205 ± 123 nmol versus 70 ± 35 nmol, $p = 0,028$). No se encontraron diferencias significativas en el resto de los parámetros analizados. La dieta ecológica podría aportar una mayor concentración de algunos compuestos fenólicos. Sin embargo, más estudios cuidadosamente diseñados deberían ser llevados a cabo para corroborar esta hipótesis y elucidar los mecanismos implicados.

antioxidants

MDPI

Article

Increase of 4-Hydroxybenzoic, a Bioactive Phenolic Compound, after an Organic Intervention Diet

Sara Hurtado-Barroso [1,2,3], Paola Quifer-Rada [4], María Marhuenda-Muñoz [1,2,3], Jose Fernando Rinaldi de Alvarenga [1,3], Anna Tresserra-Rimbau [2,5] and Rosa M. Lamuela-Raventós [1,2,3,*]

1 Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain
2 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain
3 INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08028 Barcelona, Spain
4 Department of Endocrinology & Nutrition, CIBER of Diabetes and Associated Metabolic Diseases, Biomedical Research Institute Sant Pau, Hospital de la Santa Creu i Sant Pau, 08041 Barcelona, Spain
5 Unitat de Nutrició Humana, Hospital Universitari Sant Joan de Reus, Departament de Bioquímica i Biotecnologia, Institut d'Investigació Pere Virgili (IISPV), Universitat Rovira i Virgili, 43007 Reus, Spain
* Correspondence: lamuela@ub.edu; Tel.: +34-934-034-843

Received: 24 July 2019; Accepted: 22 August 2019; Published: 24 August 2019

check for updates

Abstract: Consumption of organic products is increasing yearly due to perceived health-promoting qualities. Several studies have shown higher amounts of phytochemicals such as polyphenols and carotenoids in foods produced by this type of agriculture than in conventional foods, but whether this increase has an impact on humans still needs to be assessed. A randomized, controlled and crossover study was carried out in nineteen healthy subjects aged 18–40 years, who all followed an organic and conventional healthy diet, both for a 4-week period. Analysis of biological samples revealed a significant increase on the excretion of 4-hydroxybenzoic acid (4-HBA), a phenolic metabolite with biological activity, after the organic intervention. However, no changes were observed in the other variables analyzed.

Keywords: healthy diet; phenolic acid; 4-HBA; crossover study; carotenes; microbiota metabolites; intervention; humans; metals

1. Introduction

Organic food consumption has been increasing yearly over the last decade due to growing public awareness of its environmental benefits and alleged healthy properties [1,2]. The general belief that organic produce is healthier due to a lower use of chemical agents, such as pesticides, fertilizers and antibiotics, [3] is supported by studies reporting lower concentrations of pesticide residues in individuals consuming organic food [4–8]. Differences in nutritional composition associated with the cropping system have also been found, but more studies are needed to draw conclusions [9]. Factors known to influence the nutritional composition of food include crop variety, geographical location, climatic conditions, soil type, season and state of maturity from harvest to storage. Organic food seems to have higher amounts of bioactive compounds such as polyphenols and carotenoids than conventionally produced food [10–16]. When exposed to a stressful environment, plants activate defense mechanisms. Accordingly, a lack of synthetic protectors (pesticides, chemical fertilizers, etc.) induces organic crops to produce phytochemicals. Phenolic acids represent one third of the phenol group in a diet, but also many of them are produced from dietary polyphenols through microbiota metabolism. Approximately 90% of polyphenols are not absorbed in the small intestine reaching the

colon, where they are transformed to other compounds such as the phenolic acids [17,18]. In addition, lower concentrations of cadmium have been observed in organic versus conventional cereals [19], as well as differences in the content of fatty acids and proteins [20–23]. Among foods of animal origin, total polyunsaturated fatty acid (PUFA) and n-3 PUFA concentrations are higher in organically rather than conventionally produced milk [21]. A similar profile has been observed in meat, although the evidence is weak [23].

The few studies to evaluate the effect of organic foods on human biochemical parameters and health have employed methodologies with some limitations and provide inconclusive results [9], so further intervention studies are needed to corroborate their possible beneficial effects. Consumers of organic produce are associated with having a higher quality diet, lower body mass index (BMI), greater physical activity [24,25] and a generally healthier and more holistic lifestyle [26,27]. Thus, the question is the following: Are the consumers of organic food healthier due to their lifestyle or also because their diet has a superior nutritional value?

The aim of this study was to evaluate the effect of an intervention with organic diet versus a conventional one on biological parameters, inorganic elements, bioactive compounds, and phenolic acids and carotenes in healthy subjects.

2. Materials and Methods

2.1. Study Subjects

Twenty-one healthy volunteers aged 18–40 years were included in the intervention, 19 of whom completed the study and two dropped out alleging personal reasons. Participants had previous interest in healthy diets and organic food, and they were recruited from the Food and Nutrition Torribera Campus of the University of Barcelona and surroundings. Exclusion criteria were history of cancer, cardiovascular diseases, hypertension and dyslipidemia, chronic illness or homeostatic disorder, as well as toxic habits such as tobacco and other drugs and an excessive alcohol intake.

After approval of the protocol by the Ethics Committee of Clinical Investigation of the University of Barcelona (Barcelona, Spain), the study was registered (ISRCTN29145931). Each participant signed an informed consent prior to the start, which was conducted according to the principles of the Declaration of Helsinki.

2.2. Study Design

An open, crossover, randomized and controlled study was carried out (Figure 1). Each volunteer consumed an organic diet (OD) and a conventional diet (CD), both for 4 weeks, and received dietary advice to support adherence. Organic products represented at least 80% of the OD and no organic foods were allowed in the CD. In both diets, subjects were encouraged to follow a healthy Mediterranean diet with a similar food pattern. Additionally, during the OD intervention participants were given weekly vouchers from Ecoveritas S.A., as well as products (oil, wine, snacks and canned vegetables) from other organic food companies to facilitate dietary compliance. At the end of each intervention, the absence of differential dietary patterns was checked. Interventions were separated by a washout period of two months. The study was run in the Department of Nutrition, Food Science and Gastronomy of the Food Science and Nutrition Torribera Campus of the University of Barcelona (Spain).

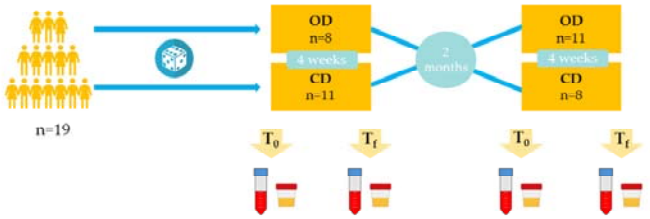


Figure 1. Study design. OD: Organic diet; CD: Conventional diet; T_0: Initial time point (before interventions); T_f: Final time point (after interventions).

2.3. Assessment of Diet and Physical Activity

Before the study, adherence to the Mediterranean diet and physical activity were measured through a 14-item questionnaire [28] and the validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire [29], respectively. Also, at baseline, participants were asked about the frequency of organic food and beverage intake. After each intervention, a 137-item semi-quantitative food frequency questionnaire was filled in with the help of the study staff to assess nutrient and food intake [30].

2.4. Anthropometric and Clinical Data Measurements

Body weight was measured using an electronic scale and height with a stadiometer. The BMI was calculated from body weight and height. Waist and hip circumferences were measured with a measuring tape accurate to 0.1 cm. The waist-hip ratio (WHR) was calculated from these parameters.

Diastolic and systolic blood pressure (DBP and SBP) and heart rate were measured in fasting conditions with an OMRON M6 monitor in triplicate at each visit.

2.5. Sample Collection

Fasting blood was collected before and after each intervention. Blood samples were collected from the arm via venipuncture using tubes containing ethylenediaminetetraacetic acid (EDTA). After centrifugation of blood samples at 1902× *g* for 15 min at 4 °C, plasma was obtained. In addition, 24 h urine was collected at each visit. Plasma and urine were stored at −80 °C.

2.6. Laboratory Evaluations

Biochemical analyses were performed by an external accredited laboratory (mdb.lad Durán Bellido) as follows. C-reactive protein (CRP) was assayed by an immunoturbidimetry method. The lipid parameters (high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol and triglycerides) were tested by an enzymatic method. Urea and uric acid were measured by enzymatic and enzymatic/chromogen methods, respectively, and creatinine by the Jaffe method (as modified by Larsen) [31]. The concentration of total proteins was quantified by a Biuret reaction to the final point and amount of albumin by a bromocresol green method.

2.7. Analysis of Inorganic Elements in Plasma

Plasma samples were digested with nitric acid (HNO_3) (Instra, J.T. Baker) in Teflon reactors. After incubation at 90 °C overnight, Milli-Q water was added to the reactors. An aliquot was transferred into assay tubes and stored at 4 °C for the chromatographic analyses. The inorganic compounds (Inorganic ventures, Christiansburg, VA, USA) used as standards were the following: Iron (Fe), arsenic (As), copper (Cu), cadmium (Cd), uranium (U), lead (Pb), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na). Fe, As, Cu, Cd, U, Pb and Zn were analyzed by ICP-MS (NexIon

350D. Perkin Elmer, Waltham, MA, USA) and Ca, Mg, K, P and Na, by ICP-OES (Optima8300. Perkin, Waltham, MA, USA). The analyses were performed in the facilities of the CCIT (Centres Científics i Tecnològics) of the University of Barcelona.

2.8. Extraction and Quantification of Phenolic Acids from Urine

Urinary phenolic compounds were extracted by solid phase extraction using a Waters Oasis HLB 96-well plate 30 µm (30 mg; Waters Oasis, Milford, MA, USA) [32]. Chromatographic analysis of phenolic compounds was performed by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), using an API 3000 triple-quadrupole mass spectrometer (Sciex, Framingham, MA, USA). The separation was carried out with Milli-Q water and acetonitrile (Panreac Quimica S.A., Barcelona, Spain) with 0.025% formic acid in both solvents (Scharlau Chemie S.A., Barcelona, Spain), according to a method validated by our group [32]. A Waters BEH C18 column 1.7 µm (50 mm × 2.1 mm) and an Acquity UPLC BEH C18 VanGuard pre-column 1.7 µm (2.1 mm × 2.0 mm) were used.

The pool of standards was prepared in synthetic urine and included 3-(4-hydroxyphenyl) propionic acid (3,4-HPPA), 4-hydroxybenzoic acid (4-HBA), 3,4-dihydroxyphenylacetic acid (3,4-DHPAA), 3-hydroxyphenylacetic acid (3-HPAA), dihydrocaffeic acid (DHCA), hippuric acid, homovanillic acid, caffeic acid (CA), m-coumaric acid (m-Cou), p-coumaric (p-Cou) and gallic acid (GA) (Sigma-Aldrich, St. Louis, MO, USA) and 4-hydroxyhippuric acid (4-HH) (Bachem Americas Inc, Torrance, CA, USA). Ethylgallate (Extrasynthese, Genay, France) was the internal standard.

2.9. Extraction and Quantification of Carotenoids from Plasma

Carotenoids were extracted from plasma samples by liquid–liquid extraction [33]. Chromatographic analysis of carotenoids was performed by high performance liquid chromatography with ultraviolet diode-array detector (HPLC-UV-DAD), using an HP 1100 HPLC system (Hewlett-105 Packard, Waldbronn, Germany) containing a quaternary pump, coupled to a DAD G1315B. The separation was carried out with Milli-Q water, methanol and methyl-tert-butyl ether (Panreac Quimica S.A., Barcelona, Spain), according to a procedure previously validated by our group [33]. A Waters reversed-phase column YMC Carotenoid S-5 µm (250 mm × 4.6 mm) and a precolumn YMC Guard Cartridge Carotenoid S-5 µm (20 mm × 4.0 mm) were used.

The standards used were α-carotene, β-carotene, and all-E-lycopene (Sigma-Aldrich, St. Louis, MO, USA) and 5-*Z*-licopene (CaroteNature GmbH, Ostermundigen, Switzerland). These were pooled and prepared in synthetic human plasma (Sigma-Aldrich, St. Louis, MO, USA).

2.10. Statistical Analysis

Normality of distribution was assessed by a Shapiro-Wilk test. A non-parametric Wilcoxon signed-rank test was used for all statistical analysis due to the small sample size and the non-normality distribution. First, baseline measures were compared to corroborate similar pre-intervention conditions. As no significant differences between interventions at baseline were observed, the final analysis was performed with post-intervention measures (n = 19). Baseline values of variables were calculated from the mean of 38 observations (2 measurements for each subject). Differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed using SPSS Version 23.0 for Windows (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Participant Characteristics

Table 1 shows the baseline characteristics of participants. Nineteen healthy subjects (9 males and 10 females) completed the study. Approximately three out of every four individuals were occasional consumers of organic products (foods and beverages). The mean age was 30 years and subjects were

physically active. The baseline adherence to the Mediterranean diet was high in 7 individuals (≥ 10 points); moderate in 11 (6–9 points) and low in 1 (≤ 5 points).

Table 1. Baseline characteristics of the participants (n = 38).

Characteristics	
Males, *n* (%)	9 (47)
Occasional intake of organic products, *n* (%)	14 (74)
Age (years)	30 ± 1
Physical activity in leisure time (METS-min/week)	3814 ± 489
14-item MedDiet score (points)	9 ± 0.3
Weight (kg)	63 ± 2
BMI (kg/m^2)	22.1 ± 0.4
Waist (cm)	76 ± 1
WHR	0.79 ± 0.01
DBP (mmHg)	75 ± 2
SBP (mmHg)	116 ± 2
Heart rate (bpm)	68 ± 2
CRP (mg/dL)	0.14 ± 0.03
HDL (mg/dL)	62 ± 3
LDL (mg/dL)	93 ± 5
Total cholesterol (mg/dL)	169 ± 6
Triglycerides (mg/dL)	69 ± 4
Urea (mg/dL)	29 ± 1
Creatinine (mg/dL)	0.80 ± 0.02
Uric acid (mg/dL)	4.60 ± 0.18
Total proteins (g/L)	72 ± 1
Albumin (g/L)	44 ± 0

Data are mean ± SEM unless otherwise specified. BMI: body mass index, WHR: waist–hip ratio, DBP: diastolic blood pressure, SBP: systolic blood pressure, CRP: C-reactive protein, HDL: high density lipoprotein, LDL: low density lipoprotein.

Baseline anthropometric (weight, BMI, waist and WHR), clinical (DBP, SBP and heart rate) and biochemical (CRP, HDL, LDL, total cholesterol, triglycerides, urea, creatinine, uric acid, total protein and albumin) measurements are also given in Table 1. The baseline concentrations of inorganic elements and bioactive compounds (phenolic acids and carotenes) are available as Supplementary Material Tables S1, S2 and S3, respectively.

3.2. Mean Dietary Composition of Participants During the Interventions

Participants followed a similar dietary pattern in both interventions (Table 2), although the OD was lower in protein (p = 0.036) and fish/seafood (p = 0.042). The mean proportion of macronutrients was the same in both diets (57% carbohydrates, 24% fats and 19% proteins). A borderline p was obtained comparing dairy products and vegetables (p = 0.051 and 0.055). However, the differences between both diets considering individual food were not significant (data not shown). In addition, a significantly lower amount of calcium and phosphorus was ingested in the OD.

Table 2. Nutrient and food intake of participants in both diets (n = 19).

	OD	CD	p
Nutrient intake			
Energy (kcal/d)	1965 ± 203	2062 ± 204	0.070
Carbohydrates (g/d)	211 ± 21	220 ± 22	0.260
Total fat (g/d)	88 ± 9	92 ± 9	0.091
SFA (g/d)	22 ± 3	23 ± 3	0.064
MUFA (g/d)	45 ± 4	46 ± 4	0.136
PUFA (g/d)	12 ± 2	12 ± 1	0.136
Protein (g/d)	68 ± 9	72 ± 9	**0.036** *
Ca (mg/d)	780 ± 111	847 ± 110	**0.024** *
Mg (mg/d)	344 ± 37	353 ± 39	0.376
P (mg/d)	1352 ± 171	1433 ± 169	**0.018** *
Fe (mg/d)	16 ± 1	16 ± 2	0.376
Food intake			
Dairy products (g/d)	192 ± 52	207 ± 50	0.051
Meat (g/d)	98 ± 20	102 ± 19	0.202
Eggs (g/d)	28 ± 3	31 ± 3	0.180
Fish and seafood (g/d)	56 ± 16	66 ± 16	**0.042** *
Vegetables (g/d)	296 ± 32	366 ± 39	0.055
Fruits (g/d)	360 ± 68	377 ± 70	0.650
Nuts (g/d)	13 ± 5	12 ± 5	0.950
Legumes (g/d)	26 ± 5	26 ± 5	0.528
Cereals (g/d)	98 ± 11	98 ± 10	0.717
Oils (g/d)	40 ± 4	40 ± 4	0.317
Cocoa (g/d)	18 ± 5	21 ± 8	0.812
Coffee (g/d)	62 ± 16	59 ± 16	0.600
Tea (g/d)	22 ± 7	17 ± 7	0.106
Wine (g/d)	54 ± 23	62 ± 28	0.634

Data are mean ± SEM. *p-value < 0.05. SFA: Saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

3.3. *Physiological Parameters of Participants After the Interventions*

Table 3 shows anthropometric, clinical and biochemical data of the participants after following the OD and CD.

Table 3. Anthropometric, clinical and biochemical measurements after the interventions (n = 19).

	OD	CD	p
Anthropometric measurements			
Weight (kg)	64 ± 2	63 ± 2	0.365
BMI	22.1 ± 0.6	22.2 ± 0.6	0.352
Waist (cm)	76 ± 1	76 ± 1	0.549
WHR	0.80 ± 0.01	0.79 ± 0.01	0.822
Clinical measurements			
DBP (mmHg)	79 ± 2	73 ± 2	0.074
SBP (mmHg)	119 ± 4	118 ± 3	0.979
Heart rate (bpm)	70 ± 3	66 ± 2	0.326
Biochemical measurements			
CRP (mg/dL)	0.17 ± 0.07	0.26 ± 0.11	0.438
HDL (mg/dL)	62 ± 4	60 ± 4	0.301
LDL (mg/dL)	92 ± 9	90 ± 7	0.653
Total cholesterol (mg/dL)	168 ± 9	164 ± 7	0.494
Triglycerides (mg/dL)	66 ± 4	68 ± 4	0.421
Urea (mg/dL)	29 ± 2	29 ± 2	0.913
Creatinine (mg/dL)	0.80 ± 0.03	0.79 ± 0.02	0.763
Uric acid (mg/dL)	4.55 ± 0.29	4.68 ± 0.26	0.456
Total proteins (g/L)	73 ± 1	71 ± 1	0.145
Albumin (g/L)	44 ± 1	43 ± 1	0.136

Data are mean ± SEM. BMI: body mass index, WHR: waist–hip ratio, DBP: diastolic blood pressure, SBP: systolic blood pressure, CRP: C-reactive protein, HDL: high density lipoprotein, LDL: low density lipoprotein.

3.4. Inorganic Elements in Plasma

No significant differences were observed in the plasmatic concentration of minerals and heavy metals between the two diets (Table 4).

Table 4. Inorganic elements in plasma after the interventions (n = 19).

	OD	CD	p
Na (ppm)	2991 ± 20	2992 ± 19	0.445
K (ppm)	839 ± 14	844 ± 11	0.778
Ca (ppm)	88 ± 1	88 ± 1	0.717
Mg (ppm)	18 ± 0	18 ± 0	0.778
P (ppm)	104 ± 3	100 ± 3	0.136
Fe (ppb)	1252 ± 127	1339 ± 107	0.601
Zn (ppb)	778 ± 98	785 ± 46	0.376
Cu (ppb)	858 ± 64	856 ± 71	0.904
As (ppb)	4.35 ± 2.27	3.99 ± 0.95	0.221
Pb (ppb)	BLD	BLD	-
Cd (ppb)	BLD	BLD	-
U (ppb)	BLD	BLD	-

Data are mean ± SEM. BLD: Below limit of detection.

3.5. Phenolic Acids in Urine

Several polyphenols, mainly phenolic acids generated by microbiota metabolism and their derivatives, were evaluated in urine after the interventions (Table 5). A significant increase was observed in 4-HBA (p = 0.028) after the OD compared to the CD, but no changes were detected in the rest of the phenols.

Table 5. Urinary phenolic acids excretion after the interventions (n = 19).

	OD	CD	p
Phenylacetic acids			
3,4-DHPAA (nmol)	90 ± 35	35 ± 9	0.42
3-HPAA (nmol)	943 ± 594	941 ± 440	0.717
Homovanillic (nmol)	154 ± 60	108 ± 27	0.868
Phenylpropionic acids			
3,4-HPPA (nmol)	10 ± 3	27 ± 12	0.407
DHCA (nmol)	1.2 ± 0.4	1.2 ± 0.4	0.955
Hydroxybenzoic and derivatives			
4-HBA (nmol)	205 ± 123	70 ± 35	**0.028** *
4-HH (nmol)	471 ± 225	212 ± 85	0.306
Hippuric (nmol)	1281 ± 235	1463 ± 211	0.231
Hydroxycinnamic and derivatives			
CA (nmol)	7 ± 2	10 ± 2	0.349
m-Cou (nmol)	0.5 ± 0.3	0.26 ± 0.07	0.501
p-Cou (nmol)	0.3 ± 0.7	0.54 ± 0.19	0.554
GA (nmol)	0.48 ± 0.45	0.07 ± 0.03	0.878

Data are mean ± SEM. * p-value < 0.05. 3,4-DHPAA: 3,4-dihydroxyphenylacetic acid, 3-HPAA: 3-hydroxyphenylacetic acid, 3,4-HPPA: 3-(4-hydroxyphenyl) propionic acid, DHCA: dihydrocaffeic acid, 4-HBA: 4-hydroxybenzoic acid, 4-HH: 4-hydroxyhippuric, CA: caffeic acid, m-Cou: m-coumaric acid, p-Cou: p-coumaric acid, GA: gallic acid.

3.6. Carotenoids in Plasma

No significant differences were observed in plasmatic concentrations of carotenes (Table 6).

Table 6. Plasmatic carotenoids after the interventions (n = 19).

	OD	CD	p
α-carotene (nmol/mL)	0.39 ± 0.09	0.27 ± 0.06	0.552
β-carotene (nmol/mL)	1.03 ± 0.24	0.95 ± 0.22	0.744
E-lycopene (nmol/mL)	0.7 ± 0.17	0.78 ± 0.18	0.913
Z-lycopene (nmol/mL)	0.15 ± 0.04	0.20 ± 0.05	0.379

Data are mean ± SEM.

4. Discussion

A randomized, controlled and crossover pilot study with nineteen healthy subjects was carried out to assess whether following an OD for 4 weeks changes health parameters and biomarkers compared to a CD.

In this study, the phenol 4-HBA increased approximately three times at the end of the OD compared to the CD (p = 0.028). 4-HBA can come from a diet, nevertheless, the intake of food rich in this phenol, such as berries, beer, etc., did not change significantly between both interventions (data not shown). However, this compound is produced from anthocyanins catabolism, as a metabolite of pelargonidin [34–36], and it can be formed by the colonic microbiota [36,37]. The metabolite 4-HBA has shown anticancer and neuroprotective effects [37–40]. Moreover, this compound is a precursor of the coenzyme Q10, showing cardioprotective properties [41,42].

No significant differences in the urinary concentration of the rest of the phenols were observed between the two diets, although vegetable intake was borderline lower in the OD. Stracke et al. carried out a study in which healthy men consumed 500 g of organic or conventional apples for four weeks. Twenty-four hours after the last intake, polyphenol concentrations in plasma and urine were not higher in the organic consumers [43].

Studies on the carotenoid content in organically grown fruits have provided inconclusive results [13,19,44]. In the present work, no effects of the OD on carotenoid levels were detected. In contrast to our results, a previous observational study reported significant differences in both carotenes and other fat-soluble micronutrients after consumption of organic food [45].

No changes in the concentration of inorganic elements in plasma were observed after either intervention. According to other authors, organic agriculture does not affect dietary copper [45,46] or zinc absorption [46]. However, a cohort from the NutriNet-Santé study presented a higher level of magnesium after following an OD, whereas no differences were found in iron absorption [45]. Higher magnesium, iron and phosphorus levels have been described in organic versus conventional plant-derived foods [47]. In contrast, concentrations of cadmium have been reported to be lower in organic food, due to the type of plant fertilizer used, but lower levels in consumers of organic produce were not observed [19,45,48]. In the present study, cadmium was not detected in plasma, nor was lead or uranium. Marchioni et al. showed that the content of cadmium and lead in coffee was influenced by temperature and mass, respectively, but not by the type of crop [49]. Although uranium is used more in conventional than in organic agriculture [50,51], a higher uranium content was not evidenced in conventional produce [52]. We found calcium and phosphate intake was lower in the OD, likely due to a lower consumption of dairy products. Although previous studies have described a higher concentration of phosphate in conventional foods due to crop fertilizers [53], here no differences were detected in the plasma levels between the two diets. Previous findings from the Environmental Defense Fund indicate that organic foods are as likely as conventional foods to contain heavy metals, because the organic standard is focused on pesticides and not these contaminants [54].

ODs are generally believed to be healthier and to provide more bioactive compounds. Some authors have observed a higher concentration of some phytochemicals in organic food, but without considering their bioavailability. In addition, when assessing the nutritional value of food, other influential factors need to be considered, including crop variety, maturity, soil and climate. On the

other hand, consumers of organic products tend to be more concerned with health-related issues than the general population, which can bias the results of observational studies.

Organic foods are appreciated for the limited use of synthetic compounds (fertilizers, pesticides and antibiotics) in their production. Nevertheless, conventional crops are also regulated in this respect, and long-term studies are required to corroborate the effect of these compounds on health. To date, evidence suggesting that organic products are more nutritive or healthier is still lacking. Therefore, further carefully designed research is needed to evaluate the effect of an OD on bioactive compounds in biological fluids and health-related biomarkers.

The strongest point of the current study is its crossover design and the evaluation of a dietary pattern instead of only one or a few foods. Also, few such clinical assays have been conducted to date, with most studies being observational. Limitations of the work include a small sample size, the short duration of interventions and some differences in dietary patterns between the two interventions. However, this may be considered a pilot study to assess the short-term effects of organic food consumption. The increase of the phenolic compound arising from microbiota metabolism (4-HBA) in consumers following the OD need to be corroborated by further research with a higher number of subjects, which may shed light on a potential mechanism and possible health beneficial effects. In addition, a better control of factors as crop variety, maturity, soil and climate would provide more reliable results.

5. Conclusions

This intervention study for only one month found a significant difference in the concentration of a phenolic acid, the 4-HBA, after the OD. No changes were observed in the rest of the bioactive compounds analyzed nor in the other health-related biomarkers considered, neither in the results of minerals and heavy metals. The relation between the organic or conventional foods consumed and the concentration of bioactive compounds in the organism should be further researched. Longer studies and with larger sample sizes could reach significant values in other biochemical and heathy variables, demonstrating the health benefits of an OD.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3921/8/9/340/s1, Table S1. Baseline concentrations of inorganic elements in plasma; Table S2. Baseline concentrations of urinary excretion of phenolic acids; Table S3. Baseline concentrations of carotenes in plasma.

Author Contributions: Conceptualization, S.H.-B., P.Q.-R., J.F.R.d.A., A.T.-R. and R.M.L.-R.; data curation, S.H.-B. and P.Q.-R.; formal analysis, S.H.-B., P.Q.-R. and A.T.-R.; investigation, S.H.-B.; methodology, S.H.-B., M.M.-M. and J.F.R.d.A.; supervision, R.M.L.-R.; writing—original draft, S.H.-B.; all participants reviewed and approved the paper; and R.M.L.-R. was the main person responsible for the project and the final content.

Funding: This study was supported by CYCIT from the Ministerio de Ciencia, Innovación y Universidades (grant number AGL2016-75329-R), the Instituto de Salud Carlos III—CIBEROBN (C03-01) and Generalitat de Catalunya (SGR 2017)—Departament d'Agricultura, Ramaderia, Pesca i Alimentació—Direcció General d'Agricultura i Ramaderia under grant 53 05012 2016.

Acknowledgments: First: we thank all the participants of the study and sponsors (Ecoveritas S.A., Conservas José Salcedo Soria S.L., Paul & Pippa Gourmet Food S.L., Artfood S.L., Can Feixes, Grupo Codorniu Aceites Borges Pont S.A. and Molí dels Torms, S.L.) who kindly provided organic wine, olive oil, snacks and canned vegetables, and weekly vouchers to shop in organic supermarkets. S.H.B. and M.M.M. received support from the Ministerio de Educación, Cultura y deporte (MECD) through predoctoral scholarship FPU (FPU14/01715 and FPU17/00513, respectively). P.Q.-R. is thankful for the Sara Borrell postdoctoral program from the Instituto de Salud Carlos III (ISCIII). J.F.R.d.A. is grateful to the Science without Borders program for the predoctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—Brazil (233576/2014-2). A.T.-R. thanks the Juan de la Cierva postdoctoral program (FJCI-2016-28694) from the Ministerio de Economía, Industria y Competitividad.

Conflicts of Interest: Dra. R.M.L.-R. reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia. Moreover, weekly vouchers and other organic products have been provided by Ecoveritas S.A. and sponsors previously named. Nevertheless, these foundations and sponsors were not involved in the study design, the collection, analysis and interpretation of data, the writing of the manuscript, or the decision to submit the manuscript for publication.

References

1. Magnusson, M.K.; Arvola, A.; Koivisto Hursti, U.-K.; Åberg, L.; Sjödén, P.-O. Choice of organic foods is related to perceived consequences for human health and to environmentally friendly behaviour. *Appetite* **2003**, *40*, 109–117. [CrossRef]
2. Dean, M.; Lampila, P.; Shepherd, R.; Arvola, A.; Saba, A.; Vassallo, M.; Claupein, E.; Winkelmann, M.; Lähteenmäki, L. Perceived relevance and foods with health-related claims. *Food Qual. Prefer.* **2012**, *24*, 129–135. [CrossRef]
3. Hoefkens, C.; Verbeke, W.; Aertsens, J.; Mondelaers, K.; Van Camp, J. The nutritional and toxicological value of organic vegetables. *Br. Food J.* **2009**, *111*, 1062–1077. [CrossRef]
4. Curl, C.L.; Fenske, R.A.; Elgethun, K. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Environ. Health Perspect.* **2003**, *111*, 377–382. [CrossRef]
5. Lu, C.; Toepel, K.; Irish, R.; Fenske, R.A.; Barr, D.B.; Bravo, R. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environ. Health Perspect.* **2006**, *114*, 260–263. [CrossRef]
6. Bradman, A.; Quirós-Alcalá, L.; Castorina, R.; Schall, R.A.; Camacho, J.; Holland, N.T.; Barr, D.B.; Eskenazi, B. Effect of Organic Diet Intervention on Pesticide Exposures in Young Children Living in Low-Income Urban and Agricultural Communities. *Environ. Health Perspect.* **2015**, *123*, 1086–1093. [CrossRef]
7. Oates, L.; Cohen, M.; Braun, L.; Schembri, A.; Taskova, R. Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. *Environ. Res.* **2014**, *132*, 105–111. [CrossRef]
8. Baudry, J.; Debrauwer, L.; Durand, G.; Limon, G.; Delcambre, A.; Vidal, R.; Taupier-Letage, B.; Druesne-Pecollo, N.; Galan, P.; Hercberg, S.; et al. Urinary pesticide concentrations in French adults with low and high organic food consumption: Results from the general population-based NutriNet-Santé. *J. Expo. Sci. Environ. Epidemiol.* **2019**, *29*, 366–378. [CrossRef]
9. Hurtado-Barroso, S.; Tresserra-Rimbau, A.; Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Organic food and the impact on human health. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 704–714. [CrossRef]
10. Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Foodomics: A new tool to differentiate between organic and conventional foods. *Electrophoresis* **2016**, *37*, 1784–1794. [CrossRef]
11. Vallverdú-Queralt, A.; Medina-Remón, A.; Casals-Ribes, I.; Amat, M.; Lamuela-Raventós, R.M. A metabolomic approach differentiates between conventional and organic ketchups. *J. Agric. Food Chem.* **2011**, *59*, 11703–11710. [CrossRef]
12. Vallverdú-Queralt, A.; Medina-Remón, A.; Casals-Ribes, I.; Lamuela-Raventos, R.M. Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chem.* **2012**, *130*, 222–227. [CrossRef]
13. Vallverdú-Queralt, A.; Martínez-Huélamo, M.; Casals-Ribes, I.; Lamuela-Raventós, R.M. Differences in the carotenoid profile of commercially available organic and conventional tomato-based products. *J. Berry Res.* **2014**, *4*, 69–77. [CrossRef]
14. Borguini, R.G. *Avaliação do Potencial Antioxidante e de Algumas Características Físico-Químicas do Tomate (Lycopersicon Esculentum) Orgânico em Comparação ao Convencional*; Biblioteca Digital de Teses e Dissertações, Universidade de São Paulo: São Paulo, Brazil, 2006.
15. Györe-Kis, G.; Deák, K.; Lugasi, A.; Csúr-Vargaa, A.; Helyes, L. Comparison of conventional and organic tomato yield from a three-year-term experiment. *Acta Aliment.* **2012**, *41*, 486–493. [CrossRef]
16. Roussos, P.A.; Gasparatos, D. Apple tree growth and overall fruit quality under organic and conventional orchard management. *Sci. Hortic.* **2009**, *123*, 247–252. [CrossRef]
17. Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* **2005**, *81*, 243S–255S. [CrossRef]
18. Thilakarathna, S.; Rupasinghe, H. Flavonoid Bioavailability and Attempts for Bioavailability Enhancement. *Nutrients* **2013**, *5*, 3367–3387. [CrossRef]
19. Barański, M.; Średnicka-Tober, D.; Volakakis, N.; Seal, C.; Sanderson, R.; Stewart, G.B.; Benbrook, C.; Biavati, B.; Markellou, E.; Giotis, C.; et al. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: A systematic literature review and meta-analyses. *Br. J. Nutr.* **2014**, *112*, 794–811. [CrossRef]
20. Palupi, E.; Jayanegara, A.; Ploeger, A.; Kahl, J. Comparison of nutritional quality between conventional and organic dairy products: A meta-analysis. *J. Sci. Food Agric.* **2012**, *92*, 2774–2781. [CrossRef]

39. Winter, A.N.; Brenner, M.C.; Punessen, N.; Snodgrass, M.; Byars, C.; Arora, Y.; Linseman, D.A. Comparison of the Neuroprotective and Anti-Inflammatory Effects of the Anthocyanin Metabolites, Protocatechuic Acid and 4-Hydroxybenzoic Acid. *Oxid. Med. Cell Longev.* **2017**, *2017*, 1–13. [CrossRef]
40. Distelmaier, F. 4-Hydroxybenzoic acid for multiple system atrophy? *Parkinsonism Relat. Disord.* **2018**, *50*, 119–120. [CrossRef]
41. Timoshchuk, S.V.; Vavilova, H.L.; Strutyns'ka, N.A.; Talanov, S.A.; Petukhov, D.M.; Kuchmenko, O.B.; Donchenko, H.V.; Sahach, V.F. Cardioprotective action of coenzyme Q in conditions of its endogenous synthesis activation in cardiac ischemia-reperfusion in old rats. *BMC Pharmacol. Toxicol.* **2009**, *55*, 58–63.
42. Kumchenko, E.B.; Petukhov, D.N.; Donchenko, G.V.; Mkhitarian, L.S.; Timoshchuk, S.V.; Strutinskaia, N.A.; Vavilova, G.L.; Sagach, V.F. Effect of precursors and modulators of coenzyme Q biosynthesis on the heart mitochondria function in aged rats. *Biomed. Khim.* **2010**, *56*, 244–256.
43. Stracke, B.A.; Rüfer, C.E.; Bub, A.; Seifert, S.; Weibel, F.P.; Kunz, C.; Watzl, B. No effect of the farming system (organic/conventional) on the bioavailability of apple (Malus domestica Bork., cultivar Golden Delicious) polyphenols in healthy men: A comparative study. *Eur. J. Nutr.* **2010**, *49*, 301–310. [CrossRef]
44. Cardoso, P.C.; Tomazini, A.P.B.; Stringheta, P.C.; Ribeiro, S.M.R.; Pinheiro-Sant'Ana, H.M. Vitamin C and carotenoids in organic and conventional fruits grown in Brazil. *Food Chem.* **2011**, *126*, 411–416. [CrossRef]
45. Baudry, J.; Ducros, V.; Druesne-Pecollo, N.; Galan, P.; Hercberg, S.; Debrauwer, L.; Amiot, M.J.; Lairon, D.; Kesse-Guyot, E. Some Differences in Nutritional Biomarkers are Detected Between Consumers and Nonconsumers of Organic Foods: Findings from the BioNutriNet Project. *Curr. Dev. Nutr.* **2019**, *3*, nzy090. [CrossRef]
46. Mark, A.B.; Kápolna, E.; Laursen, K.H.; Halekoh, U.; Rasmussen, S.K.; Husted, S.; Larsen, E.H.; Bügel, S. Consumption of organic diets does not affect intake and absorption of zinc and copper in men-evidence from two cross-over trials. *Food Funct.* **2013**, *4*, 409–419. [CrossRef]
47. Rembiałkowska, E. Review Quality of plant products from organic agriculture. *J. Sci. Food Agric.* **2007**, *87*, 2757–2762. [CrossRef]
48. Kratz, S.; Schick, J.; Schnug, E. Trace elements in rock phosphates and P containing mineral and organo-mineral fertilizers sold in Germany. *Sci. Total Environ.* **2016**, *542*, 1013–1019. [CrossRef]
49. Marchioni, C.; de Oliveira, F.M.; de Magalhães, C.S.; Luccas, P.O. Assessment of Cadmium and Lead Adsorption in Organic and Conventional Coffee. *Anal. Sci.* **2015**, *31*, 165–172. [CrossRef]
50. Schnug, E.; Haneklaus, N. *Uranium in Phosphate Fertilizers—Review and Outlook. Uranium—Past and Future Challenges*; Springer International Publishing: Cham, Switzerland, 2015; pp. 123–130.
51. De Kok, L.J.; Luit, J.; Schnug, E. *Loads and Fate of Fertilizer-Derived Uranium*; Backhuys Publishers, 2008; p. 229.
52. Mie, A.; Andersen, H.R.; Gunnarsson, S.; Kahl, J.; Kesse-Guyot, E.; Rembiałkowska, E.; Quaglio, G.; Grandjean, P. Human health implications of organic food and organic agriculture: A comprehensive review. *Environ. Health* **2017**, *16*, 111. [CrossRef]
53. Syers, J.K.; Johnston, A.E.; Curtin, D. Efficiency of Soil and Fertilizer Phosphorus Use Reconciling Changing Concepts of Soil Phosphorus Behaviour with Agronomic Information. 2008. Available online: http://www.fao.org/3/a-a1595e.pdf (accessed on 10 June 2019).
54. Environmental Defense Fund (EDF). Consumer Reports Study Finds "Concerning Levels" of Heavy Metals in Baby and Toddler Foods. Available online: https://www.edf.org/media/consumer-reports-study-finds-concerning-levels-heavy-metals-baby-and-toddler-foods (accessed on 10 June 2019).

4.4. Publicación 5: Los carotenos podrían mejorar el perfil lipídico a través de la modulación de los ácidos grasos volátiles producidos por la microbiota: resultados de un estudio piloto en humanos sanos.

Artículo titulado "Carotenes could improve lipid profile, modulating volatile fatty acids: Results from a pilot study in healthy adults" Enviado a la revista científica "*Antioxidants*" (Índice de impacto: 4,520 (2018); Q1, D1).

Además de los compuestos bioactivos procedentes de la dieta, la microbiota también genera productos biológicamente activos a partir de reacciones metabólicas. Las investigaciones en esta área han evidenciado la relación entre la composición microbiana y sus productos metabólicos y la dieta. Los VFA son generados desde la microbiota a partir de sustratos dietéticos (fibra, principalmente, y péptidos) y han demostrado propiedades beneficiosas en la salud, particularmente mediante la regulación del metabolismo energético. Por otra parte, los carotenos son fitoquímicos relacionados con el consumo de hortalizas y frutas que también se relacionan con la modulación de algunos procesos metabólicos. Tanto los VFA como los carotenos están implicados en la regulación de la síntesis del colesterol en la sangre, sin embargo, no se conoce si hay alguna interacción entre dichos compuestos. Por lo tanto, el objetivo de este estudio piloto llevado a cabo en 19 sujetos sanos fue evaluar si hay alguna asociación entre los VFA liberados desde la microbiota y excretados mediante las heces y el consumo dietético de carotenos, cuantificados en el plasma. Además, se estudió la relación entre los compuestos bioactivos desde la microbiota y la dieta y el perfil lipídico en sangre. Los sujetos fueron analizados en dos períodos diferentes de un mes de duración, y de esta forma se obtuvieron 38 observaciones en total (19 sujetos x 2 observaciones). Los compuestos bioactivos (VFA y carotenos) fueron analizados mediante métodos cromatográficos y los parámetros lipídicos se cuantificaron en plasma a partir de métodos enzimáticos. La excreción de propionato fue directamente asociada con la concentración de carotenos en plasma (p = 0,012), y ambos se relacionaron con el incremento del colesterol HDL en plasma (p = 0,017 y 0,033, respectivamente). Sin embargo, los efectos sobre el colesterol plasmático fueron más notorios en el caso del butirato, debido a que además de su relación con el incremento en el colesterol HDL (p = 0,021), también se asoció con una disminución significativa en el nivel del colesterol LDL y del total (p = 0,011 y 0,043, respectivamente). Entre los carotenos, elevadas concentraciones de α-caroteno se relacionaron positivamente con el colesterol HDL en plasma (p = 0,009). Además, los carotenos también se asociaron

con el acetato y otros VFA minoritarios. Por lo tanto, los resultados del presente estudio indican una posible interacción entre los VFA y los carotenos, que podría estar relacionada con la modulación del perfil lipídico.

antioxidants

MDPI

Article

Carotenes could improve lipid profile, modulating volatile fatty acids: Results from a pilot study in healthy adults.

Sara Hurtado-Barroso[1,3], Anna Tresserra-Rimbau[2,4], Jose Fernando Rinaldi de Alvarenga[1,3], María Marhuenda-Muñoz[1,3], Juan J. Moreno[1,3] and Rosa M. Lamuela-Raventós[1,3,*]

[1] Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain; sara.hurtado_17@ub.edu (S.H.-B.); jrinaldi@ub.edu (J.F.R.A); mmarhuendam@ub.edu (M.M.-M.); jjmoreno@ub.edu (J.J.M).

[2] Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029, Madrid, Spain

[3] INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08028 Barcelona, Spain

[4] Unitat de Nutrició Humana, Hospital Universitari Sant Joan de Reus, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, Hospital Universitari Sant Joan de Reus, Institut d'Investigació Pere Virgili (IISPV), Universitat Rovira i Virgili, 43007 Reus, Spain, anna.tresserra@iispv.cat (A.T.-R.).

* Correspondence: lamuela@ub.edu (R.M.L-R); Tel.: +34-934-034-843

Abstract: The release of volatile fatty acids (VFAs) may be correlated with carotenes, described as biomarkers of vegetable and fruit intake. Both groups of biological compounds seem to be involved in the regulation of lipid metabolism. Nineteen healthy subjects were included in this pilot study, and they follow up consisted of two one-month periods, generating a total of 38 observations. VFAs were analyzed from feces by Headspace Gas Chromatography (HS-GC-MS). Plasmatic carotenes were measured by HPLC-DAD (High-performance liquid chromatography-diode array detector). Lipidic parameters were assayed in plasma by an enzymatic method. Greater release of propionate was particularly related to higher concentrations of carotenes (p= 0.012) and an increase of high density lipoprotein cholesterol (HDL) in plasma (p=0.017). Moreover, this improvement in HDL cholesterol was also associated with major levels of plasmatic carotenes (p=0.033), particularly with α-carotene (p=0.009). In addition, the effect of butyrate on the lipid profile was highlighted, and other VFAs (acetate, isovalerate and heptanoate) seem to be related to plasmatic carotene concentrations. The study suggests an association between VFAs and carotenes and their improvement of the lipid profile.

Keywords: volatile fatty acids, acetate, propionate, butyrate, carotene, lycopene, LDL and HDL cholesterol.

1. Introduction

The relationship between diet, gut microbiota, and health have been widely described [1–3]. VFAs are the major end products of bacterial metabolism in the human large intestine [4]. They are mainly generated by dietary fiber, and they are able to modulate the gut microbiota [5]. Nevertheless, less than 1% of VFAs is formed from proteins and amino acids [6]. The role of VFAs in energy metabolism has been previously reported [7,8]. The main VFAs produced are acetate, propionate, and butyrate, and their colonic concentrations vary according to dietary and microbial compositions as well as the site of fermentation [9]. Acetate represents around 50% of the total VFAs and it has been found to promote energy metabolisms by increasing fasting fat oxidation and resting energy expenditure [10]. Propionate is mainly involved in the regulation of hepatic lipogenesis and appetite as well as the improvement of insulin sensitivity, triggering a decrease in the risk of obesity [11,12].

Butyrate seems to be involved in the improvement of chronic pathologies, such as cardiovascular diseases and cancer [13,14]. Other VFAs are also produced in the colon, such as valerate, caproate, and heptanoate, as well as the branched-chain fatty acids (BCFAs) isobutyrate, isovalerate, and isocaproate; however, they are produced in very low amounts [15]. BCFAs, mainly consumed from dairy products, are thought to be synthesized from the catabolic products of the branched chain amino acids (valine, leucine, and isoleucine) and stand out to be normal constituents of the human neonatal gut [16–18]. Additionally, BCFAs seem to be related to energy metabolism [19].

On the other hand, dietary phytochemicals can modulate the gut microbiota, resulting in consequences in health [20]. Carotenoids have been described as biomarkers of vegetable and fruit intake, and they can decrease the risk of several chronic diseases, such as some cancer sites, type 2 diabetes, cardiovascular diseases, and macular degeneration related to age [21,22]. However, the association between dietary carotenoids and gut microbiota still remains unknown. These compounds are poorly absorbed (10-40%) and may reach the colon and be metabolized by the microbiota [23]. Some authors have described the influence of these phytochemicals in improving the gut microbiota [23,24], but further studies are needed in this field. On the other hand, carotenoids and VFAs seem to modify the lipid profile, decreasing the risk of cardiovascular disease incidence [8,13,25–29]. Thus, the aim of the present study was to evaluate the relationship between the release of VFAs and plasmatic carotenes as well as their effects on the lipid profile in a healthy human.

2. Materials and Methods

2.1. Study population

A pilot study was performed on 19 healthy subjects aged between 18 and 40 years. In each case, diet was monitored twice and followed up over the course of a month. The two periods of following a diet were separated by two months of wash out. Thus, the study was comprised of a total of 38 observations. Participants were recruited from the University of Barcelona and surrounding communities between June 2016 and February 2017. Exclusion criteria were a history of chronic disease or altered biochemical parameters, toxic habits (tobacco, alcohol, or drugs), and chronic use of medication, supplements and/or antibiotics.

All participants provided a written informed consent prior to the study. The study was carried out according to the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Clinical Investigation of the University of Barcelona (Barcelona, Spain).

2.2. Questionnaires and biological samples collection

Dietary intake of the month previous to the study was obtained through a validated 137-items semi-quantitative food frequency questionnaire filled in by a trained interviewer [30]. Information from the food frequency questionnaire was used to calculate intake of energy and nutrients.

Biological samples of plasma, urine, and feces were collected (at the beginning and at the end of the study). Plasma was obtained from fasting blood collected via venipuncture in the arm using ethylenediaminetetraacetic acid (EDTA) tubes and following centrifugation (1902 g for 15 min at 4 °C). 24-hours urine and a recent sample of feces were also provided the same day.

2.3. Anthropometric and clinical parameters

Body weight was measured with an electronic scale and height with a Seca 213 portable stadiometer; the participant was wearing light clothes and no shoes. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Waist circumference was measured with a measuring tape accurate to 0.1 cm.

Systolic and diastolic blood pressure (SBP and DBP) and heart rate were measured in fasting conditions with an OMRON M6 monitor in triplicate at the beginning and at the end of the study.

Biochemistry analyses were performed by an external laboratory (mdb.lad Durán Bellido). C-reactive protein (CRP) was analyzed with the immunoturbidimetry method. HDL, low density lipoprotein (LDL), total cholesterol, and triglycerides were analyzed by enzymatic method. Urea and uric acid were analyzed by enzymatic and enzymatic/chromogen methods, respectively. Creatinine was assayed by reaction kinetics [31]. Total proteins and albumin were assayed through Biuret reaction to final point and bromocresol green methods, respectively.

2.4. VFA analysis in feces

Feces were acidified and homogenized with formic acid 1M (500 mL/ 100mg of feces). The slurry mixture (100 µL) was added alongside 20 µL of 2-ethyl butyric acid 0.85 mM (internal standard) into a headspace vial (Agilent, Santa Clara, CA, USA).

Chromatographic analysis of SCFAs was performed by HS-GC-MS based on a previously described method [32], using a TRACE GC Ultra system (Thermo Fisher Scientific) after incubating the samples for 20 min at 60ºC. Initial oven temperature was 80ºC, achieving 125ºC and 225ºC, with rates of 50ºC/min and 6ºC/min, respectively. The injector temperature was 250ºC. An Agilent HP-FFAP column 0.3 µm (25 m x 0.2 mm) was used for the separation of the compounds. A volume of 1 mL of volatile analytes was injected by an inlet split with a flow rate of 10 mL/min. Constant flow of the carrier gas (Helium) at 1mL/min was transferred. A blank sample (Milli-Q water) was injected between each sample to avoid interference. Samples were assayed in triplicate.

A volatile free acid mix of 10 mM (Supelco, Bellefonte, PA, USA) containing acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and n-heptanoic acids was used as a standard. 2-Ethyl butyric acid (Sigma-Aldrich, St. Louis, MO, USA) was used as an internal standard. Acetic and propionic were quantified without considering the internal standard. A synthetic matrix was not used because the matrix effect was not significant.

2.5. Extraction and quantification of carotenoids from plasma

Carotenoids were extracted by liquid-liquid extraction from plasma samples [33]. Chromatographic analysis of carotenoids was performed by HPLC-DAD, using an HP 1100 HPLC system (Hewlett-105 Packard, Waldbronn, DE) that contained a quaternary pump coupled to a DAD G1315B. The separation was carried out with Milli-Q water, methanol, and methyl-tert-butyl ether (Panreac Quimica S.A., Barcelona, Spain), according to a procedure previously validated in our group [33]. A reversed-phase column YMC Carotenoid S-5 µm (250 mm x 4.6 mm) and a precolumn YMC Guard Cartridge Carotenoid S-5 µm (20 mm × 4.0 mm) were used.

The standards used were α-carotene, β-carotene and all-*E*-lycopene (Sigma-Aldrich, St. Louis, MO, USA), and 5-Z-lycopene (CaroteNature GmbH, Ostermundigen, Switzerland). These were pooled and prepared in synthetic human plasma (Sigma-Aldrich, St. Louis, MO, USA).

2.6. Statistical analysis

Normality was checked by a Shapiro-Wilk test. The variables were log-transformed due to their non-normal distribution. Linear regression was used to assay the relationship between VFAs and the rest of the parameters (carotenes and biochemical markers), with adjustments by sex, age (years), and energy intake (kcal/day). A p-value below 0.05 was considered statistically significant. Analyses were performed with the Stata software, version 14.2 (Stata Corp; College Station, TX, USA).

3. Results

133 *3.1. Characteristics of study subjects*

134 Table 1 shows characteristics of participants, including anthropometric, clinical, and biochemical
135 parameters. Additionally, food and nutrients intake are described. The concentrations of carotenes
136 and VFAs are also included.

137 **Table 1. Characteristics of the study population.**

Characteristic	
Males, n (%)	9 (50)
Age (years)	29 ± 6
Anthropometric and clinical measurements	
BMI	22.3 ± 2.6
WHR	0.80 ± 0.06
SBP (mmHg)	119 ± 15
DBP (mmHg)	75 ± 11
Heart rate (bpm)	67 ± 11
Biochemical measurements	
CRP (mg/dL)	0.23 ± 0.42
LDL cholesterol (mg/dL)	92 ± 35
HDL cholesterol (mg/dL)	60 ± 16
Total cholesterol (mg/dL)	165 ± 36
Triglycerides (mg/dL)	67 ± 18
Urea (mmoles/L)	4.84 ± 1.26
Creatinine (μmoles/L)	70 ± 10
Uric acid (μmoles/L)	275 ± 72
Total proteins (g/L)	72 ± 4
Albumin (g/L)	44 ± 3
Nutrient intake	
Energy (kcal/d)	2067 ± 868
Carbohydrates (g/d)	218 ± 93
Total fat (g/d)	93 ± 37
SFA (g/d)	23 ± 11
MUFA (g/d)	47 ± 18
PUFA (g/d)	12 ± 6
Protein (g/d)	72 ± 37
Carotenes	
α-carotene (nmol/mL)	0.30 ± 0.26
β-carotene (nmol/mL)	0.68 ± 0.79
E-lycopene (nmol/mL)	1.24 ± 0.82
5-Z-lycopene (nmol/mL)	0.29 ± 0.21
Total carotenes[1] (nmol/mL)	2.42 ± 1.66
VFA	
Acetate (mM)	4.25 ± 3.19

Propionate (mM)	1.78 ± 1.30
Butyrate (mM)	2.40 ± 1.50
Isobutyrate (mM)	0.34 ± 0.18
Valerate (mM)	0.40 ± 0.22
Isovalerate (mM)	0.44 ± 0.24
Caproate (mM)	0.16 ± 0.10
Isocaproate (mM)	0.08 ± 0.03
Heptanoate (mM)	0.11 ± 0.04
Total VFAs[2]	9.57 ± 5.58

Mean±SD. [1]Total carotenes represents the sum of all carotenes presented in the table; [2]Total VFAs represents the sum of all volatile fatty acids presented in the table. BMI: body mass index, WHR: waist–hip ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, CRP: C-reactive protein, LDL: low density lipoprotein, HDL: high density lipoprotein, SFA: Saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, VFA: volatile fatty acid.

138 *3.2. Relationship between carotenoids in plasma and VFAs in feces*

139 In the study cohort, propionate was significantly and directly associated with α and β-carotenes and
140 *E*-lycopene (p= 0.018, 0.031, and 0.021). Acetate, isobutyrate, and isovalerate were associated with the
141 plasmatic concentrations of *E*-lycopene (p= 0.036, 0.046 and 0.028) and valerate was related to
142 α-carotene (p=0.028). On the contrary, heptanoate was inversely related to β-carotenes and
143 *E*-lycopene (p= 0.014 and 0.047, respectively). Total carotenes analyzed were directly linked to
144 acetate (p=0.036), propionate (p=0.012), and isovalerate (p=0.039), and they were linked inversely to
145 heptanoate (p=0.046) (Table 2).

146 **Table 2. Association between plasmatic carotenes and VFAs in feces.**

	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Isobutyrate (mM)	Valerate (mM)	Isovalerate (mM)	Caproate (mM)	Isocaproate (mM)	Heptanoate (mM)
α-carotene (nmol/mL)	0.21 (0.275)	**0.52 (0.018*)**	0.27 (0.233)	0.22 (0.421)	**0.52 (0.028*)**	0.31 (0.234)	0.21 (0.500)	-0.18 (0.576)	-0.15 (0.694)
β-carotene (nmol/mL)	0.17 (0.122)	**0.28 (0.031*)**	0.14 (0.346)	0.17 (0.284)	0.24 (0.097)	0.18 (0.246)	-0.16 (0.390)	-0.34 (0.072*)	**-0.54 (0.014)**
***E*-lycopene (nmol/mL)**	**0.27 (0.036*)**	**0.37 (0.021*)**	0.11 (0.506)	**0.38 (0.046*)**	0.32 (0.061)	**0.40 (0.028*)**	-0.05 (0.815)	-0.39 (0.095)	**-0.54 (0.047*)**
5-*Z*-lycopene (nmol/mL)	0.21 (0.457)	0.33 (0.331)	0.07 (0.831)	0.38 (0.349)	0.18 (0.620)	0.47 (0.222)	-0.29 (0.532)	-0.88 (0.063)	-1.03 (0.065)
Total carotenes[1]	**0.24 (0.036)**	**0.35 (0.012)**	0.18 (0.220)	0.31 (0.071)	0.29 (0.057)	**0.33 (0.039)**	-0.05 (0.800)	-0.35 (0.085)	**-0.47 (0.046)**

Data are expressed as coefficient β (p-*value*) and adjusted by sex, age and energy intake. * p-value < 0.05. [1]Total carotenes is the sum of all carotenes presented in the table.

147 *3.3. Relationship between lipidic biomarkers and VFAs*

148 Total cholesterol and LDL decrease as well as HDL increase were directly related with higher release
149 of butyrate (p=0.043, 0.011 and 0.021). Moreover, higher levels of HDL were associated with a higher
150 concentration of propionate in feces (p= 0.017) (Table 3).

151 **Table 3. Association between plasmatic lipidic markers and SCFAs in feces.**

	Acetate (mM)	Propionate (mM)	Butyrate (mM)	Isobutyrate (mM)	Valerate (mM)	Isovalerate (mM)	Caproate (mM)	Isocaproate (mM)	Heptanoate (mM)
LDL cholesterol (mg/dL)	-0.04 (0.619)	-0.06 (0.523)	**-0.23 (0.011*)**	-0.15 (0.156)	-0.07 (0.466)	-0.16 (0.116)	-0.21 (0.075)	<0.01 (0.995)	-0.13 (0.386)
HDL cholesterol (mg/dL)	0.09 (0.079)	**0.15 (0.017*)**	**0.15 (0.021*)**	0.06 (0.435)	0.12 (0.083)	0.07 (0.363)	0.08 (0.345)	-0.08 (0.270)	-0.07 (0.523)
Total cholesterol (mg/dL)	0.01 (0.770)	<0.01 (0.932)	**-0.10 (0.043*)**	-0.05 (0.424)	<-0.01 (0.966)	-0.06 (0.340)	-0.11 (0.116)	-0.04 (0.569)	-0.12 (0.165)
Triglycerides (mg/dL)	0.01 (0.860)	0.03 (0.702)	-0.06 (0.391)	0.01 (0.903)	0.04 (0.625)	-0.01 (0.890)	-0.07 (0.429)	-0.01 (0.925)	-0.05 (0.651)

Data are expressed as coefficient β (*p-value*) and adjusted by sex, age and energy intake. * p-value < 0.05

152 *3.4. Relationship between lipidic biomarkers and carotenes*

153 Table 4 shows that a higher concentration of plasmatic carotenes, particularly α-carotene, was
154 associated with an improvement of HDL-cholesterol (p= 0.033 and 0.009, respectively).

155 **Table 4.** Association between plasmatic lipidic markers and carotenes.

	α-carotene (nmol/mL)	β-carotene (nmol/mL)	*E*-lycopene (nmol/mL)	5-*Z*-lycopene (nmol/mL)	Total carotenes[1]
LDL cholesterol (mg/dL)	-0.09 (0.146)	-0.03 (0.785)	-0.02 (0.812)	-0.03 (0.156)	-0.14 (0.519)
HDL cholesterol (mg/dL)	**0.13 (0.009*)**	0.12 (0.154)	0.09 (0. 210)	0.02 (0.582)	**0.17 (0.033*)**
Total cholesterol (mg/dL)	-0.02 (0.666)	0.02 (0.735)	0.02 (0.781)	-0.02 (0.464)	-0.04 (0.527)
Triglycerides (mg/dL)	-0.06 (0.209)	-0.08 (0.337)	<0.01 (0.970)	-0.02 (0.617)	-0.09 (0.240)

Data are expressed as coefficient β (*p-value*) and adjusted by sex, age and energy intake. * p-value < 0.05.

[1]Total carotenes is the sum of all carotenes presented in the table.

156 **4. Discussion**

157 In this study, a direct association between propionate and carotenes has been observed, reaching
158 significance with the total and with α- and β-carotenes and *E*-lycopene. In addition, an increase of

acetate in feces was linked to total carotenes, particularly to *E*-lycopene in plasma, but butyrate levels seem not to be affected by the carotene concentrations.

Higher propionate and carotene concentrations increased HDL-cholesterol, and butyrate was related to lower LDL, total cholesterol, and higher HDL. These results suggest that butyrate could play a role in cardiovascular health, as has been previously pointed out by several studies, since this VFA stands out as the preferred substrate and energy source for colonocytes, and its role regulating lipid metabolism have been previously described [34,35]. Moreover, the enhancement of HDL directly associated with the release of propionate also was observed by Venter et al. [36]. Propionate seems to be an effective inhibitor of acetate as substrate for cholesterol synthesis, particularly when the last one is present in high concentrations [28,37]. However, a recent study carried out in overweight and obese men showed a greater amount of acetate in the circulation after colonic infusion of high concentrations of acetate plus propionate in fasting conditions [10]. Some authors show that propionate can reduce obesity and other metabolic diseases, reducing lipogenesis and cholesterol synthesis in the liver and improving insulin sensitivity [11,38].

The research in this issue is limited and uncertain, and the mechanisms still remain unknown. However, previous studies suggest that within the VFAs absorbed in the colon, butyrate is the primary energy substrate for colonocytes, and acetate and propionate are transported to the liver and peripheral organs to regulate the metabolism. VFAs bind to the free fatty acids receptors (FFAR) 2 and 3, with a higher affinity for acetate, and propionate can also act as a histone deacetylase (HDAC) inhibitor and regulate many physiological processes through signaling via G-protein-coupled receptors (GPCRs) [29,34,39].

Moreover, HDL-cholesterol increased with higher levels of carotenes, particularly α-carotene. Similar results have been observed with carotenoids regulating the lipid metabolism, particularly the cholesterol concentration [25,26,40–43]. The cardioprotective function of lycopene increasing the HDL-cholesterol or decreasing LDL-cholesterol was observed by some authors [25,41,42]. In addition, other carotenoids, such as α-carotene and β-carotene, seem to be involved in the cholesterol metabolism [26,40,43]. A study performed using a macrophage cell line showed 63% and 73% lower cholesterol synthesis from acetate after incubation with 10 μM of β-carotene and lycopene, respectively [40]. Silva et al., studied the effect of a hypercholesterolemic diet supplemented with β-carotene in rats and reported a lower cholesterol concentration in the serum and liver as well as a larger excretion via feces. This finding suggested a decrease in cholesterol absorption in the intestine [26]. Also, the HDL-cholesterol synthesis may be increased by activation of the enzymes paraoxonase-1 (PON-1) and lecithin cholesterol acyltransferase (LCAT) [43].

Regarding the minority of VFAs, a direct relation was observed between valerate and α-carotene. However, total carotene, as well as β-carotene and *E*-lycopene, were inversely associated with heptanoate. In addition, BCFAs seem to be related to carotenes. Higher releases of isovalerate were linked to total carotenes, particularly to *E*-lycopene. The increase of this carotene has been associated with isobutyrate levels. Butyrate and caproate synthesis mainly from the elongation of acetate, as well as valerate and heptanoate from the elongation of propionate, has been described [44,45]. This process is called chain elongation and is produced by bacteria from ethanol and volatile fatty acids [45]. However, VFA selectivity depends on different factors, such as substrate, microorganisms, and pH [46–48]. Thus, the opposite effects of heptanoate could be due to a main synthesis of other fatty acids, particularly valerate.

E-lycopene is the most abundant carotene consumed by the Mediterranean population [49], and it is the carotene mostly closely related to VFAs. Although many papers describe the health effects of carotenes, little research has been conducted to evaluate, in an up to date manner, their association with VFAs [50]. In a randomized crossover trial, twenty-two healthy young men following a low-carotenoid diet consumed 330 mL of tomato or carrot juice per day for 2 weeks, and no relevant changes in VFAs were observed in their fecal content [51]. A study carried out in rats showed a significant increase in butyrate, but not in acetate and propionate, after consuming ~30 mL/day of

high lycopene content tomato juice for 3 weeks (intake of 3-4 mg/day of lycopene) [52]. Hwang et al. showed a higher amount of propionate and a lower concentration of acetate and butyrate in the cecum of rats after consumption of different diets supplemented with cherry tomatoes [53].

This work has some limitations. This is a pilot study carried out in a small sample of healthy humans. The cross-sectional nature of the study does not allow us to assess causality. Another limitation of the study was the lack of analyses of carotenes in feces, but the plasmatic concentration of them are representative of vegetable and fruit intake [21]. On the other hand, this work did not consider the gut microbiota composition, which could help to explain the involved mechanisms and trigger more reliable results.

As a strength, we highlight that, to our knowledge, no other studies have comprehensively assessed the association between some carotenes and VFAs in healthy adults. In fact, there is little research investigating the effects of dietary bioactive compounds on VFAs, particularly focused in the interaction between polyphenols and microbiota. We also want to note the comprehensive analyses of different human samples: plasma and feces.

Further carefully designed studies should be carried out to clarify the specific roles of VFAs on health and their interactions with phytochemicals from diet, such as carotenoids. A larger sample of subjects should be analyzed to establish whether there is an association between carotenes from diet and fermentations by gut microbiota and VFAs, as well as their biological plausibility.

5. Conclusions

The results of this study suggest an association between fecal propionate and the plasmatic concentrations of carotenes and HDL-cholesterol. The relationship between carotenes and HDL-cholesterol was also significant. Moreover, carotenes seem to interact with acetate and minority VFAs. Contrary to positive relations that are mainly found, lower concentrations of heptanoate seem to be related to higher levels of these phytochemicals. In addition, butyrate could have a protective role in the maintenance of cardiovascular health, modulating the levels of cholesterol, LDL, and HDL. Therefore, phytochemicals, such as carotenes, could play a role in enhancing the production of VFAs through microbiota, particularly propionate, triggering a better lipid profile.

Author Contributions: Conceptualization, R.M.L-R., J.J.M. and A.T-R.; methodology, S.H-B., J.F.R.d.A., and M.M-M; formal analysis, S.H-B. and A.T-R.; investigation, S.H-B.; data curation, S.H-B. and A.T-R.; writing—original draft preparation, S.H-B.; supervision, R.M.L-R. All participants reviewed and approved the paper.

Funding: This research was funded by CYCIT from the Ministerio de Ciencia, Innovación y Universidades, grant number AGL2016-75329-R; the Instituto de Salud Carlos III – CIBEROBN, C03-01 and Generalitat de Catalunya, SGR 2017– Departament d'Agricultura, Ramaderia, Pesca i Alimentació – Direcció General d'Agricultura i Ramaderia, grant 53 05012 2016.

Acknowledgments: First, we would like to thank all the participants of the study. S.H.B. and M.M.M. received support from the Ministerio de Educación, Cultura y Deporte (MECD) through the predoctoral scholarship FPU (FPU14/01715 and FPU17/00513, respectively). A.T-R. thanks the Juan de la Cierva postdoctoral program (FJCI-2016-28694) from the Ministerio de Economía, Industria y Competitividad. J.F.R.A. is grateful to the Science without Borders program for the predoctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)—Brazil (233576/2014-2).

Conflicts of Interest: Dra. R.M.L.-R. reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia, but this foundation was not involved in the study design, the collection, analysis, or interpretation of data, the writing of the manuscript, or the decision to submit the manuscript for publication.

References

1. Singh, R. K.; Chang, H.-W.; Yan, D.; Lee, K. M.; Ucmak, D.; Wong, K.; Abrouk, M.; Farahnik, B.; Nakamura, M.; Zhu, T. H.; Bhutani, T.; Liao, W. Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* **2017**, *15*, 73, doi:10.1186/s12967-017-1175-y.
2. Shen, T.-C. D. Diet and Gut Microbiota in Health and Disease. In *Nestle Nutrition Institute workshop series*; 2017; Vol. 88, pp. 117–126.
3. Chassaing, B.; Vijay-Kumar, M.; Gewirtz, A. T. How diet can impact gut microbiota to promote or endanger health. *Curr. Opin. Gastroenterol.* **2017**, *33*, 417–421, doi:10.1097/MOG.0000000000000401.
4. Macfarlane, S.; Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **2003**, *62*, 67–72, doi:10.1079/PNS2002207.
5. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345, doi:10.1016/j.cell.2016.05.041.
6. Louis, P.; Flint, H. J. Formation of propionate and butyrate by the human colonic microbiota. *Environ. Microbiol.* **2017**, *19*, 29–41, doi:10.1111/1462-2920.13589.
7. Cummings, J. H. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763, doi:10.1136/GUT.22.9.763.
8. den Besten, G.; van Eunen, K.; Groen, A. K.; Venema, K.; Reijngoud, D.-J.; Bakker, B. M. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **2013**, *54*, 2325–2340, doi:10.1194/jlr.R036012.
9. Fredstrom, S. B.; Lampe, J. W.; Jung, H.-J. G.; Slavin, J. L. Apparent Fiber Digestibility and Fecal Short-Chain Fatty Acid Concentrations With Ingestion of Two Types of Dietary Fiber. *J. Parenter. Enter. Nutr.* **1994**, *18*, 14–19, doi:10.1177/014860719401800114.
10. Canfora, E. E.; van der Beek, C. M.; Jocken, J. W. E.; Goossens, G. H.; Holst, J. J.; Olde Damink, S. W. M.; Lenaerts, K.; Dejong, C. H. C.; Blaak, E. E. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci. Rep.* **2017**, *7*, 2360, doi:10.1038/s41598-017-02546-x.
11. Weitkunat, K.; Schumann, S.; Nickel, D.; Kappo, K. A.; Petzke, K. J.; Kipp, A. P.; Blaut, M.; Klaus, S. Importance of propionate for the repression of hepatic lipogenesis and improvement of insulin sensitivity in high-fat diet-induced obesity. *Mol. Nutr. Food Res.* **2016**, *60*, 2611–2621, doi:10.1002/mnfr.201600305.
12. Chambers, E. S.; Viardot, A.; Psichas, A.; Morrison, D. J.; Murphy, K. G.; Zac-Varghese, S. E. K.; MacDougall, K.; Preston, T.; Tedford, C.; Finlayson, G. S.; Blundell, J. E.; Bell, J. D.; Thomas, E. L.; Mt-Isa, S.; Ashby, D.; Gibson, G. R.; Kolida, S.; Dhillo, W. S.; Bloom, S. R.; Morley, W.; Clegg, S.; Frost, G. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* **2015**, *64*, 1744–1754, doi:10.1136/gutjnl-2014-307913.
13. Chen, Y.; Xu, C.; Huang, R.; Song, J.; Li, D.; Xia, M. Butyrate from pectin fermentation inhibits

intestinal cholesterol absorption and attenuates atherosclerosis in apolipoprotein E-deficient mice. *J. Nutr. Biochem.* **2018**, *56*, 175–182, doi:10.1016/j.jnutbio.2018.02.011.

14. Encarnação, J. C.; Abrantes, A. M.; Pires, A. S.; Botelho, M. F. Revisit dietary fiber on colorectal cancer: butyrate and its role on prevention and treatment. *Cancer Metastasis Rev.* **2015**, *34*, 465–478, doi:10.1007/s10555-015-9578-9.

15. Cummings, J. H.; Pomare, E. W.; Branch, W. J.; Naylor, C. P.; Macfarlane, G. T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **1987**, *28*, 1221–1227, doi:10.1136/gut.28.10.1221.

16. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Thomas Brenna, J. Branched-chain fatty acid content of foods and estimated intake in the USA. *Br. J. Nutr.* **2014**, *112*, 565–572, doi:10.1017/S0007114514001081.

17. Ran-Ressler, R. R.; Glahn, R. P.; Bae, S.; Brenna, J. T. Branched-Chain Fatty Acids in the Neonatal Gut and Estimated Dietary Intake in Infancy and Adulthood. In *Nestle Nutrition Institute workshop series*; 2013; Vol. 77, pp. 133–143.

18. Liu, L.; Wang, Z.; Park, H. G.; Xu, C.; Lawrence, P.; Su, X.; Wijendran, V.; Walker, W. A.; Kothapalli, K. S. D.; Brenna, J. T. Human fetal intestinal epithelial cells metabolize and incorporate branched chain fatty acids in a structure specific manner. *Prostaglandins, Leukot. Essent. Fat. Acids* **2017**, *116*, 32–39, doi:10.1016/j.plefa.2016.12.002.

19. Su, X.; Magkos, F.; Zhou, D.; Eagon, J. C.; Fabbrini, E.; Okunade, A. L.; Klein, S. Adipose tissue monomethyl branched-chain fatty acids and insulin sensitivity: Effects of obesity and weight loss. *Obesity* **2015**, *23*, 329–334, doi:10.1002/oby.20923.

20. Carrera-Quintanar, L.; López Roa, R. I.; Quintero-Fabián, S.; Sánchez-Sánchez, M. A.; Vizmanos, B.; Ortuño-Sahagún, D. Phytochemicals That Influence Gut Microbiota as Prophylactics and for the Treatment of Obesity and Inflammatory Diseases. *Mediators Inflamm.* **2018**, *2018*, 1–18, doi:10.1155/2018/9734845.

21. Woodside, J. V.; Draper, J.; Lloyd, A.; McKinley, M. C. Use of biomarkers to assess fruit and vegetable intake. *Proc. Nutr. Soc.* **2017**, *76*, 308–315, doi:10.1017/S0029665117000325.

22. Bohn, T. Bioactivity of Carotenoids - Chasms of Knowledge. *Int. J. Vitam. Nutr. Res.* **2017**, *87*, 5–9, doi:10.1024/0300-9831/a000400.

23. Lyu, Y.; Wu, L.; Wang, F.; Shen, X.; Lin, D. Carotenoid supplementation and retinoic acid in immunoglobulin A regulation of the gut microbiota dysbiosis. *Exp. Biol. Med.* **2018**, *243*, 613–620, doi:10.1177/1535370218763760.

24. Liu, H.; Liu, M.; Fu, X.; Zhang, Z.; Zhu, L.; Zheng, X.; Liu, J. Astaxanthin Prevents Alcoholic Fatty Liver Disease by Modulating Mouse Gut Microbiota. *Nutrients* **2018**, *10*, 1298, doi:10.3390/nu10091298.

25. McEneny, J.; Wade, L.; Young, I. S.; Masson, L.; Duthie, G.; McGinty, A.; McMaster, C.; Thies, F. Lycopene intervention reduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. *J. Nutr. Biochem.* **2013**, *24*, 163–168, doi:10.1016/j.jnutbio.2012.03.015.

26. Silva, L. S. e; de Miranda, A. M.; de Brito Magalhães, C. L.; dos Santos, R. C.; Pedrosa, M. L.; Silva, M. E. Diet supplementation with beta-carotene improves the serum lipid profile in rats fed a cholesterol-enriched diet. *J. Physiol. Biochem.* **2013**, *69*, 811–820, doi:10.1007/s13105-013-0257-4.

27. Skiepko, N.; Chwastowska-Siwiecka, I.; Kondratowicz, J.; Mikulski, D. Fatty acid profile, total cholesterol, vitamin content, and TBARS value of turkey breast muscle cured with the addition of lycopene. *Poult. Sci.* **2016**, *95*, 1182–1190, doi:10.3382/ps/pew005.

28. Demigné, C.; Morand, C.; Levrat, M. A.; Besson, C.; Moundras, C.; Rémésy, C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br. J. Nutr.* **1995**, *74*, 209–19, doi:10.1079/bjn19950124.

29. Chambers, E. S.; Preston, T.; Frost, G.; Morrison, D. J. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. *Curr. Nutr. Rep.* **2018**, *7*, 198–206, doi:10.1007/s13668-018-0248-8.

30. Fernández-Ballart, J. D.; Piñol, J. L.; Zazpe, I.; Corella, D.; Carrasco, P.; Toledo, E.; Perez-Bauer, M.; Martínez-González, M. Á.; Salas-Salvadó, J.; Martín-Moreno, J. M. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br. J. Nutr.* **2010**, *103*, 1808–1816, doi:10.1017/S0007114509993837.

31. Larsen, K. Creatinine assay by a reaction-kinetic principle. *Clin. Chim. Acta* **1972**, *41*, 209–217, doi:10.1016/0009-8981(72)90513-X.

32. Zhao, G.; Nyman, M.; Jönsson, J. Å. Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. *Biomed. Chromatogr. Biomed. Chromatogr* **2006**, *20*, 674–682, doi:10.1002/bmc.580.

33. Colmán-Martínez, M.; Martínez-Huélamo, M.; Miralles, E.; Estruch, R.; Lamuela-Raventós, R. M. A New Method to Simultaneously Quantify the Antioxidants: Carotenes, Xanthophylls, and Vitamin A in Human Plasma. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1–10, doi:10.1155/2016/9268531.

34. Abdul Rahim, M. B. H.; Chilloux, J.; Martinez-Gili, L.; Neves, A. L.; Myridakis, A.; Gooderham, N.; Dumas, M.-E. Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. *Acta Diabetol.* **2019**, *56*, 493–500, doi:10.1007/s00592-019-01312-x.

35. Mathew, O. P.; Ranganna, K.; Mathew, J.; Zhu, M.; Yousefipour, Z.; Selvam, C.; Milton, S. G. Cellular Effects of Butyrate on Vascular Smooth Muscle Cells are Mediated through Disparate Actions on Dual Targets, Histone Deacetylase (HDAC) Activity and PI3K/Akt Signaling Network. *Int. J. Mol. Sci.* **2019**, *20*, 2902, doi:10.3390/ijms20122902.

36. Venter, C. S.; Vorster, H. H.; Cummings, J. H. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am. J. Gastroenterol.* **1990**, *85*, 549–53.

37. Wolever, T. M. S.; Spadafora, P.; Eshuis, H. Interaction between colonic acetate and propionate in humans. *Am. J. Clin. Nutr.* **1991**, *53*, 681–687, doi:10.1093/ajcn/53.3.681.

38. Lin, H. V.; Frassetto, A.; Kowalik Jr, E. J.; Nawrocki, A. R.; Lu, M. M.; Kosinski, J. R.; Hubert, J. A.; Szeto, D.; Yao, X.; Forrest, G.; Marsh, D. J. Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. *PLoS One* **2012**, *7*, e35240, doi:10.1371/journal.pone.0035240.

39. Sivaprakasam, S.; Prasad, P. D.; Singh, N. Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. *Pharmacol. Ther.* **2016**, *164*, 144–151, doi:10.1016/j.pharmthera.2016.04.007.

40. Fuhrman, B.; Elis, A.; Aviram, M. Hypocholesterolemic Effect of Lycopene and β-Carotene Is Related to Suppression of Cholesterol Synthesis and Augmentation of LDL Receptor Activity in Macrophages. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 658–662, doi:10.1006/bbrc.1997.6520.

41. Cheng, H. M.; Koutsidis, G.; Lodge, J. K.; Ashor, A.; Siervo, M.; Lara, J. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. *Atherosclerosis* **2017**, *257*, 100–108, doi:10.1016/j.atherosclerosis.2017.01.009.

42. Palozza, P.; Catalano, A.; Simone, R. E.; Mele, M. C.; Cittadini, A. Effect of Lycopene and Tomato Products on Cholesterol Metabolism. *Ann. Nutr. Metab.* **2012**, *61*, 126–134, doi:10.1159/000342077.

43. Daniels, J.-A.; Mulligan, C.; McCance, D.; Woodside, J. V; Patterson, C.; Young, I. S.; McEneny, J. A randomised controlled trial of increasing fruit and vegetable intake and how this influences the carotenoid concentration and activities of PON-1 and LCAT in HDL from subjects with type 2 diabetes. *Cardiovasc. Diabetol.* **2014**, *13*, 16, doi:10.1186/1475-2840-13-16.

44. Steinbusch, K. J. J.; Hamelers, H. V. M.; Plugge, C. M.; Buisman, C. J. N. Biological formation of caproate and caprylate from acetate: fuel and chemical production from low grade biomass. *Energy Environ. Sci.* **2011**, *4*, 216–224, doi:10.1039/C0EE00282H.

45. Grootscholten, T. I. M.; Kinsky dal Borgo, F.; Hamelers, H. V. M.; Buisman, C. J. N. Promoting chain elongation in mixed culture acidification reactors by addition of ethanol. *Biomass and Bioenergy* **2013**, *48*, 10–16, doi:10.1016/J.BIOMBIOE.2012.11.019.

46. Roghair, M.; Liu, Y.; Adiatma, J. C.; Weusthuis, R. A.; Bruins, M. E.; Buisman, C. J. N.; Strik, D. P. B. T. B. Effect of n-Caproate Concentration on Chain Elongation and Competing Processes. *ACS Sustain. Chem. Eng.* **2018**, *6*, 7499–7506, doi:10.1021/acssuschemeng.8b00200.

47. Grootscholten, T. I. M.; Steinbusch, K. J. J.; Hamelers, H. V. M.; Buisman, C. J. N. High rate heptanoate production from propionate and ethanol using chain elongation. *Bioresour. Technol.* **2013**, *136*, 715–718, doi:10.1016/j.biortech.2013.02.085.

48. Ilhan, Z. E.; Marcus, A. K.; Kang, D.-W.; Rittmann, B. E.; Krajmalnik-Brown, R. pH-Mediated Microbial and Metabolic Interactions in Fecal Enrichment Cultures. *mSphere* **2017**, 2, doi:10.1128/mSphere.00047-17.

49. Collins, J. K.; Perkins-Veazie, P.; Roberts, W. Lycopene: From Plants to Humans. *HortScience* **2006**, *41*, 1135–1144, doi:10.21273/HORTSCI.41.5.1135.

50. Djuric, Z.; Bassis, C. M.; Plegue, M. A.; Ren, J.; Chan, R.; Sidahmed, E.; Turgeon, D. K.; Ruffin,

M. T.; Kato, I.; Sen, A.; Sen, A. Colonic Mucosal Bacteria Are Associated with Inter-Individual Variability in Serum Carotenoid Concentrations. *J. Acad. Nutr. Diet.* **2018**, *118*, 606–616.e3, doi:10.1016/j.jand.2017.09.013.

51. Schnäbele, K.; Briviba, K.; Bub, A.; Roser, S.; Pool-Zobel, B. L.; Rechkemmer, G. Effects of carrot and tomato juice consumption on faecal markers relevant to colon carcinogenesis in humans. *Br. J. Nutr.* **2008**, *99*, 606–13, doi:10.1017/S0007114507819143.

52. Periago, M. J.; Martín-Pozuelo, G.; González-Barrio, R.; Santaella, M.; Gómez, V.; Vázquez, N.; Navarro-González, I.; García-Alonso, J. Effect of tomato juice consumption on the plasmatic lipid profile, hepatic HMGCR activity, and fecal short chain fatty acid content of rats. *Food Funct.* **2016**, *7*, 4460–4467, doi:10.1039/C6FO00344C.

53. Hwang, D.; Koh, J.-H.; Lee, J.; Kim, Y.; Lim, Y.-H. Cherry tomato supplementation increases the area of the intestinal mucosa and the number of muscle layers in rats. *Food Res. Int.* **2014**, *64*, 298–304, doi:10.1016/j.foodres.2014.06.041.

4.5. Publicación 6: Efecto del consumo de hortalizas y frutas sobre la prognosis en supervivientes de cáncer: una revisión sistemática y meta-análisis.

Artículo titulado "Effect of vegetable and fruit consumption on prognosis among cancer survivors: A systematic review and meta-analysis" Enviado a la revista científica "*Advances in Nutrition*" (Índice de impacto: 7,240 (2018); Q1, D1).

El envejecimiento de la población y otros factores de riesgo (como por ejemplo dietas no saludables, tabaquismo, exceso de alcohol e inactividad física) incrementan la incidencia de cáncer. Sin embargo, la tasa de supervivencia ha aumentado en los últimos años principalmente por la diagnosis temprana y los avances en la medicina en cuanto a los tratamientos y cuidado de los pacientes. Los efectos beneficiosos de consumir hortalizas y frutas en la prevención de la incidencia de cáncer han sido ampliamente estudiados y el nivel de evidencia de los resultados ha sido descrito en el informe redactado por el WCRF/AICR. Sin embargo, las investigaciones en pacientes para mejorar su prognosis son muy escasas (a excepción del cáncer de mama). Además de las posibles diferencias que pueden existir en cuanto a las recomendaciones a nivel de prevención primaria y terciaria (prognosis), también existen diferencias importantes según el tipo de cáncer. Debido a las especulaciones sobre las propiedades quimioproctectoras de los nutrientes y fitoquímicos que contienen las hortalizas y frutas, se ha llevado a cabo una revisión sistemática y meta-análisis para evaluar el efecto de su consumo sobre la prognosis del cáncer (recurrencia y mortalidad). Para ello se realizó una búsqueda bibliográfica exhaustiva en las bases de datos PubMed y Scopus y se seleccionaron 28 estudios de cohorte. Un elevado consumo de hortalizas fue inversamente correlacionado con la mortalidad total en pacientes de cáncer de cabeza y cuello (HR = 0,75, 95% CI, 0,65-0,87) y de ovario (HR= 0,78, 95% CI, 0,66-0,91). Además, en las pacientes de cáncer de ovario el consumo de frutas también parece proteger de la mortalidad por todas las causas (HR= 0,82, 95% CI, 0,70-0,96). Los resultados no fueron claros en el caso del linfoma no-Hodgkin (NHL), y en el resto de los cánceres la evidencia no fue suficiente. Sin embargo, el consumo de al menos 5 raciones (equivalente a 400 gramos) de hortalizas y frutas al día, acorde con las recomendaciones estipuladas por la OMS, parece ser extrapolable a los supervivientes de cáncer, particularmente de cabeza y cuello y de ovario. Las investigaciones en el resto de los tipos de cáncer son muy escasas, y para poder evaluar la asociación entre el consumo de hortalizas y frutas y la prognosis, deberían llevarse a cabo más estudios.

Advances in Nutrition: an International Review Journal

Effect of vegetable and fruit consumption on prognosis among cancer survivors: A systematic review and meta-analysis

–Manuscript Draft–

Manuscript Number:	ADVANCES-19-0246R1
Full Title:	Effect of vegetable and fruit consumption on prognosis among cancer survivors: A systematic review and meta-analysis
Short Title:	Vegetable and fruit intake and cancer prognosis
Article Type:	Review
Keywords:	cancer; survival; mortality; recurrence; prognosis; vegetables; fruits; cohort; meta-analysis
Corresponding Author:	Raul Zamora-Ros, PhD Institut d'Investigacio Biomedica de Bellvitge Barcelona, SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut d'Investigacio Biomedica de Bellvitge
Corresponding Author's Secondary Institution:	
First Author:	Sara Hurtado-Barroso
First Author Secondary Information:	
Order of Authors:	Sara Hurtado-Barroso
	Marta Trius-Soler
	Rosa M. Lamuela-Raventós
	Raul Zamora-Ros, PhD
Order of Authors Secondary Information:	
Abstract:	The number of cancer survivors is growing rapidly worldwide, especially long-term survivors. Although a healthy diet with a high vegetable and fruit consumption is a key factor in primary cancer prevention, there is a lack of specific dietary recommendations for cancer survivors, except in the case of breast cancer (World Cancer Research Fund (WCRF) / American Institute Cancer Research (AICR) report). We therefore carried out a systematic review and meta-analysis of studies reporting on the associations between vegetable and fruit intake and cancer recurrence and mortality and all-cause mortality in cancer patients. After a comprehensive search of PubMed and Scopus databases, the results of 28 selected articles were analysed. A high vegetable intake was inversely associated with overall mortality in survivors of head and neck (hazard ratio (HR) = 0.75, 95% confidence interval (CI), 0.65-0.87) and ovarian cancer (HR= 0.78, 95% CI, 0.66-0.91). In ovarian cancer patients, fruit intake was also inversely associated with lower all-cause mortality (HR= 0.82, 95% CI, 0.70-0.96). Null results were obtained with non-Hodgkin lymphoma patients. The evidence was insufficient for survivors of other cancers, although these associations generally tended to be protective. Therefore, more studies are needed to clarify the role of dietary vegetables and fruits in the prognosis of these different types of cancer. To date, the general recommendation for consuming at least 5 servings of vegetables and fruits per day is also appropriate for cancer survivors, particularly those with head and neck, and ovarian tumours.
Additional Information:	
Question	Response
Designate alternate corresponding author	None

Effect of vegetable and fruit consumption on prognosis among cancer survivors: A systematic review and meta-analysis

Sara Hurtado-Barroso[1-3]#, Marta Trius-Soler[1,2]#, Rosa M. Lamuela-Raventós[1-3] and Raul Zamora-Ros[4]*

1 Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, 08028, Barcelona, Spain.

2 INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08028, Barcelona, Spain

3 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Madrid, 28029, Spain.

4 Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), 08908 L'Hospitalet de Llobregat (Barcelona), Spain.

# Both authors equally contributed.

* Correspondence: rzamora@idibell.cat; Tel.: +34-932607401

The word count for the entire manuscript: 4,951

The number of figures: 4 figures (Figure 1-4) and 1 figure as supplementary material (Figure S1).

The number of tables: 3 tables (Table 1-3) and 6 tables as supplementary material (Tables S1-S6).

Running title: Vegetable and fruit intake and cancer prognosis

Funding: This research was funded by the Instituto de Salud Carlos III through the grant CP15/00100 and PI18/00191 (Co-funded by European Regional Development Fund. ERDF, a way to build Europe), the CYCIT from the Ministerio de Ciencia, Innovación y Universidades (grant number AGL2016-75329-R), the Instituto de Salud Carlos III, ISCIII (CIBEROBN) and Generalitat de Catalunya (GC) 2017. We thank CERCA Program / Generalitat de Catalunya for institutional support. S.H.B. is grateful for the predoctoral scholarship FPU (FPU14/01715) from the Ministerio de Educación. M.T-S. is thankful for the APIF 2018-2019 fellowship from the University of Barcelona. R.Z-R would like to thank the "Miguel Servet" program (CP15/00100) from the Institute of Health Carlos III (Co-funded by the European Social Fund (ESF) - ESF investing in your future).

Conflicts of Interest: Dra. R.M.L.-R. reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia. Moreover, weekly vouchers and other organic products have been provided by Ecoveritas S.A. and sponsors previously named. Nevertheless, these foundations and sponsors were not involved in the study design, the

collection, analysis and interpretation of data, the writing of the manuscript, or the decision to submit the manuscript for publication. The rest of authors declare no conflict of interest.

Abbreviations: AICR American Institute Cancer Research; CI Confidence Interval; GRADE Grading of Recommendations Assessment, Development and Evaluation; HR Hazard Ratio; MOOSE Meta-analysis of Observational Studies in Epidemiology; NHL Non-Hodgkin Lymphoma; NOS Newcastle Ottawa Scale; PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RR risk ratio; STROBE Strengthening the Reporting of Observational Studies in Epidemiology; WCRF World Cancer Research Fund; WHO World Health Organization.

Abstract: The number of cancer survivors is growing rapidly worldwide, especially long-term survivors. Although a healthy diet with a high vegetable and fruit consumption is a key factor in primary cancer prevention, there is a lack of specific dietary recommendations for cancer survivors, except in the case of breast cancer (World Cancer Research Fund (WCRF) / American Institute Cancer Research (AICR) report). We therefore carried out a systematic review and meta-analysis of studies reporting on the associations between vegetable and fruit intake and cancer recurrence and mortality and all-cause mortality in cancer patients. After a comprehensive search of PubMed and Scopus databases, the results of 28 selected articles were analysed. A high vegetable intake was inversely associated with overall mortality in survivors of head and neck (hazard ratio (HR) = 0.75, 95% confidence interval (CI), 0.65-0.87) and ovarian cancer (HR= 0.78, 95% CI, 0.66-0.91). In ovarian cancer patients, fruit intake was also inversely associated with lower all-cause mortality (HR= 0.82, 95% CI, 0.70-0.96). Null results were obtained with non-Hodgkin lymphoma patients. The evidence was insufficient for survivors of other cancers, although these associations generally tended to be protective. Therefore, more studies are needed to clarify the role of dietary vegetables and fruits in the prognosis of these different types of cancer. To date, the general recommendation for consuming at least 5 servings of vegetables and fruits per day is also appropriate for cancer survivors, particularly those with head and neck, and ovarian tumours.

Keywords: cancer; survival; mortality; recurrence; prognosis; vegetables; fruits, cohort, meta-analysis.

1. Introduction

The global burden of cancer is increasing because of an aging and expanding population and a growing prevalence of unhealthy habits (1). In parallel, advances in early detection, treatment and supportive care have led to a rapid and steady rise in the number of cancer survivors worldwide (2). The proportion of people predicted to survive a diagnosis of cancer is increasing by ~ 3% per year (3), the majority now surviving 5 years or more (4). Indeed, in 2011, 50% of UK cancer patients had a 10-year survival rate (5). However, there is considerable variation according to the cancer site and stage (6). A cancer survivor is considered to be anyone who has been diagnosed with cancer, completed treatment with curative-intent (but not maintenance treatment) and is disease-free (no evidence of active cancer) (7).

One third of deaths from cancer are due to behavioural and dietary risk factors (e.g., high body fatness, low vegetable and fruit intake, lack of physical activity, and tobacco and alcohol consumption) (8). The role of diet and nutrition in the cancer burden is well-established (9), approximately 5% of cancers being exclusively attributed to dietary factors (10), without taking into account obesity (20%) and alcohol (4%). Islami et al. reported that 6.9% of cancers in a Chinese population and 1.9% of cancers in a US population were attributable to a low vegetable and fruit intake (11,12). In European and US cohorts, adherence to a healthy dietary pattern, such as the Mediterranean diet and WCRF/AICR dietary recommendations, has been inversely associated with overall cancer risk (13,14). In addition, an updated meta-analysis concluded that greater adherence to the Mediterranean diet was associated with a lower risk and mortality of several cancer types, especially colorectal cancer (15). Other dietary quality indexes and dietary patterns have shown similar results (16,17).

Healthy diets are based largely on plant-based foods, above all vegetables and fruits, which are low in fat, especially saturated fat, high in fibre, and contain many vitamins, minerals and

phytochemical compounds (such as carotenoids, polyphenols and sulphur compounds) (18). Vegetable consumption has been associated with a reduced overall cancer mortality (17). However, vegetable and fruit intake was not related to cancer survival in breast cancer patients (19,20). Additionally, the WCRF/AICR report concluded that there is limited evidence linking a higher consumption of foods containing fibre with increased breast cancer survival (2). Among vegetable classes, the strongest associations with reduced cancer incidence have been found for green-yellow and cruciferous vegetables (21), which may be due to the chemopreventive properties of carotenoids and isothiocyanates, respectively (22). Among fruits, citrus fruits may have a relevant protective role against several cancers because of their high content of flavanones and vitamin C (23–27).

Apart from the WCRF/AICR recommendations for breast cancer survivors (2), there are no dietary guidelines for cancer survivors beyond those recommended for primary cancer prevention. There is therefore a need for specific dietary recommendations for cancer survivors. In this context, the aim of this work was to review the literature and conduct a meta-analysis, when it was possible, of epidemiological studies reporting associations between vegetable and fruit intake and prognosis in cancer, evaluating cancer recurrence, site-specific cancer mortality, and overall mortality in cancer survivors.

2. Materials and Methods

2.1. Data sources and search method

The literature search was performed by S.H-B. and M.T-S. using PubMed and Scopus databases (from their inception to March 2019). The following search terms were used: (cancer OR neoplasm OR carcinoma) AND (mortality OR survival OR recurrence OR prognosis OR

outcome OR death) AND (vegetable OR fruit). The search was restricted to the English language. In addition, a human filter was used in the PubMed database. References to reviews and recovered articles were also checked. This work was conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (28) and MOOSE (Meta-analysis of Observational Studies in Epidemiology) (29) guidelines.

2.2. Inclusion and exclusion criteria

The study selection was carried out by 2 authors (S.H-B. and M.T-S.). Full-text articles were selected according to the following inclusion criteria: 1) cohort study design, 2) vegetable and fruit intake pre- and post-diagnosis as exposure, 3) overall mortality, site-specific cancer mortality, cancer recurrence and prognosis as end-points, and 4) risk (HR or risk ratio (RR) with 95% CI) estimated and adjusted by confounding variables. Exclusion criteria were 1) duplicated studies, 2) *in vitro* or animal studies, 3) clinical trials, ecological studies, editorials, reviews and meta-analyses, 4) outcomes associated with incidence risk, 5) non- vegetable and fruit foods and dietary patterns, 6) breast cancer survivors, and 7) no RR or HR (with 95% CI).

2.3. Data extraction

Discrepancies in data from the selected studies were discussed by S.H-B and M.T-S. For each study, the model adjusted for the highest number of confounding variables was extracted. Studies were classified and aggregated by cancer site (bladder, colorectal, head and neck (including oral cavity, pharynx and larynx), gastric, lung, melanoma, non-Hodgkin lymphoma (NHL), oesophagus, ovarian, pancreas, and prostate tumours). Data from each study included in the systematic review and meta-analysis were the following: 1) cancer site, 2) outcome

(cancer and overall mortality and recurrence), 3) identification of cohort (country, name of study), 4) follow-up, 5) sample characterization (size, number of cases, age and sex of subjects), 6) dietary assessment and timeframe, 7) exposures and their extreme categories (such as highest vs. lowest), 8) risk estimated as HR/RR (95% CI), 9) adjustments for confounding variables, and 10) author and year of study.

2.4. Study quality assessment

The quality of each study was independently checked and discussed by S.H-B. and M.T-S. Any discrepancies in the study inclusion, data extraction and quality assessment were resolved with the support of a third person (R.Z-R). To evaluate the risk of bias in individual studies, two validated scales were used: the STROBE-nut (extension of Strengthening the Reporting of Observational Studies in Epidemiology) and the NOS (Newcastle Ottawa Scale) checklists. STROBE-nut is focused on epidemiological studies related to nutrition and shares some common items with the original STROBE Statement (30). In the present work, 30 items were used to evaluate study quality. A scale of 9 points was used for the NOS Statement for cohort studies: selection (up 4 points), comparability (up 2 points) and outcome (up 3 points) (31). From the NOS scale, study quality was considered high when the score was equal to or higher than 7, moderate between 3 and 6 points, and low if the score was equal to or lower than 2.

2.5. Evidence quality assessment

The GRADE (Grading of Recommendations Assessment, Development and Evaluation) scale was used to evaluate the overall strength of evidence for each outcome (32). GRADE items were evaluated for each association and classified into very low, low, moderate or high. The

level of evidence was low due to the non-randomized controlled trial design of studies included in this meta-analysis, and outcomes were downgraded for risk of bias, inconsistency (heterogeneous studies, i.e. I^2>50%), indirectness (self-reported outcome or non-representativeness of cohorts), imprecision (higher CI threshold than HR or RR) and/or publication bias. Upgrading for large effect, dose response and no plausible confounding was also applied.

2.6. Statistical analysis

Prior to the analyses, the studies were classified by cancer site and outcome (i.e. cancer recurrence, site-specific cancer mortality, and overall mortality). The meta-analysis was performed by pooling the multivariable-adjusted RRs or HR of the highest dietary intake categories (e.g. total vegetables and fruits, total vegetables, and total fruits) compared to the lowest one if at least three studies reported data for the same exposure, cancer site, and outcome. Heterogeneity across studies was evaluated by the I^2 test and classified as low (~25%), moderate (~50%) or high (~75%) (33). A fixed-effects model was used when I2 was > 50% and a random-effects model when I^2 was ≤ 50%. Meta-analyses were performed with the metan function of the Stata software, version 14 (Stata Corp; College Station, TX, USA).

3. Results

3.1. Literature search and study characteristics

A total of 8,322 articles were identified from two databases (PubMed and Scopus). An additional 26 articles were included from other sources (reviews and manual searching). After

removing the duplicates, 6,035 articles remained potentially eligible. Among these, 5,998 were excluded after title and abstract screening due to exclusion criteria (reviews/meta-analysis, editorials, ecological studies, in vitro or animal studies, other outcomes and non-vegetable exposure). Thereafter, the full text of 86 articles was evaluated in detail. Finally, a total of 28 papers were included in the systematic review and meta-analysis (Figure 1). Of these, 16 studies were used for the qualitative review (34–49) and 12 for the quantitative meta-analysis (50–61).

3.2. Cohort studies

The studies included in the systematic review and meta-analysis examining the association of total vegetable and fruit intake with cancer prognosis are summarized in Tables 1-3, which focus on aerodigestive, genital and urinary, and other cancers, respectively. Results for vegetable and fruit subtypes are shown in Supplementary Tables S1-S3. A total of 18,278 males and females aged between 16 and 84 years from European, North American, East Asian and Australian cohorts were included in the systematic review and meta-analysis. The follow-up periods varied from 9.1 months to 16 years. Dietary information assessment was predominantly before cancer diagnosis. Regarding vegetable and fruit consumption, exposures were analysed mainly by comparing the highest with the lowest categories.

3.2.1. Vegetable and fruit intake and prognosis in aerodigestive cancer patients

Head and neck. After meta-analysing five cohort studies (34,50,54–56), an inverse association between total vegetable consumption and overall mortality (HR: 0.75; 95% CI, 0.65-0.87, I^2=0%) was observed in head and neck cancer patients, including oral cavity, pharynx and larynx (Figure 2A). A stronger association was detected for both all-cause and site-specific cancer mortality with post-diagnosis total vegetable intake than with pre-diagnosis intake in oral cavity and oropharynx cancer (Table 1). However, no association between all-cause

mortality and fruits or citrus fruits was observed (Figure 2B, and Supplementary Table S1). No studies were found on the consumption of vegetable subtypes.

Digestive tract. In a Norwegian study, a high consumption of fruits and berries was related to lower colorectal cancer recurrence (HR= 0.3; 95% CI= 0.1-0.9), but not in the other studies (Table 1). Total vegetable intake was not linked with prognosis in colorectal cancer survivors. Lower consumption of green leafy vegetables was associated with a higher risk of all-cause mortality in colon cancer patients (HR= 2.06; 95% CI= 1.10-3.86), but not in rectal cancer patients (Supplementary Table S1). The consumption of 3 or more servings of raw vegetables per week was related to a lower risk of site-specific cancer mortality in gastric cancer patients. There was no association between green vegetable intake and overall mortality in pancreatic cancer patients. A Chinese study reported that higher consumption of preserved vegetables was significantly correlated with a higher risk of all-cause mortality in esophagus cancer patients.

Respiratory tract. No association was found between vegetable and fruit consumption and overall survival in lung cancer patients (Table 1). However, high cruciferous vegetable intake was linked to lower site-specific cancer mortality (HR= 0.69; CI 95%=0.49-0.95) in female lung cancer patients from the Shanghai Women's Health Study (Supplementary Table S1).

3.2.2. Vegetable and fruit intake and prognosis in genital and urinary cancer patients

Ovary. The 4 meta-analyzed studies on ovarian cancer patients (57–60) showed an inverse association between total vegetable and total fruit intake and overall mortality (HR = 0.78; 95% CI, 0.66-0.91, I^2= 0% and HR= 0.82; 95% CI, 0.70-0.96, I^2= 0%, respectively) (Figures 3A and 3B). Regarding vegetable subgroups, no association between cruciferous vegetable consumption and overall mortality was detected (Figure S1). Null results were also found for the intake of other vegetable or fruit subgroups (e.g. green leafy, yellow and red vegetables and citrus fruits) (Supplementary Table S2).

Prostate. In prostate cancer patients, consumption of total fruits was inversely associated with all-cause mortality, but not with site-specific cancer mortality (Table 2). Raw vegetable intake was also inversely associated with all-cause and cancer-specific mortality (Supplementary Table S2), whereas no association was detected for the consumption of either total or cooked vegetables.

Bladder. In two studies on bladder cancer patients, the intake of total vegetables, fruits or both was not related to either all-cause mortality or cancer recurrence (Supplementary Table 2). No association with the consumption of cruciferous vegetables was found either (Supplementary Table S2).

3.2.3. Vegetable and fruit intake and prognosis in patients with other cancer sites

Non-Hodgkin lymphoma. Three studies have evaluated the relationship with overall mortality in patients with NHL (52,53,61), showing null results for intake of fruits (HR:0.83; 95% CI, 0.59-1.16; I^2=62.2%) and vegetables (HR:1.00; 95% CI, 0.88-1.13; I^2=0%) (Figures 4A and 4B, respectively). Heterogeneity in one of the meta-analyses was moderate-to-high, which was due to a statistically significant inverse association in one of the studies, the other studies giving null results. Protective effects were observed for green leafy vegetables and citrus fruits, but not other vegetable subtypes, such as cruciferous, bean, red and yellow vegetables.

Melanoma. The only study in melanoma patients, the Connecticut Skin Self-Examination Case-Control Study, found no association between daily consumption of fruits or weekly consumption of green salad and site-specific cancer survival.

3.3. Quality studies and overall strength of evidence

The quality of all individual studies was high (≥7 points) (Supplementary Table S4). More details about the items considered in the STROBE-nut scale are provided as supplementary

material (Supplementary Table S5). Few cohort studies, mainly prospective, were identified for each outcome, classified by cancer site. Thus, according to the GRADE scale, the evidence available for the association between vegetable and fruit intake and cancer prognosis is of low-certainty. Despite the low number of studies quantified in each analysis (between 3 and 5), heterogeneity was not observed. Nor were risk of bias or indirectness detected. Insufficient or null studies examined the association between vegetable consumption and prognosis or mortality in the other cancer sites.

4. Discussion

The aim of this systematic review and meta-analysis was to examine the available evidence for the relationship between vegetable and fruit consumption and cancer prognosis, except for breast cancer (2). Summarizing the findings, a higher survival was associated with vegetable and fruit intake in ovarian cancer patients, and with vegetable intake in head and neck cancer patients.

A 25% lower risk in all-cause mortality was observed in survivors of head and neck cancer consuming more vegetables, the association being strongest with the highest categories of total vegetable intake (8-10 servings/day) (54–56). Sandoval et al. observed a more pronounced risk reduction in all-cause and oral and oropharynx cancer mortality when considering the post-diagnosis vegetable intake compared to the pre-diagnosis intake (54). This suggests it is more useful to assess the diet after cancer diagnosis and treatment as it has more impact on prognosis. Although no significant association was found when comparing the highest and lowest categories of total vegetable intake with oral and oropharynx cancer recurrence, a 73% lower risk was observed among moderate consumers of vegetables (5-7 servings/week reported after the diagnosis) (54). In addition, a protective effect has been described for a whole-food diet rich

in vegetables and fruits regarding overall mortality in head and neck cancer patients (62). However, in the present meta-analysis, no association was found between head and neck cancer prognosis and fruit intake, in contrast with Crosignani et al., who reported a lower all-cause mortality in men consuming at least 8 vs. 1 or fewer servings/week of non-citrus fruits (56). Similar inverse associations have been observed between the intake of non-starchy vegetables and oral cancer risk (including mouth, pharynx, larynx and nasopharynx sites) in healthy populations, but not with fruit consumption (63).

There are few studies on prognosis in digestive tract cancer survivors (oesophagus (38), stomach (35,42), pancreas (40) and colorectum (39,43–45)), suggesting basically null results. However, the lowest consumption of green leafy vegetables (almost never vs. almost every day) was directly associated with a higher all-cause mortality in male colorectal cancer patients, particularly with colon cancer (39). Also, a higher consumption of fruits and berries was inversely related to colorectal cancer recurrence (44). The results of the WCRF/AICR report were similarly suggestive of an inverse relationship between a low vegetable and fruit intake and both incident colorectal and gastric cancer risks (63).

Vegetable and fruit consumption may be inversely related to the incidence of lung cancer (64,65), particularly in smoker populations (63,66). Although an uncertain relationship was found between lung cancer prognosis and total vegetable and fruit intake (46,47), Wu et al. reported 31% lower site-specific cancer mortality in female non-smoker lung cancer patients consuming ~120 g of cruciferous vegetables per day (41). The risk of lung cancer incidence was also lower among high consumers of cruciferous vegetables (66).

Our meta-analysis revealed that a higher vegetable and fruit intake was associated with a 22% and 18% lower overall mortality, respectively, in ovarian cancer patients. The strongest associations were observed in ovarian cancer patients consuming ≥5 portions of vegetables and

fruits per day (59). Almost identical protective effects were found with a higher adherence to the Healthy Eating Index (58). However, the results for the relationship between vegetable and fruit subgroup intake and ovarian cancer prognosis were inconclusive (57–60). Similarly, null results were described between vegetable and fruit intake and incident ovarian cancer risk in the WCRF/AICR report (63).

In an Italian cohort of prostate cancer patients, diets rich in fruits, mainly non-citrus fruits, and in both vegetables and fruits were associated with a lower risk of all-cause mortality (48). Regarding dietary patterns, Western diets were directly associated with overall and cause-specific cancer mortality, while Prudent and Mediterranean diets were not significantly related to overall mortality (67,68). No association was found between vegetable and fruit intake and the incidence of prostate cancer in earlier studies (69,70). Despite that, since cardiovascular disease is the major cause of death in prostate cancer survivors (71), a dietary pattern rich in vegetables, fruits and wholegrains is also typically recommended to cardiovascular prevention (72).

Vegetable and fruit consumption was not associated with prognosis in bladder cancer (36,49). The WCRF/AICR reports suggest the risk of bladder cancer is inversely related to the intake of non-starchy vegetables and fruits, although with limited evidence (63). Several meta-analyses have addressed this topic, also with inconsistent results (69,73–76).

Although a US-based study found an inverse association between vegetable and fruit consumption and total mortality in NHL cancer survivors (52), the overall results of our meta-analysis were null. Likewise, uncertain results were obtained for the intake of green leafy vegetables and citrus fruits (52,61). Regarding NHL incidence, previous meta-analyses suggest that a high intake of vegetables, as well as vegetables and fruits, was significantly related to a

20% lower NHL risk in healthy subjects (77,78). Although fruit intake was not associated with NHL risk (77,78), a diet rich in citrus fruits resulted in a 15% lower risk (78).

Food processing and cooking or its absence is another factor to be considered when assessing the effect of vegetable intake. Among studies assessing the relationship between raw vegetable consumption and cancer prognosis (36,42,48), two retrospective studies observed an association with 26% and 36% lower overall mortality in patients with gastric and prostate cancer, respectively (42,48). These results are similar to those reported for the incidence of upper gastrointestinal cancer, but not prostate cancer (79). The consumption of ≥1 serving per week of preserved vegetables was associated with higher all-cause mortality in patients with oesophagus cancer (38). Likewise, salt-preserved foods are related with a higher risk incidence of gastric and nasopharynx cancer (63). Mechanistically, high salt levels can i) modify the viscosity and integrity of the mucosa protecting the upper digestive tract; ii) increase the formation of carcinogenic N-nitroso compounds; iii) stimulate Helicobacter pylori colonization; and iv) produce cellular damage (80).

A high consumption of vegetables and fruits might counteract the significant risk of micronutrients deficiencies in cancer patients, due to the alteration of the digestion process and absorption of vitamins and minerals (71). In addition, vegetables and fruits are rich in phytochemicals with chemoprotective functions in the different stages of cancer (81–84). These involve the targeting of cellular signalling transduction pathways responsible for tumour progression, mainly by downregulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (81,85). Phytochemicals in vegetables and fruits also have antioxidant properties and are able to repair DNA damage and modulate phase I and II metabolic reactions (86,87). These compounds seem induce apoptosis, anti-proliferation, anti-invasion and autophagy by mechanisms such as caspase activation, cell cycle arrest and the inhibition of

neovascularization (81,88), through protein kinase pathways (81,89). In addition, phytochemicals can activate the p53 signalling pathway in response to stress signals and regulate the expression of tumour suppressor miRNAs (90–93). Vegetables and fruits are also rich in dietary fibre, which has been associated with a decrease in overall mortality in some cancers (2,94,95). The protective effect of dietary fibre lies mainly in its active modulation by the gut microbiota, resulting in the production of short-chain fatty acids with anticarcinogenic properties (96). Also relevant is that fibre may reduce the intestinal transit time and secondary bile acid production, and increase the faecal bulk (69). Furthermore, dietary fibre reduces the postprandial glucose and insulin response, as well as inflammation and oxidative stress biomarkers (97–99). Lastly, polyphenols and fibre can act on the microbiota and have been associated with a lower risk of cancer (96,100).

A limitation of the present study is that it is based on low-level evidence according to the GRADE scale, due to the observational design and the small number of studies carried out for each outcome and cancer site. Only cohort studies, mainly prospective, were included in the systematic review and meta-analysis. The low number of studies quantified in each subgroup (maximum of 4) precluded the performance of analyses of sensitivity. Nevertheless, according to the NOS scale, the quality of all the studies included was high (7-9 scores), which was corroborated by the items evaluated from the nut-STROBE scale. In general, studies were adjusted for the main potential confounders and the follow-up was of adequate length. Moreover, the population sample in most of the studies was representative of the general population, and the events were registered with medical certificates or report linkage. Another potential limitation is dietary measurement error, although vegetable and fruit consumption was assessed by validated dietary questionnaires, mainly those of food frequency. Consumption categories (quantiles) were based on the intake of each cohort, with considerable variability between studies, which complicates the comparison of results. Most studies only reported the

pre-diagnosis diet and therefore potential dietary changes after cancer diagnosis and treatment were not accounted for. In addition, some studies limited the analysis to smokers or non-smokers, or to males or females, and the results from these specific cohorts cannot be generalised. Another limitation was the disparity between studies in classifying vegetables when evaluating subgroup intake, in contrast with fruits, which are clearly divided into citrus and non-citrus. A homogeneous classification of vegetables should be established to facilitate interpretation of study results. Nevertheless, this meta-analysis supposes an advantage compared with previous studies. This investigation is focused on the associations between the intake of vegetables and fruits, as well as their subgroups, and cancer prognosis. To date, only recommendations of vegetable and fruit intake for breast cancer survivors have been published (2).

5. Conclusions

In the last decades, dietary recommendations have been mainly focused on the primary prevention of non-communicable diseases, for which the World Health Organization (WHO) suggests a consumption of 400 g/day (5 servings/day) of vegetables and fruits (101). In addition, a systematic and dose-response meta-analysis concluded that the consumption of 550-600 g/day (7-7.5 servings/day) of vegetables and fruits was associated with a ~14% lower risk of total incident cancer (102). To date, dietary recommendations for cancer prevention are also given to survivors, despite possible differences in the associations with cancer incidence and cancer prognosis. Both the American Cancer Society (ACS) nutritional and physical activity guidelines for cancer survivors and the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines on nutrition in cancer patients are in parallel with the current public health lines for adults, which recommend to follow a diet high in vegetables, fruits and wholegrains.

Those recommendations are based on the consideration that survivors have a high risk to suffer a second primary cancer or other chronic diseases (71,103).

According to our findings, the current recommendations based on the consumption of 5 or more servings of vegetables or fruits per day seem to be suitable to ovarian cancer patients. However, a higher amount of vegetables (8 or more servings per day) may increase survival in the case of head and neck cancer patients. It is important to bear in mind that none of the studies presented have detected a harmful relationship with the consumption of vegetables and fruits in cancer patients. Despite that, further studies taking into account diet after cancer diagnosis are warranted to establish specific dietary recommendations for other site-specific cancer survivors.

Overall, the results of our study are in agreement with the recommendations of the WCRF/AICR report (63) on cancer prevention and with the WHO recommendations for the general population (101). The general current advice for cancer survivors would be to consume at least 2 to 3 cups of vegetables and 1.5 to 2 cups of fruits per day in order to reduce the risk of cancer recurrence and mortality, as well as overall mortality.

Acknowledgments: Conceptualization, R.Z-R. and R.M.L-R.; methodology, M.T-S. and S.H-B.; analysis, M.T-S. and S.H-B.; writing—original draft preparation, S.H-B.; writing—review and editing, M.T-S., R.Z-R. and R.M.L-R. All authors have read and approved the final manuscript.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin [Internet]. 2015 [cited 2019 Jun 5];65:87–108. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25651787

2. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Survivors of breast and other cancers. Available at dietandcancerreport.org [Internet]. Available from: https://www.wcrf.org/sites/default/files/Cancer-Survivors.pdf

3. Guzzinati S, Virdone S, De Angelis R, Panato C, Buzzoni C, Capocaccia R, Francisci S, Gigli A, Zorzi M, Tagliabue G, et al. Characteristics of people living in Italy after a cancer diagnosis in 2010 and projections to 2020. BMC Cancer [Internet]. 2018 [cited 2019 Jun 5];18:169. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29426306

4. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, Bonaventure A, Valkov M, Johnson CJ, Estève J, et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet [Internet]. 2018 [cited 2019 Jun 5];391:1023–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29395269

5. Cancer Research UK (2017) Cancer Stats – Cancer Worldwide Statistics for the UK. Available from URL http://www.cancerresearchuk.org/health-professional/cancer-statistics; [Cited June 05, 2019]. [Internet]. [cited 2019 Jun 5]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics-for-the-uk

6. American Cancer Society. Cancer Facts & Figures 2019. Atlanta: American Cancer Society; 2019. [Internet]. Available from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-facts-and-figures-2019.pdf

7. Marzorati C, Riva S, Pravettoni G. Who Is a Cancer Survivor? A Systematic Review of Published Definitions. J Cancer Educ [Internet]. 2017 [cited 2019 Jun 5];32:228–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26854084

8. (OMS) OM de la S. Cancer [Internet]. [cited 2019 Aug 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer

9. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst [Internet]. 1981 [cited 2019 May 30];66:1191–308. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7017215

10. Colditz GA, Wei EK. Preventability of cancer: the relative contributions of biologic and social and physical environmental determinants of cancer mortality. Annu Rev Public Health [Internet]. 2012 [cited 2019 May 30];33:137–56. Available from: http://www.annualreviews.org/doi/10.1146/annurev-publhealth-031811-124627

11. Islami F, Chen W, Yu XQ, Lortet-Tieulent J, Zheng R, Flanders WD, Xia C, Thun MJ, Gapstur SM, Ezzati M, et al. Cancer deaths and cases attributable to lifestyle factors and infections in China, 2013. Ann Oncol Off J Eur Soc Med Oncol [Internet]. 2017 [cited 2019 May 30];28:2567–74. Available from: http://academic.oup.com/annonc/article/doi/10.1093/annonc/mdx342/3920777/Cancer-deaths-and-cases-attributable-to-lifestyle

12. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, Jacobs EJ, McCullough ML, Patel A V., Ma J, Soerjomataram I, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin [Internet]. 2018 [cited 2019 May 30];68:31–54. Available from: http://doi.wiley.com/10.3322/caac.21440

13. Couto E, Boffetta P, Lagiou P, Ferrari P, Buckland G, Overvad K, Dahm CC, Tjønneland A, Olsen A, Clavel-Chapelon F, et al. Mediterranean dietary pattern and cancer risk in the EPIC cohort. Br J Cancer [Internet]. 2011 [cited 2019 May 30];104:1493–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21468044

14. Jankovic N, Geelen A, Winkels RM, Mwungura B, Fedirko V, Jenab M, Illner AK, Brenner H, Ordóñez-Mena JM, Kiefte de Jong JC, et al. Adherence to the WCRF/AICR Dietary Recommendations for Cancer Prevention and Risk of Cancer in Elderly from Europe and the United States: A Meta-Analysis within the CHANCES Project. Cancer Epidemiol Biomarkers Prev [Internet]. 2017 [cited 2019 May 30];26:136–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27793797

15. Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G. Adherence to Mediterranean Diet and Risk of Cancer: An Updated Systematic Review and Meta-Analysis. Nutrients [Internet]. 2017 [cited 2019 May 30];9:1063. Available from: http://www.mdpi.com/2072-6643/9/10/1063

16. Milajerdi A, Namazi N, Larijani B, Azadbakht L. The Association of Dietary Quality Indices and Cancer Mortality: A Systematic Review and Meta-analysis of Cohort Studies. Nutr Cancer [Internet]. Taylor & Francis; 2018 [cited 2019 May 30];70:1091–105. Available from: https://www.tandfonline.com/doi/full/10.1080/01635581.2018.1502331

17. Schwedhelm C, Boeing H, Hoffmann G, Aleksandrova K, Schwingshackl L. Effect of diet on mortality and cancer recurrence among cancer survivors: a systematic review and meta-analysis of cohort studies. Nutr Rev [Internet]. 2016 [cited 2019 May 30];74:737–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27864535

18. Yalcin H, Çapar TD. Bioactive Compounds of Fruits and Vegetables. Springer, Boston, MA; 2017 [cited 2019 Jul 3]. p. 723–45. Available from: http://link.springer.com/10.1007/978-1-4939-7018-6_21

19. Peng C, Luo W-P, Zhang C-X. Fruit and vegetable intake and breast cancer prognosis: a meta-analysis of prospective cohort studies. Br J Nutr [Internet]. 2017 [cited 2019 May 30];117:737–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28366183

20. He J, Gu Y, Zhang S. Consumption of vegetables and fruits and breast cancer survival: a systematic review and meta-analysis. Sci Rep [Internet]. Nature Publishing Group; 2017 [cited 2019 May 30];7:599. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28377568

21. Amiot-Carlin M-J. [Fruit and vegetable consumption: what benefits, what risks?]. Rev Prat [Internet]. 2019 [cited 2019 May 30];69:139–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30983210

22. Baena Ruiz R, Salinas Hernández P. Cancer chemoprevention by dietary phytochemicals: Epidemiological evidence. Maturitas [Internet]. 2016 [cited 2019 May 30];94:13–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378512216301888

23. Farooqi AA, Wang Z, Hasnain S, Attar R, Aslam A, Mansoor Q, Ismail M. Citrus fruits and their bioactive ingredients: leading four horsemen from front. Asian Pac J Cancer Prev [Internet]. 2015 [cited 2019 Jul 4];16:2575–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25824799

24. Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Laganà G, Daglia M, Meneghini S, Nabavi SM. Flavanones: Citrus phytochemical with health-promoting properties. BioFactors [Internet]. 2017 [cited 2019 Jul 4];43:495–506. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28497905

25. Zhao W, Liu L, Xu S. Intakes of citrus fruit and risk of esophageal cancer. Medicine (Baltimore) [Internet]. 2018 [cited 2019 Jul 4];97:e0018. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29595629

26. Bae J-M, Kim EH. Dietary intakes of citrus fruit and risk of gastric cancer incidence: an adaptive meta-analysis of cohort studies. Epidemiol Health [Internet]. 2016 [cited 2019 Jul 4];38:e2016034. Available from: http://e-epih.org/journal/view.php?doi=10.4178/epih.e2016034

27. Cirmi S, Navarra M, Woodside J V., Cantwell MM. Citrus fruits intake and oral cancer risk: A systematic review and meta-analysis. Pharmacol Res [Internet]. 2018 [cited 2019 Jul 4];133:187–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29753688

28. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med [Internet]. 2009 [cited 2019 May 30];6:e1000097. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19621072

29. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA [Internet]. 2000 [cited 2019 May 30];283:2008–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10789670

30. Lachat C, Hawwash D, Ocké MC, Berg C, Forsum E, Hörnell A, Larsson C, Sonestedt E, Wirfält E, Åkesson A, et al. Strengthening the Reporting of Observational Studies in Epidemiology—Nutritional Epidemiology (STROBE-nut): An Extension of the STROBE Statement. PLOS Med [Internet]. 2016 [cited 2019 May 30];13:e1002036. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27270749

31. GA Wells, B Shea, D O'Connell, J Peterson, V Welch, M Losos PT. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Internet]. [cited 2019 May 30]. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

32. Ryan R, Hill S. How to GRADE the quality of the evidence. Cochrane Consumers and Communications Group, available at http://cccrg.cochrane.org/author-resources. Version 3.0 December 2016 [Internet]. [cited 2019 May 30]. Available from: https://health-policy-systems.biomedcentral.com/articles/10.1186/s12961-019-0435-0

33. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ [Internet]. BMJ Publishing Group; 2003 [cited 2019 May 30];327:557–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12958120

34. Duffy SA, Ronis DL, McLean S, Fowler KE, Gruber SB, Wolf GT, Terrell JE. Pretreatment Health Behaviors Predict Survival Among Patients With Head and Neck Squamous Cell Carcinoma. J Clin Oncol [Internet]. 2009 [cited 2019 May 30];27:1969–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19289626

35. Ferronha I, Castro C, Carreira H, Bento MJ, Carvalho I, Peleteiro B, Lunet N. Prediagnosis lifestyle exposures and survival of gastric cancer patients: a cohort study from

Portugal. Br J Cancer [Internet]. Nature Publishing Group; 2012 [cited 2019 May 30];107:537–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22699821

36. Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB, McCann SE. Intake of cruciferous vegetables modifies bladder cancer survival. Cancer Epidemiol Biomarkers Prev [Internet]. 2010 [cited 2019 May 30];19:1806–11. Available from: http://cebp.aacrjournals.org/cgi/doi/10.1158/1055-9965.EPI-10-0008

37. Gould Rothberg BE, Bulloch KJ, Fine JA, Barnhill RL, Berwick M. Red meat and fruit intake is prognostic among patients with localized cutaneous melanomas more than 1mm thick. Cancer Epidemiol [Internet]. 2014 [cited 2019 May 30];38:599–607. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1877782114001453

38. Shi F, Yan F, Jin M, Chang H, Zhou Q, Zhao L, Hu Z, Song Q, Li J, He Y, et al. Pre-diagnosis consumption of preserved vegetables and prognosis of invasive oesophageal squamous cell carcinoma: a prospective cohort study in one high-risk area in China. J Int Med Res [Internet]. 2018 [cited 2019 May 30];46:4306–14. Available from: http://journals.sagepub.com/doi/10.1177/0300060518775585

39. Tamakoshi A, Nakamura K, Ukawa S, Okada E, Hirata M, Nagai A, Matsuda K, Kamatani Y, Muto K, Kiyohara Y, et al. Characteristics and prognosis of Japanese colorectal cancer patients: The BioBank Japan Project. J Epidemiol [Internet]. 2017 [cited 2019 May 30];27:S36–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28214186

40. Pelucchi C, Galeone C, Polesel J, Manzari M, Zucchetto A, Talamini R, Franceschi S, Negri E, La Vecchia C. Smoking and body mass index and survival in pancreatic cancer patients. Pancreas [Internet]. 2014 [cited 2019 May 30];43:47–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24177141

41. Wu Q-J, Yang G, Zheng W, Li H-L, Gao J, Wang J, Gao Y-T, Shu X-O, Xiang Y-B. Pre-diagnostic cruciferous vegetables intake and lung cancer survival among Chinese women. Sci Rep [Internet]. 2015 [cited 2019 May 30];5:10306. Available from: http://www.nature.com/articles/srep10306

42. Huang X-E, Tajima K, Hamajima N, Kodera Y, Yamamura Y, Xiang J, Tominaga S, Tokudome S. Effects of Dietary, Drinking, and Smoking Habits on the Prognosis of Gastric Cancer. Nutr Cancer [Internet]. 2000 [cited 2019 May 30];38:30–6. Available from: http://www.tandfonline.com/doi/abs/10.1207/S15327914NC381_5

43. Kunzmann AT, Coleman HG, Huang W-Y, Cantwell MM, Kitahara CM, Berndt SI. Fruit and vegetable intakes and risk of colorectal cancer and incident and recurrent adenomas in the PLCO cancer screening trial. Int J cancer [Internet]. 2016 [cited 2019 May 30];138:1851–61. Available from: http://doi.wiley.com/10.1002/ijc.29922

44. Almendingen K, Hofstad B, Vatn MH. Dietary habits and growth and recurrence of colorectal adenomas: results from a three-year endoscopic follow-up study. Nutr Cancer [Internet]. 2004 [cited 2019 May 30];49:131–8. Available from: http://www.tandfonline.com/doi/abs/10.1207/s15327914nc4902_3

45. Dray X, Boutron-Ruault M-C, Bertrais S, Sapinho D, Benhamiche-Bouvier A-M, Faivre J. Influence of dietary factors on colorectal cancer survival. Gut [Internet]. 2003 [cited 2019 May 30];52:868–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12740344

46. Li W, Tse LA, Au JSK, Yu KS, Wang F, Yu IT. Prognostic value of alcohol consumption and some other dietary habits for survival in a cohort of Chinese men with lung cancer. Chin J Cancer [Internet]. 2017 [cited 2019 Jun 18];36:21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28187735

47. Skuladottir H, Tjoenneland A, Overvad K, Stripp C, Olsen JH. Does high intake of fruit and vegetables improve lung cancer survival? Lung Cancer [Internet]. 2006 [cited 2019 May 30];51:267–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169500205003259

48. Taborelli M, Polesel J, Parpinel M, Stocco C, Birri S, Serraino D, Zucchetto A. Fruit and vegetables consumption is directly associated to survival after prostate cancer. Mol Nutr Food Res [Internet]. 2017 [cited 2019 May 30];61:1600816. Available from: http://doi.wiley.com/10.1002/mnfr.201600816

49. Jochems SHJ, van Osch FHM, Reulen RC, van Hensbergen M, Nekeman D, Pirrie S, Wesselius A, van Schooten F-J, James ND, Wallace DMA, et al. Fruit and vegetable intake and the risk of recurrence in patients with non-muscle invasive bladder cancer: a prospective cohort study. Cancer Causes Control [Internet]. 2018 [cited 2019 May 30];29:573–9. Available from: http://link.springer.com/10.1007/s10552-018-1029-9

50. Lang S, Schimansky S, Beynon R, Penfold C, Davies A, Waylen A, Thomas S, Pring M, Pawlita M, Waterboer T, et al. Dietary behaviors and survival in people with head and neck cancer: Results from Head and Neck 5000. Head Neck [Internet]. 2019 [cited 2019 May 30]; Available from: http://doi.wiley.com/10.1002/hed.25660

51. Shen G-P, Xu F-H, He F, Ruan H-L, Cui C, Chen L-Z, Zeng Y-X, Jia W-H. Pretreatment lifestyle behaviors as survival predictors for patients with nasopharyngeal carcinoma. Yeudall A, editor. PLoS One [Internet]. 2012 [cited 2019 Jul 4];7:e36515. Available from: https://dx.plos.org/10.1371/journal.pone.0036515

52. Han X, Zheng T, Foss F, Holford TR, Ma S, Zhao P, Dai M, Kim C, Zhang Y, Bai Y, et al. Vegetable and fruit intake and non-Hodgkin lymphoma survival in Connecticut women.

Leuk Lymphoma [Internet]. 2010 [cited 2019 May 30];51:1047–54. Available from: http://www.tandfonline.com/doi/full/10.3109/10428191003690364

53. Leo QJN, Ollberding NJ, Wilkens LR, Kolonel LN, Henderson BE, Le Marchand L, Maskarinec G. Nutritional factors and non-Hodgkin lymphoma survival in an ethnically diverse population: the Multiethnic Cohort. Eur J Clin Nutr [Internet]. 2016 [cited 2019 May 30];70:41–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26330148

54. Sandoval M, Font R, Mañós M, Dicenta M, Quintana MJ, Bosch FX, Castellsagué X. The role of vegetable and fruit consumption and other habits on survival following the diagnosis of oral cancer: a prospective study in Spain. Int J Oral Maxillofac Surg [Internet]. 2009 [cited 2019 May 30];38:31–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0901502708003238

55. Dikshit RP, Boffetta P, Bouchardy C, Merletti F, Crosignani P, Cuchi T, Ardanaz E, Brennan P. Lifestyle habits as prognostic factors in survival of laryngeal and hypopharyngeal cancer: a multicentric European study. Int J cancer [Internet]. 2005 [cited 2019 May 30];117:992–5. Available from: http://doi.wiley.com/10.1002/ijc.21244

56. Crosignani P, Russo A, Tagliabue G, Berrino F. Tobacco and diet as determinants of survival in male laryngeal cancer patients. Int J cancer [Internet]. 1996 [cited 2019 May 30];65:308–13. Available from: http://doi.wiley.com/10.1002/%28SICI%291097-0215%2819960126%2965%3A3%3C308%3A%3AAID-IJC5%3E3.0.CO%3B2-3

57. Playdon MC, Nagle CM, Ibiebele TI, Ferrucci LM, Protani MM, Carter J, Hyde SE, Neesham D, Nicklin JL, Mayne ST, et al. Pre-diagnosis diet and survival after a diagnosis of ovarian cancer. Br J Cancer [Internet]. 2017 [cited 2019 May 30];116:1627–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28463959

58. Thomson CA, E Crane T, Wertheim BC, Neuhouser ML, Li W, Snetselaar LG, Basen-Engquist KM, Zhou Y, Irwin ML. Diet quality and survival after ovarian cancer: results from the Women's Health Initiative. J Natl Cancer Inst [Internet]. 2014 [cited 2019 May 30];106:dju314-dju314. Available from: https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/dju314

59. Dolecek TA, McCarthy BJ, Joslin CE, Peterson CE, Kim S, Freels SA, Davis FG. Prediagnosis food patterns are associated with length of survival from epithelial ovarian cancer. J Am Diet Assoc [Internet]. 2010 [cited 2019 May 30];110:369–82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002822309019488

60. Nagle CM, Purdie DM, Webb PM, Green A, Harvey PW, Bain CJ. Dietary influences on survival after ovarian cancer. Int J Cancer [Internet]. 2003 [cited 2019 May 30];106:264–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12800204

61. Ollberding NJ, Aschebrook-Kilfoy B, Caces DBD, Smith SM, Weisenburger DD, Chiu BC-H. Dietary intake of fruits and vegetables and overall survival in non-Hodgkin lymphoma. Leuk Lymphoma [Internet]. 2013 [cited 2019 May 30];54:2613–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23488609

62. Arthur AE, Peterson KE, Rozek LS, Taylor JM, Light E, Chepeha DB, Hébert JR, Terrell JE, Wolf GT, Duffy SA, et al. Pretreatment dietary patterns, weight status, and head and neck squamous cell carcinoma prognosis. Am J Clin Nutr [Internet]. 2013 [cited 2019 May 30];97:360–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23269814

63. Aicr, WCRF. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Wholegrains, vegetables and fruit and the risk

of cancer. [Internet]. Available from: https://www.wcrf.org/sites/default/files/Wholegrains-veg-and-fruit.pdf

64. Wang Y, Li F, Wang Z, Qiu T, Shen Y, Wang M. Fruit and vegetable consumption and risk of lung cancer: A dose–response meta-analysis of prospective cohort studies. Lung Cancer [Internet]. 2015 [cited 2019 Jul 8];88:124–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25747805

65. Wang M, Qin S, Zhang T, Song X, Zhang S. The effect of fruit and vegetable intake on the development of lung cancer: a meta-analysis of 32 publications and 20 414 cases. Eur J Clin Nutr [Internet]. 2015 [cited 2019 Jul 8];69:1184–92. Available from: http://www.nature.com/articles/ejcn201564

66. Vieira AR, Abar L, Vingeliene S, Chan DSM, Aune D, Navarro-Rosenblatt D, Stevens C, Greenwood D, Norat T. Fruits, vegetables and lung cancer risk: a systematic review and meta-analysis. Ann Oncol [Internet]. 2016 [cited 2019 Jul 8];27:81–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26371287

67. Yang M, Kenfield SA, Blarigan EL Van, Batista JL, Sesso HD, Ma J, Stampfer MJ, Chavarro JE. Dietary patterns after prostate cancer diagnosis in relation to disease-specific and total mortality. Cancer Prev Res (Phila) [Internet]. NIH Public Access; 2015 [cited 2019 May 30];8:545. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4452958/

68. Kenfield SA, DuPre N, Richman EL, Stampfer MJ, Chan JM, Giovannucci EL. Mediterranean Diet and Prostate Cancer Risk and Mortality in the Health Professionals Follow-up Study. Eur Urol [Internet]. 2014 [cited 2019 May 30];65:887–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0302283813008300

69. Bradbury KE, Appleby PN, Key TJ. Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Clin Nutr [Internet]. 2014 [cited 2019 Jul 7];100:394S–398S. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24920034

70. Meng H, Hu W, Chen Z, Shen Y. Fruit and vegetable intake and prostate cancer risk: A meta-analysis. Asia Pac J Clin Oncol [Internet]. 2014 [cited 2019 Jul 7];10:133–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23551391

71. Rock CL, Doyle C, Demark-Wahnefried W, Meyerhardt J, Courneya KS, Schwartz AL, Bandera E V., Hamilton KK, Grant B, McCullough M, et al. Nutrition and physical activity guidelines for cancer survivors. CA Cancer J Clin. Wiley; 2012;62:242–74.

72. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation [Internet]. 2019 [cited 2019 Sep 23];140:e596–e646. Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000000678

73. Yao B, Yan Y, Ye X, Fang H, Xu H, Liu Y, Li S, Zhao Y. Intake of fruit and vegetables and risk of bladder cancer: a dose–response meta-analysis of observational studies. Cancer Causes Control [Internet]. 2014 [cited 2019 Jul 7];25:1645–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25248495

74. Liu H, Wang X-C, Hu G-H, Guo Z-F, Lai P, Xu L, Huang T-B, Xu Y-F. Fruit and vegetable consumption and risk of bladder cancer. Eur J Cancer Prev [Internet]. 2015 [cited 2019 Jul 7];24:508–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25642791

75. Vieira AR, Vingeliene S, Chan DSM, Aune D, Abar L, Navarro Rosenblatt D, Greenwood DC, Norat T. Fruits, vegetables, and bladder cancer risk: a systematic review and meta-analysis. Cancer Med [Internet]. 2015 [cited 2019 Jul 7];4:136–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25461441

76. Xu C, Zeng X-T, Liu T-Z, Zhang C, Yang Z-H, Li S, Chen X-Y. Fruits and Vegetables Intake and Risk of Bladder Cancer. Medicine (Baltimore) [Internet]. 2015 [cited 2019 Jul 7];94:e759. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25929912

77. Chen G-C, Lv D-B, Pang Z, Liu Q-F. Fruits and vegetables consumption and risk of non-Hodgkin's lymphoma: A meta-analysis of observational studies. Int J Cancer [Internet]. 2013 [cited 2019 Jul 6];133:190–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23238796

78. Sergentanis TN, Psaltopoulou T, Ntanasis-Stathopoulos I, Liaskas A, Tzanninis I-G, Dimopoulos M-A. Consumption of fruits, vegetables, and risk of hematological malignancies: a systematic review and meta-analysis of prospective studies. Leuk Lymphoma [Internet]. 2018 [cited 2019 Jul 6];59:434–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28847196

79. Link LB, Potter JD. Raw versus cooked vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev [Internet]. 2004 [cited 2019 Jun 17];13:1422–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15342442

80. Aicr; WCRF World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Preservation and processing of foods. [Internet]. Available from: https://www.wcrf.org/sites/default/files/Preservation-and-processing-of-foods.pdf

81. Chen H, Liu RH. Potential Mechanisms of Action of Dietary Phytochemicals for Cancer Prevention by Targeting Cellular Signaling Transduction Pathways. J Agric Food Chem [Internet]. 2018 [cited 2019 Jun 18];66:3260–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29498272

82. Appari M, Babu KR, Kaczorowski A, Gross W, Her I. Sulforaphane, quercetin and catechins complement each other in elimination of advanced pancreatic cancer by miR-let-7 induction and K-ras inhibition. Int J Oncol [Internet]. 2014 [cited 2019 Jun 18];45:1391–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25017900

83. Chen J, Song Y, Zhang L. Lycopene/tomato consumption and the risk of prostate cancer: a systematic review and meta-analysis of prospective studies. J Nutr Sci Vitaminol (Tokyo) [Internet]. 2013 [cited 2019 Jun 18];59:213–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23883692

84. Katz E, Nisani S, Chamovitz DA. Indole-3-carbinol: a plant hormone combatting cancer. F1000Research [Internet]. 2018 [cited 2019 Jun 18];7:689. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29904587

85. Prasad S, Ravindran J, Aggarwal BB. NF-κB and cancer: how intimate is this relationship. Mol Cell Biochem [Internet]. 2010 [cited 2019 Jun 18];336:25–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19823771

86. Obermeier MT, White RE, Yang CS. Effects of bioflavonoids on hepatic P450 activities. Xenobiotica [Internet]. 1995 [cited 2019 Jun 21];25:575–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7483658

87. Uno T, Ogura C, Izumi C, Nakamura M, Yanase T, Yamazaki H, Ashida H, Kanamaru K, Yamagata H, Imaishi H. Point mutation of cytochrome P450 2A6 (a polymorphic variant

CYP2A6.25) confers new substrate specificity towards flavonoids. Biopharm Drug Dispos [Internet]. 2015 [cited 2019 Jun 21];36:552–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26222491

88. Arumugam A, Abdull Razis AF. Apoptosis as a Mechanism of the Cancer Chemopreventive Activity of Glucosinolates: a Review. Asian Pac J Cancer Prev [Internet]. 2018 [cited 2019 Jun 18];19:1439–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29936713

89. Burotto M, Chiou VL, Lee J-M, Kohn EC. The MAPK pathway across different malignancies: A new perspective. Cancer [Internet]. 2014 [cited 2019 Jun 21];120:3446–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24948110

90. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell [Internet]. 1995 [cited 2019 Jun 21];80:293–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7834749

91. van Ginkel PR, Yan MB, Bhattacharya S, Polans AS, Kenealey JD. Natural products induce a G protein-mediated calcium pathway activating p53 in cancer cells. Toxicol Appl Pharmacol [Internet]. 2015 [cited 2019 Jun 21];288:453–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26341291

92. Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. J Pathol [Internet]. 2011 [cited 2019 Jun 21];223:102–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21125669

93. Hagiwara K, Gailhouste L, Yasukawa K, Kosaka N, Ochiya T. A robust screening method for dietary agents that activate tumour-suppressor microRNAs. Sci Rep [Internet]. 2015 [cited 2019 Jun 21];5:14697. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26423775

94. Song M, Wu K, Meyerhardt JA, Ogino S, Wang M, Fuchs CS, Giovannucci EL, Chan AT. Fiber Intake and Survival After Colorectal Cancer Diagnosis. JAMA Oncol [Internet]. 2018 [cited 2019 May 30];4:71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29098294

95. Xu H, Ding Y, Xin X, Wang W, Zhang D. Dietary fiber intake is associated with a reduced risk of ovarian cancer: a dose-response meta-analysis. Nutr Res [Internet]. 2018 [cited 2019 Jul 7];57:1–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30122191

96. Kawabata K, Yoshioka Y, Terao J. Role of Intestinal Microbiota in the Bioavailability and Physiological Functions of Dietary Polyphenols. Molecules [Internet]. 2019 [cited 2019 Jun 21];24:370. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30669635

97. Stewart ML, Zimmer JP. Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: a double-blind, randomized, controlled trial. Nutrition [Internet]. 2018 [cited 2019 Jun 21];53:59–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29655779

98. Solà R, Valls RM, Godàs G, Perez-Busquets G, Ribalta J, Girona J, Heras M, Cabré A, Castro A, Domenech G, et al. Cocoa, Hazelnuts, Sterols and Soluble Fiber Cream Reduces Lipids and Inflammation Biomarkers in Hypertensive Patients: A Randomized Controlled Trial. Biondi-Zoccai G, editor. PLoS One [Internet]. 2012 [cited 2019 Jun 21];7:e31103. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22383996

99. Xu X, Zhu Y, Li J, Wang S. Dietary fiber, glycemic index, glycemic load and renal cell carcinoma risk. Carcinogenesis [Internet]. 2019 [cited 2019 Jun 21];40:441–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30859214

100. Edwards CA, Havlik J, Cong W, Mullen W, Preston T, Morrison DJ, Combet E. Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota. Nutr Bull [Internet]. Wiley-Blackwell; 2017 [cited 2019 Feb 18];42:356–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29200959

101. World Health Organization (WHO)/Food and Agriculture Organization (FAO) of the United Nations. Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series. Vol. 916 [Internet]. 2003. Available from: https://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf;jsessionid=3879 36955093F88C3F42370BEA1219D2?sequence=1

102. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, Greenwood DC, Riboli E, Vatten LJ, Tonstad S. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. Int J Epidemiol [Internet]. Oxford University Press; 2017 [cited 2019 May 30];46:1029–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28338764

103. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, Fearon K, Hütterer E, Isenring E, Kaasa S, et al. ESPEN guidelines on nutrition in cancer patients. Clin Nutr. Churchill Livingstone; 2017;36:11–48.

Figure titles and legends

Figure 1. Flow chart of the study selection for the systematic review and meta-analysis.

Figure 2. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in head and neck cancer patients, comparing the highest vs. lowest intake of vegetable (A) and fruit (B) intake category.

Figure 3. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in ovarian cancer patients, comparing the highest vs. lowest vegetable (A) and fruit (B) intake categories.

Figure 4. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in non-Hodgkin lymphoma patients, comparing the highest vs. lowest vegetable (A) and fruit (B) intake categories.

Figure S1. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in ovarian cancer patients, comparing the highest vs. lowest cruciferous intake categories.

Table 1. Summary of studies included in the systematic review and meta-analysis evaluating association between total vegetable and fruit consumption and aerodigestive cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year [Ref.]
Head and neck	All-cause mortality	UK HN5000	3.2 (mean)	2,202 (445)	M/F ≥16	FFQ Pre-diagnosis (over the last previous year of diagnosis)	**Vegetables** T3 (>1 portions/day) vs. T1 (<5 portions/week) **Fruits** T3 (>1 portions/day) vs. T1 (<1 portions/week)	0.79 (0.61-1.03) 0.91 (0.67-1.23)	Age, gender, site, stage, comorbidity, treatment intent, education, relationship status, income, smoking, alcohol and fried food	Lang *et al.*, 2019 (50)
Nasopharyngeal	All-cause mortality	China ns	3.22 (median)	1533 (243)	M/F 46.1 (mean)	FFQ Pre-diagnosis (pretreatment evaluation)	**Fruits** Daily or more vs. fewer than monthly servings	0.78 (0.53-1.14)	Age, sex, marital status, education level, clinical stage, smoking status, alcohol intake and BMI	Shen *et al.*, 2012 (51)
Head and neck	All-cause mortality	US UM HN-SPORE	2.7 (median)	504 (166)	M/F 58.8 (mean)	Willet FFQ Pre-diagnosis (1 year prior the	**Vegetables** Lowest (none to 2-4 per week) vs. highest **Fruits**	0.82 (0.59-1.15) 1.26	Age, sex, race, sleep score, educational level, marital status, cancer site, tumor stage,	Duffy *et al.*, 2009 (34)

		program				newly	Lowest (none to 1-3 per	(0.88-1.81)	comorbidities, treatment			
						diagnosis)	month) vs. highest		received, smoking status,			
									alcohol consumption and			
									physical activity			
Oral cavity and oropharynx	All-cause mortality	Spain	3.13 (mean)	146 (74)	M/F	Questionnaire or a trained interviewer	**Vegetables** T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 0.54 (0.30-0.98) **Post:** 0.14 (0.04-0.50)	Age, gender, clinical stage, and tumor site	Sandoval *et al.*, 2009 (54)		
		ns			Categories: <50, 50-59, 60-69 and ≥70	Pre-diagnosis (baseline)	**Fruits** T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 1.26 (0.73-2.18) **Post:** 0.77 (0.36-1.64)				
						Post-diagnosis (1 year after diagnosis)	**Vegetables** T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 0.61 (0.29-1.31) **Post:** 0.14 (0.03-0.69)				
	Cancer cause-specific mortality		3.13 (mean)	146 (49)			**Fruits** T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 1.04 (0.52-2.10) **Post:** 0.89				

								(0.34-2.32)		
	Recurrence		3.63 (mean)	146 (47)			**Vegetables** T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 0.56 (0.27-1.15) **Post:** 0.43 (0.16-1.18)		
							Fruits T3 (≥8) vs. T1 (≤4) servings/week	**Pre:** 0.75 (0.37-1.52) **Post:** 0.68 (0.29-1.59)		
Laryngeal/ Hypopharyngeal	All-cause mortality	Switzerland, France, Italy and Spain Population-based case-control study	8 (mean)	931 (755)	M/F Categories: <50, 50-59, 60-69 and ≥70	Dietary history questionnaire Pre-diagnosis (1 year before symptoms)	**Vegetables** Q4 (≥773.42) vs. Q1 (≤148.57) g/day **Fresh fruit** Q4 (321.42) vs. Q1 (0) g/day	0.8 (0.6-0.9) 1.0 (0.7-1.2)	Age, gender, centre, site of primary tumor, alcohol drinking, cigarette smoking, caloric intake without alcohol and vegetable or fruit intake	Dikshit *et al*., 2005 (55)
Laryngeal	All-cause mortality	Italy	Up 10	215 (136)	M	Dietary questionnaire	**Vegetables** T3 (5414) vs. T1 (1415)	0.57 (0.35-0.94)	Age at diagnosis, clinical stage, occurrence of new	Crosignani *et al*., 1996 (56)

		Population-based case-control study		59 (median)		g/week Pre-diagnosis (1 year before diagnosis)			primaries and total calorie intake		
Gastric	All-cause mortality	Portugal	1.2 (median)	568 (345)	M/F	FFQ	**Vegetables and fruits** T1 (<2.67) vs. T3 (>4.30)	0.98 (0.75-1.28)	Age, sex, education, extent of diseases and total energy intake		Ferronha *et al.*, 2012 (35)
		Population based case-control study		63 (median)		Pre-diagnosis (previous year or the year before onset of symptoms)	servings/day				
Gastric	Cancer cause-specific mortality	Japan	Up to 10	877 (241)	M/F	Questionnaire given by a trained interviewer on food and beverage intake	**Fruits** Highest vs. Lowest Cut off: 3 times/week.	0.98 (0.73-1.31)	Age, gender, and pathological type and stage of cancer		Huang *et al.*, 2000 (42)
		ns*		40-79		Pre-diagnosis					

						(before examination and diagnosis, in the first visit to the hospital)				
Colorectal	Recurrence	US PLCO intervention group	10	1,667 (738)	M/F 55-74	137-item FFQ Pre-diagnosis (1 year before diagnosis)	**Vegetables** T3 (≥1.47) vs. T1 (<1.06) FPED cup equivalents/1,000 kcal/day **Fruits (excluding juice)** T3 (≥0.9) vs. T1 (<0.48) FPED cup equivalents/1,000 kcal/day **Vegetables and fruits** T3 (≥2.8) vs. T1 (<1.99) FPED cup equivalents/1,000 kcal/day	0.93 (0.69-1.25) 0.96 (0.70-1.32) 0.87 (0.64-1.20)	Age, sex, center, race, energy intake, year of follow-up screening, adenoma at T0, T3 or T5, adequate screening at TO, T3 or T5, processed meat intake per 1,000kcal and smoking history	Kunzmann *et al.*, 2016 (43)
Colorectal	Recurrence	Norway	3	87 (53)	M/F	5-Day Dietary Record	**Vegetables** Highest vs. lowest	1.2 (0.5-2.9)	Colorectal cancer in a first-degree relative, BMI	Almendingen *et al.*, 2004 (44)

		Cases of a placebo-controlled follow-up and intervention study		65 (median)		Post-diagnosis (3 years after inclusion)	Cut off: 110 g/day **Fruits and berries** Highest vs. lowest Cut off: 200 g/day	0.3 (0.1-0.9)		and type of intervention		
Colorectal	All-cause mortality	France Cases of a case-control study	10 5 (RR of all-cause mortality)	148 (46 deaths after 5 years and 70 after 10 years)	M/F 30-79	Questionnaire by meal and Questionnaire by list of foods Pre-diagnosis (1 year before diagnosis)	**Vegetables** T3 vs. T1 **Fruits** T3 vs. T1	1.09 (0.49-2.45) 0.84 (0.37-1.88)	Age, sex, tumor stage, tumor location, and energy intake		Dray *et al.*, 2003 (45)	
Lung	All-cause mortality	China Population from case–	9.1 months (median)	1052 (869) ≤80	M	FFQ Post-diagnosis (patients were	**Vegetables and fruits** Occasional consumer (<1 serving/day) vs. frequent consumer (≥1 serving/day)	0.86 (0.72-1.02)	District of residence, age at diagnosis, BMI, cancer history in first-degree relatives, education level,		Li *et al.*, 2017 (46)	

		control							family income, stage at		
		study				asked about			diagnosis, smoking		
						consumption of			status, smoking		
						past year)			pack-years, and treatment		
Lung	All-cause	Denmark	From	286 (ns)	M/F	192-item FFQ	**Vegetables**	0.84	Sex, age, extent of	Skuladotiir *et*
	mortality		1993-199				T3 (160-536) vs. T1	(0.59-1.21)	disease, duration of	*al*., 2006 (47)
		Diet, Cancer	7 to death	Current	50-64	Pre-diagnosis	(16-88)	0.81	smoking and potato and	
		and Health	or 2004	smokers			**Fruits (excluding juices)**	(0.58-1.15)	fruit/vegetable intake	
							T3 (143-671) vs. T1 (0-51)			
							g/day			

*Retrospective cohort study; BMI: body mass index; FFQ: food frequency questionnaire; HN5000: Head & Neck 5000; M/F: male and female; ns: not specified; PLCO:The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial

Table 2. Summary of studies included in the systematic review and meta-analysis evaluating association between total vegetable and fruit consumption and genital and urinary cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year [Ref.]
Ovarian	All-cause mortality	Australians	5.9 (mean)	811 (547)	F 18-79	135-item FFQ Pre-diagnosis (> 1 year before diagnosis)	**Vegetables** T3 (≥5) vs. T1 (<3) servings/day **Fruits** T3 (≥4) vs. T1 (none or <2) servings/day	0.88 (0.68-1.13) 0.82 (0.63-1.07)	Age at diagnosis, International Federation of Gynaecology and Obstetrics (FIGO) stage, amount of residual disease, grade, tumor subtype, smoking status, BMI, physical activity index, marital status, and daily caloric intake	Playdon *et al.*, 2017 (57)
Ovarian	All-cause mortality	US WHI	From 1995 to 2012	636 (354)	F 50-79	WHI FFQ Pre-diagnosis (>1 year before diagnosis)	**Vegetables** T3 (5) vs. T1 (2.5) points **Fruits** T3 (5) vs. T1 (2.5) points	0.70 (0.47-1.04) 0.82 (0.55-1.21)	Age at diagnosis, stage at diagnosis, race/ethnicity, diabetes, physical activity, total energy intake, waist circumference, family history of ovarian cancer, and clinical trial arms	Thomson *et al.*, 2014 (58)
	Cancer cause-specific mortality			636 (305)			**Vegetables** T3 (5) vs. T1 (2.5) points	0.76 (0.48-1.20)		

						Fruits	0.84					
						T3 (5) vs. T1 (2.5)	(0.54-1.28)					
						points						
Ovarian	All-cause	US	Up 10	341 (176)	F	60-item FFQ	**Vegetables**	0.66	Age group, race, stage, grade,			Dolecek
	mortality						T3 (≥14) vs. T1 (<7)	(0.43-1.01)	residual lesions, smoking status,			*et al.*,
		ns			18-74	**Pre-diagnosis**	Servings/week		BMI, oral contraceptive use,			2010 (59)
						(3-5 years	**Fruits**	0.67	parity, and total energy intake			
						before	T3 (≥14) vs. T1 (<7)	(0.44-1.04)				
						diagnosis)	Servings/week					
							Vegetables and	0.61				
							fruits	(0.38-0.98)				
							T3 (≥35) vs. T1					
							(<21)					
							Servings/week					
Ovarian	All-cause	Australia	7.3	609 (394)	F	119-item FFQ	**Vegetables**	0.75	FIGO stage, age, grade, total			Nagle *et*
	mortality		(mean)				T3 (>5.56) vs. T1	(0.57-0.99)	energy intake and BMI			*al.*, 2003
		ns			18-79	**Pre-diagnostic**	(<3.9) Servings/day					(60)
						(1 year before	**Fruits**	0.89				
						diagnostic)	T3 (>4.49) vs. T1	(0.67-1.18)				

							(<2.79)				
							Servings/day				
Prostate	All-cause	Italy	7.5	777 (263)	M	78-item FFQ	**Vegetables**	0.81	Area of residence at diagnosis,	Taborelli
	mortality		(median)				Q4 (≥203) vs. Q1	(0.56-1.16)	calendar period, age at diagnosis,	*et al.*,
					66	Pre-diagnosis	(<102) g/day		years of education, Gleason score,	2017 (48)
		ns*			(median)	(2 years before	**Fruits**	0.63	BMI, smoking habits and total	
						diagnosis)	Q4 (≥380) vs. Q1	(0.44-0.90)	energy intake	
							(<152) g/day			
							Vegetables	0.66		
							(cut off=146) **and**	(0.47-0.93)		
							fruits (cut off=247)			
	Cancer						**Vegetables**	0.82		
	cause-specific		5.7	777 (81)			Q4 (≥203) vs. Q1	(0.42-1.58)		
	mortality		(median)				(<102) g/day			
							Fruits	0.78		
							Q4 (≥380) vs. Q1	(0.38-1.61)		
							(<152) g/day			
							Vegetables	0.89		
							(cut off=146) **and**	(0.48-1.63)		

							fruits (cut off=247)			
Bladder	First recurrence	UK Part of BCPP	3.7 (mean)	Pre-diagnosis: 728 (241) Post-diagnosis: 389 (144)	M/F 69 (mean)	16-item FFQ Pre-diagnosis (1 year before diagnosis) Post-diagnosis (1 year after diagnosis)	**Vegetables** T3 (>2.5) vs. T1 (<1.5) portions/day **Fruits** T3 (>1.5) vs. T1 (<1) portions/day **Vegetables and fruits** T3 (>4) vs. T1 (<2.5) portions/day **Vegetables** T3 (>2.5) vs. T1	**Pre:** 1.02 (0.74-1.41) **Post:** 0.77 (0.50-1.18) **Pre:** 0.85 (0.63-1.14) **Post:** 1.07 (0.78-1.47) **Pre:** 1.07 (0.78-1.47) **Post:** 0.65 (0.42-1.01) **Pre:** 0.97 (0.86-1.11) **Post:** 0.96 (0.74-1.09) **Pre:** 1.05 (0.91-1.20)	Age, sex, smoking status, tumor stage, grade, size, and multiplicity (and additionally adjusted for re-resection of a bladder tumor (second transurethral resection) in the time to multiple recurrences analysis)	Jochems *et al.*, 2018 (49)
	Multiple recurrence			Pre-diagnosis: 728 (391) Post-diagnosis: 389 (221)						

						(<1.5)	**Post:**			
						portions/day	1.02			
							(0.87-1.20)			
						Fruits	**Pre:** 1.02			
						T3 (>1.5) vs. T1	(0.90-1.15)			
						(<1)	**Post:**			
						portions/day	1.00			
							(0.85-1.18)			
						Vegetables and				
						fruits				
						T3 (>4) vs. T1				
						(<2.5)				
						portions/day				
Bladder	All-cause	USA	8	239 (179)	M/F	44-item FFQ	**Vegetables**	0.91	Age at diagnosis, total meat intake, pack-years of smoking, tumor stage and radiation therapy	Tang *et al.*, 2010 (36)
	mortality		(mean)			Pre-diagnosis	T3 (>85.5) vs. T1	(0.62-1.36)		
		RPCI		239 (101)	Categories: <60, 60-70 and >70	(few years prior diagnosis)	(<52) servings/months	0.91		
	Cancer						**Fruits**	(0.62-1.33)		

cause-specific		T3 (>51) vs. T1	
mortality	6.42	(<27.5)	1.06
	(median)	servings/months	(0.63-1.78)
		Vegetables	
		T3 (>85.5) vs. T1	1.09
		(<52)	(0.66-1.81)
		servings/months	
		Fruits	
		T3 (>51) vs. T1	
		(<27.5)	
		servings/months	

BCPP: Bladder Cancer Prognosis Programme; BMI: body mass index; FFQ: food frequency questionnaire; M/F: male and female; ns: not specified; RPCI: Roswell Park Cancer Institute; WHI: Women's Health Initiative

Table 3. Summary of studies included in the systematic review and meta-analysis evaluating association between total vegetable and fruit consumption and other cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year [Ref.]
NHL	All-cause mortality	US ns	8.2 (mean)	301 (91)	M/F 20-75	117-item FFQ Pre-diagnosis (1 year before diagnosis)	**Vegetables** T3 (>102.1) vs. T1 (<66.1) g/1000 kcal/day **Fruits** T3 (>138.1) vs. T1 (<69.5) g/1000 kcal/day **Vegetables and fruits** T3 (>239.7) vs. T1 (<147.8) g/1000 kcal/day	0.9 (0.5-1.5) 0.9 (0.5-1.6) 1.1 (0.6-2.1)	Age, sex, education, smoking status and total energy intake	Ollberding *et al.*, 2013 (61)
NHL	All-cause mortality	US Population-based case-control study	7.7 (median)	568 (250)	F 21-84	FFQ Pre-diagnosis (1 year before diagnosis)	**Vegetables** Highest vs. Lowest Cut off: 3	0.58 (0.38-0.89)	Age, education, stage, B-symptom, initial treatment and total energy intake	Han *et al.*, 2010 (52)

			servings/day	0.91
			Fruits	(0.70-
			Highest vs. lowest	1.18)
			Cut off: 2	
			servings/day	0.68
Cancer			**Vegetables and**	(0.49-
cause-	568		**fruits**	0.95)
specific	(148)		Highest vs. lowest	
mortality			Cut off: 5	0.58
			servings/day	(0.33-
			Vegetables	1.03)
			Highest vs. lowest	
			Cut off: 3	1.04
			servings/day	(0.74-
			Fruits	1.45)
			Highest vs. lowest	
			Cut-off: 2	0.58
			servings/day	(0.33-
			Vegetables and	1.03)

							fruits				
							Highest vs. lowest				
							Cut off: 5 servings/day				
NHL	All-cause mortality	US MEC	4.5 (mean)	2,339 (1348)	M/F 45-75 (mean at diagnosis: 71.8)	56-item FFQ Pre-diagnosis (baseline)	**Vegetables** T3 (≥179.9) vs. T1 (<120.8) g/4184 KJ/day	0.98 (0.85-1.12)	Age at cohort entry, age at diagnosis, sex, BMI, education, comorbidity, NHL type, stage, treatment, smoking status and alcohol intake	Leo *et al.*, 2016 (53)	
							Fruits T3 (≥201.3) vs. T1 (<98.6) g/4184 KJ/day	1.03 (0.90-1.19)			
	Cancer cause-specific mortality			2,339 (903)			**Vegetables** T3 (≥179.9) vs. T1 (<120.8) g/4184 KJ/day	0.98 (0.83-1.16)			
							Fruits T3 (≥201.3) vs. T1 (<98.6)	1.04 (0.88-1.24)			

							g/4184 KJ/day				
Melanoma	Cancer cause-specific mortality	US From Connecticut Skin Self-Examination Case-Control Study	16 years (median)	249 (92)	M/F	Dietary interview Pre-diagnosis	**Fruits** At least daily vs. less than daily	0.66 (0.42-1.04)	Breslow thickness, age at diagnosis, sex, ulceration and microsatelliosis	Gould Rothberga et al., 2014 (37)	
					≤65 and >65 (at the time of diagnosis)						

BMI: body mass index; **FFQ**: food frequency questionnaire; **M/F**: male and female; **MEC**: Multiethnic Cohort Study; **NHL**: Non-Hodgkin lymphoma; **ns**: not specified

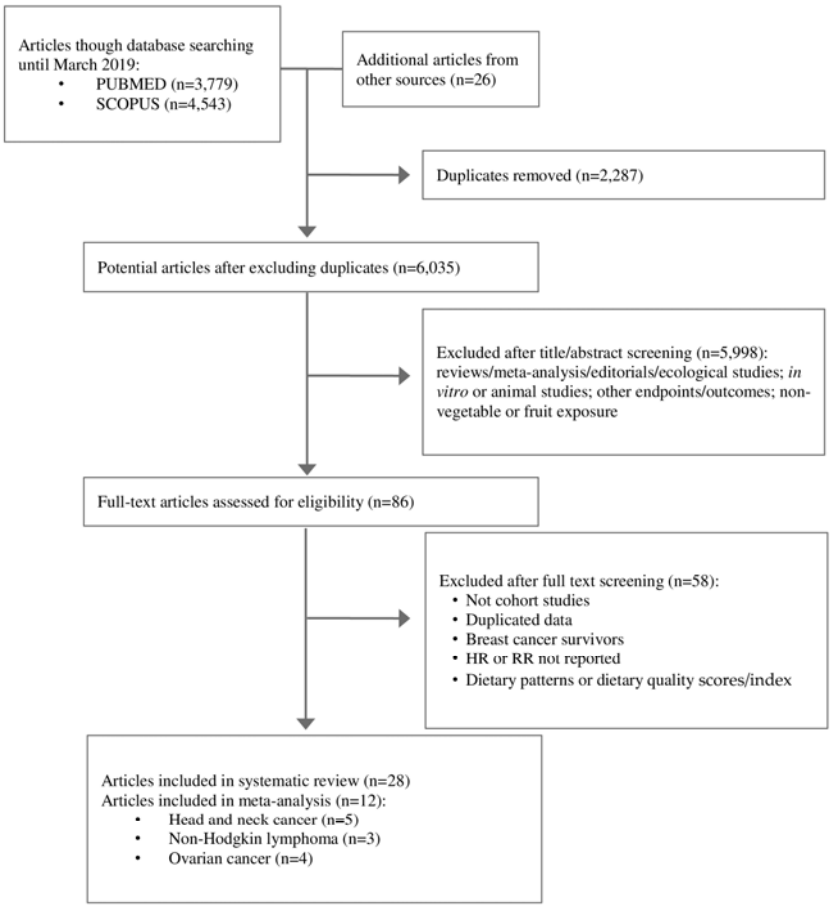


Figure 1. Flow chart of the study selection for the systematic review and meta-analysis.

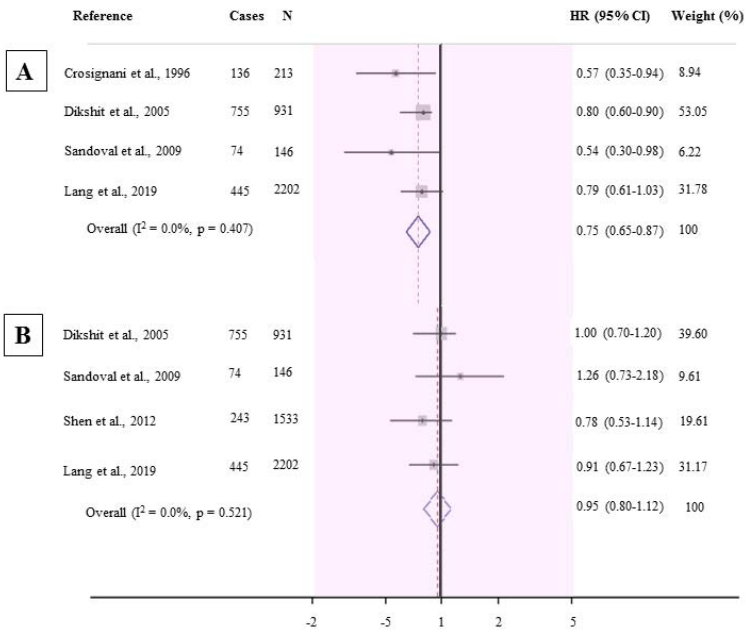


Figure 2. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in head and neck cancer patients, comparing the highest vs. lowest intake of vegetable (A) and fruit (B) intake category.

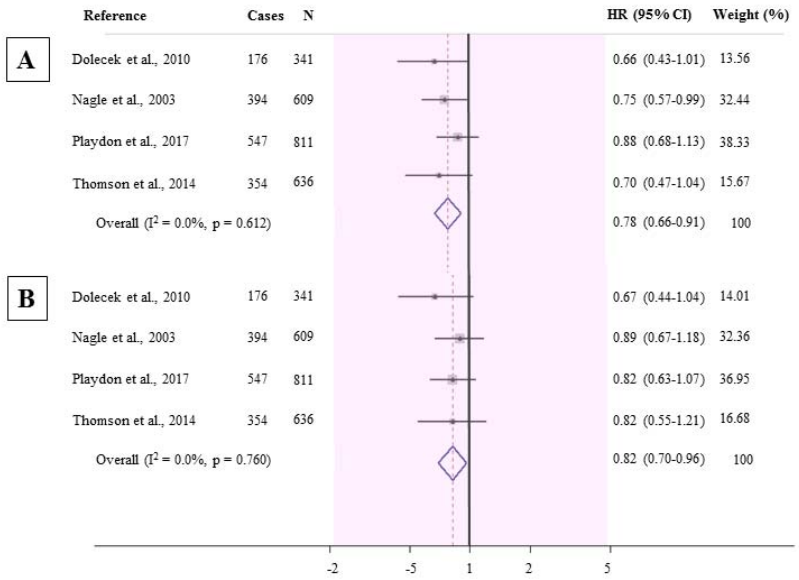


Figure 3. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in ovarian cancer patients, comparing the highest vs. lowest vegetable (A) and fruit (B) intake categories.

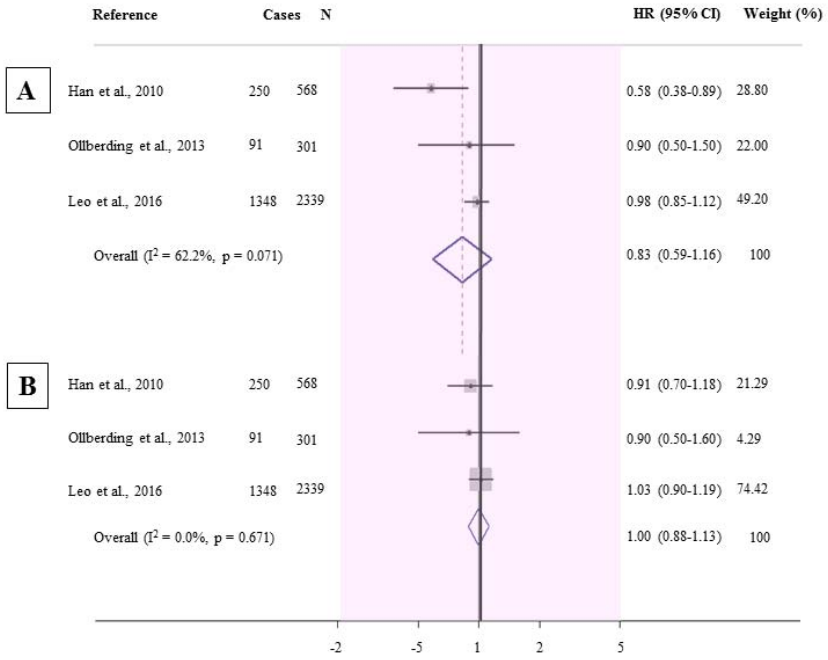


Figure 4. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in non-Hodgkin lymphoma patients, comparing the highest vs. lowest vegetable (A) and fruit (B) intake categories.

Supplementary Material

Figure S1. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in ovarian cancer patients, comparing the highest vs. lowest cruciferous intake categories.

Table S1. Summary of studies included in the systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and aerodigestive cancer prognosis.

Table S2. Summary of studies included in the systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and genital and urinary cancer prognosis.

Table S3. Summary of studies included in this systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and other cancer prognosis.

Table S4. PRISMA checklist.

Table S5. Quality assessment of studies included in this systematic review and meta-analysis according to the Newcastle Ottawa Scale.

Table S6. Quality assessment of studies included in this systematic review and meta-analysis according to the STROBE-nut Statement.

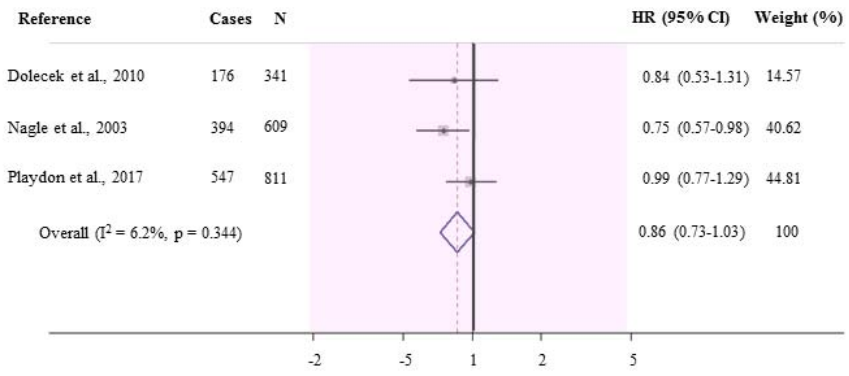


Figure S1. Forest plot showing pooled risk ratios (RRs) with 95% CI for overall mortality risk in ovarian cancer patients, comparing the highest vs. lowest cruciferous intake categories.

Table S1. Summary of studies included in the systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and aerodigestive cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year (Ref.)
Laryngeal	All-cause mortality	Italy Population-based case-control study	Up 10	215 (136)	M 59 (median)	Dietary questionnaire Pre-diagnosis (1 year before diagnosis)	**Citrus fruits** T3 (1680) vs. T1 (0) g/week **Other fruits** T3 (4620) vs. T1 (580) g/week	0.76 (0.49-1.19) 0.65 (0.39-1.07)	Age at diagnosis, clinical stage, occurrence of new primaries and total calorie intake	Crosignani *et al.*, 1996 (56)
Oesophagus	All-cause mortality	China ns	4.1 (median)	185 (ns)	M/F >70	Modified FFQ Pre-diagnosis (1 year before diagnosis)	**Preserved vegetables** Highest vs. lowest Cut-off: ≥1 time/week	1.58 (1.01-2.47)	Age and sex	Shi *et al.*, 2018 (38)
Gastric	Cancer cause-specific mortality	Japan ns*	Up to 10	877 (241)	M/F 40-79	Questionnaire for a trained interviewer about beverage and food intake Pre-diagnosis (before examination and diagnosis, in the first hospital visit))	**Raw vegetables** Highest vs. lowest Cut -off: >3 times/week. **Carrot** Highest vs. lowest Cut-off: >3 times/week **Pumpkin** Highest vs. lowest Cut-off: >3 times/week **Cabbage** Highest vs. lowest Cut point: >3 times/week **Lettuce** Highest vs. lowest Cut point: >3 times/week	0.74 (0.56-0.99) 0.67 (0.45-1.01) 1.00 (0.62-1.62) 0.88 (0.61-1.29) 0.82 (0.57-1.17)	Age, gender, and pathological type and stage of cancer	Huang *et al.*, 2000 (42)
Colorectal	Colorectal all-cause mortality Colon all-cause mortality Rectal all-cause mortality	Japan Cases of CRC from BBJ	7.4 (median)	1,709 (559)	M/F ≥20	Dietary interview Post-diagnosis (within 90 days after diagnosis)	**Green leafy vegetable consumption** Q1 (almost never) vs. Q4 (almost every day) **Green leafy vegetable consumption** Q1 (almost never) vs. Q4 (almost every day) **Green leafy vegetable consumption** Q1 (almost never) vs. Q4 (almost every day)	1.87 (1.22-2.88) 2.06 (1.10-3.86) 1.40 (0.70-2.83)	Age and entry year	Tamakoshi *et al.*, 2017 (39)
Pancreas	All-cause mortality	Italy From hospital-based case-	10 months (median) Up to 27	648 (612)	M/F 2 cohorts of 59 and 63	Dietary interview Pre-diagnosis (up to the time of	**Green vegetables** Highest vs. lowest Cut-off: ≥7 portions/week	0.96 (0.81-1.14)	Age and calendar period at diagnosis, study centre, and sex	Pelucchi *et al.*, 2014 (40)

			control studies		years (median)	diagnosis)				
Lung	All-cause mortality	Denmark Diet, Cancer and Health	From 1993-1997 to death or 2004	286 (ns) Current smokers	M/F 50-64	192-item FFQ Pre-diagnosis	**Leafy** T3 vs. T1 **Fruiting** T3 vs. T1 **Root** T3 vs. T1 **Cabbage** T3 vs. T1 **Onion** T3 vs. T1 **Stalk** T3 vs. T1	0.77 (0.46-1.28) 1.01 (0.59-1.74) 0.86 (0.54-1.36) 0.99 (0.65-1.50) 0.94 (0.62-1.43) 0.68 (0.44-1.07)	Sex, extent of disease, duration of smoking, intake of fruit, potatoes and the other subcategories studied	Skuladottir *et al.*, 2006 (47)
Lung	Cancer cause-specific mortality	China SWHS study	13.2 (mean)	547 (393)	F 59.2±8.5	Semi-quantitative 77-item FFQ Pre-diagnosis (at baseline and the first follow-up conducted 2-3 years after baseline)	**Cruciferous vegetables** Q4 (≥118.7) vs. Q1 (<53.2) g/day	0.69 (0.49-0.95)	Age at diagnosis, total energy intake, BMI, tea drinking, cigarette smoking, intakes of fruits and non-cruciferous vegetables	Wu *et al.*, 2015 (41)

*Retrospective cohort study; M: male; F: female; M/F: male and female; BMI: body mass index; FFQ: food frequency questionnaire; ns: not specified.

Table S2. Summary of studies included in the systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and genital and urinary cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year (Ref.)
Ovarian	All-cause mortality	Australia ns	5.9 (mean)	811 (547)	F 18-79	135-item FFQ Pre-diagnosis (> 1 year before diagnosis)	**Green leafy vegetables** T3 (≥0.67) vs. T1 (none or <0.33) servings/day **Cruciferous vegetables** T3 (≥1.5) vs. T1 (none or <0.75) servings/day **Red/yellow vegetables** T3 (≥2) vs. T1 (none or <1) servings/day	0.79 (0.62-0.99) 0.99 (0.77-1.29) 0.91 (0.69-1.20)	Age at diagnosis, International Federation of Gynaecology and Obstetrics (FIGO) stage, amount of residual disease, grade, tumour subtype, smoking status, BMI, physical activity index, marital status, and daily caloric intake	Playdon *et al.*, 2017 (57)
Ovarian	All-cause mortality Cancer cause-specific mortality	US WHI	From 1995 to 2012	636 (354) 636 (305)	F 50-79	WHI FFQ Pre-diagnosis (>1 year before diagnosis)	**Dark green and orange vegetables, legumes** T3 (5) vs. T1 (>2.5) points **Dark green and orange vegetables, legumes** T3 (5) vs. T1 (>2.5) points	1.10 (0.71-1.72) 1.17 (0.74-1.87)	Age at diagnosis, stage at diagnosis, race/ethnicity, diabetes, physical activity, total energy intake, waist circumference, family history of ovarian cancer, and clinical trial arms	Thomson *et al.*, 2014 (58)
Ovarian	All-cause mortality	US ns	Up to 10	341 (176)	F 18-74	60-item FFQ Pre-diagnosis (3-5 years before diagnosis)	**Yellow vegetables** T3 (≥3) vs. T1 (<1) servings/week **Green vegetables** T3 (≥7) vs. T1 (<3) servings/week **Cruciferous vegetables** T3 (≥3) vs. T1 (<1) servings/week **Citrus fruits** T3 (≥7) vs. T1 (<3) servings/week **Other fruits** T3 (≥7) vs. T1 (<3) servings/week	0.61 (0.39-0.94) 0.71 (0.47-1.07) 0.84 (0.53-1.31) 0.82 (0.53-1.26) 0.71 (0.46-1.09)	Age group, race, stage, grade, residual lesions, smoking status, BMI, oral contraceptive use, parity, and total energy intake	Dolecek *et al.*, 2010 (59)
Ovarian	All-cause mortality	Australia ns	7.3 (mean)	609 (394)	F 18-79	119-item FFQ Pre-diagnostic (1 year before diagnosis)	**Cruciferous vegetables** T3 (≥0.83) vs. T1 (<0.41) **Yellow vegetables** T3 (≥0.99) vs. T1 (>0.6) Serves/day	0.75 (0.57-0.98) 0.98 (0.73-1.31)	FIGO stage, age, grade, total energy intake and BMI	Nagle *et al.*, 2003 (60)
Prostate	All-cause mortality Cancer cause-	Italy ns* 	7.5 (median) 5.7 (median)	777 (263) 777 (81)	M 66 (median)	78-item FFQ Pre-diagnosis (2 years before diagnosis)	**Raw vegetables** Q4 (≥114) vs. Q1 (<53) g/day **Cooked vegetables** Q4 (≥92) vs. Q1 (<35) g/day **Citrus fruits** Q4 (≥38) vs. Q1 (<5) g/day **Non-citrus fruits** Q4 (≥340) vs. Q1 (<120) g/day **Raw vegetables**	0.64 (0.44-0.91) 1.18 (0.82-1.68) 0.77 (0.53-1.10) 0.61 (0.43-0.87) 0.49	Area of residence at diagnosis, calendar period, age at diagnosis, years of education, Gleason score, BMI, smoking habits, and total energy intake	Taborelli et al., 2016 (48)

	specific mortality					Q4 (≥114) vs. Q1 (<53) g/day	(0.26-0.91)		
						Cooked vegetables			
						Q4 (≥92) vs. Q1 (<35)	1.55 (0.79-3.05)		
						Citrus fruits			
						Q4 (≥38) vs. Q1 (<5) g/day	1.34 (0.72-2.51)		
						Non-citrus fruits			
						Q4 (≥240) vs. Q1 (<120) g/day	0.66 (0.33-1.33)		
Bladder	All-cause mortality	USA	8 (mean)	239 (179)	M/F,	**44-item FFQ**	**Cruciferous**	0.87	Age at diagnosis, total meat intake, pack-years of smoking, tumour stage and radiation therapy	Tang *et al.*, 2010 (36)
		RPCI	or			Pre-diagnosis	T3 (>14) vs. T1 (>5.5)	(0.60-1.26)		
			6.42		Categories:	(few years before	servings/months			
			(median)	239 (101)	<60, 60-70 and >70	diagnosis)	**Raw cruciferous**	0.89		
							T3 (>3) vs. T1 (>1)	(0.53-1.48)		
	Cancer cause-specific mortality						servings/months			
							Cruciferous	0.89		
							T3 (>14) vs. T1 (>5.5)	(0.53-1.48)		
							servings/months			
							Raw cruciferous	0.73		
							T3 (>3) vs. T1 (>1)	(0.44-1.21)		
							servings/months			

*Retrospective cohort study; M: male; F: female; M/F: male and female; BMI: body mass index; FFQ: food frequency questionnaire; ns: not specified.

Table S3. Summary of studies included in this systematic review and meta-analysis evaluating the association between vegetable and fruit subgroup consumption and other cancer prognosis.

Cancer Site	Outcome	Country Cohort name	Follow-up (years)	Sample Size (cases)	Sex Age (years)	Dietary assessment Timeframe	Exposure categorization	HR/RR (95% CI)	Adjustments	Author, Year (Ref.)
NHL	All-cause mortality	US ns	8.2 (mean)	301 (91)	M/F 20-75	117-item FFQ Pre-diagnosis (1 year before diagnosis)	**Cruciferous vegetables** T3 (>10.7) vs. T1 (<3.7) g/1000kcal/day **Green leafy vegetables** T3 (>18.1) vs. T1 (<6.3) g/1000kcal/day **Carotene-rich vegetables** T3 (>7.0) vs. T1 (<2.8) g/1000kcal/day **Citrus fruits** T3 (>57.2) vs. T1 (<17.0) g/1000kcal/day **Carotene-rich fruits** T3 (>17.2) vs. T1 (<6.1) g/1000kcal/day	0.8 (0.5-1.3) 0.8 (0.5-1.3) 0.7 (0.4-1.2) 1.2 (0.7-2.1) 0.9 (0.5-1.7)	Age, sex, education, smoking status and total energy intake	Ollberding *et al.*, 2013 (61)
NHL	All-cause mortality Cancer cause-specific mortality	US Population-based case-control study	7.7 (median)	568 (250) 568 (148)	F 21-84	FFQ Pre-diagnosis (1 year before diagnosis)	**Bean vegetables** Highest vs. Lowest Cut-point: 0.3 **Cruciferous vegetables** Highest vs. Lowest Cut-point: 0.2 **Green leafy vegetables** Highest vs. Lowest Cut-point: 0.3 servings/day **Red vegetables** Highest vs. Lowest Cut-point: 0.3 servings/day **Yellow vegetables** Highest vs. Lowest Cut-point: 0.3 servings/day **Citrus fruits** Highest vs. Lowest Cut-point: 1.1 servings/day **Bean vegetables** Highest vs. Lowest Cut-point: 0.3 servings/day **Cruciferous vegetables** Highest vs. Lowest Cut-point: 0.2 servings/day **Green leafy vegetables** Highest vs. Lowest Cut-point: 0.3 servings/day **Red vegetables**	1.14 (0.85-1.54) 0.91 (0.67-1.24) 0.71 (0.51-0.98) 1.03 (0.76-1.38) 0.93 (0.69-1.55) 0.73 (0.54-0.99) 1.05 (0.71-1.54) 0.75 (0.49-1.14) 0.82 (0.54-1.23) 1.11	Age, education, stage, B-symptom, initial treatment and total energy intake	Han *et al.*, 2010 (52)

						Highest vs. Lowest	(0.76-1.62)			
						Cut-point: 0.3 servings/day				
						Yellow vegetables				
						Highest vs. Lowest	**1.11** (0.77-1.61)			
						Cut-point: 0.3 servings/day				
						Citrus fruits				
						Highest vs. Lowest	**0.81** (0.54-1.20)			
						Cut-point: 1.1 servings/day				
Melanoma	Cancer cause-specific mortality	US From Connecticut Skin Self-Examination Case-Control Study	16 years (median)	249 (92)	M/F Categories: ≤65 and >65	Dietary interview Pre-diagnosis (at the time of diagnosis)	**Green salad** Weekly or more vs. Less than weekly	0.87 (0.52-1.44)	**Breslow** thickness, age at diagnosis, sex, ulceration and microsatellitosis	Gould Rothbergа *et al.*, 2014 (37)

NHL: Non-Hodgkin lymphoma; F: female; M/F: male and female; FFQ: food frequency questionnaire; ns: not specified.

Table S4. PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1,2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8-9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	12
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	18-19
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	19
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Table S5. Quality assessment of studies included in this systematic review and meta-analysis according to the Newcastle Ottawa Scale.

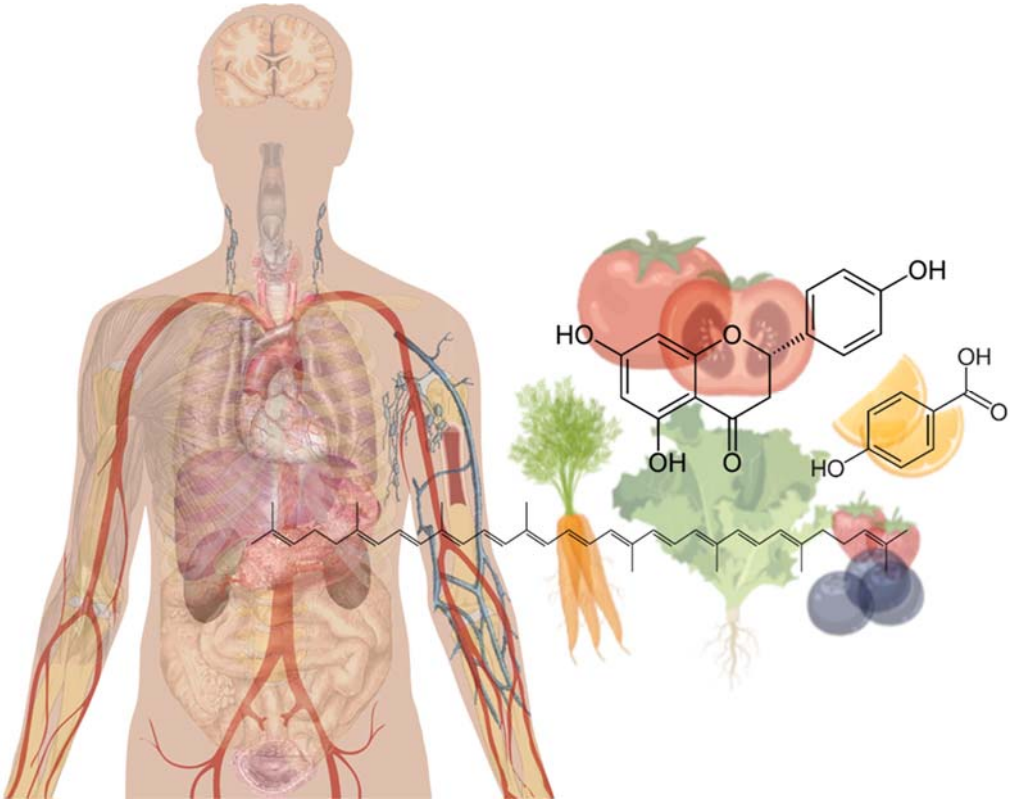
Study	Topic			Study	Topic		
	Selection (****)	Comparability (**)	Outcome (***)		Selection (****)	Comparability (**)	Outcome (***)
Almercingen et al., 2004	***	**	***	Li et al., 2017	****	**	**
Crosignani et al., 1996	****	**	***	Nagle et al., 2003	***	**	***
Dixshit et al., 2005	***	**	***	Ollbering et al., 2013	****	**	***
Dolecek et al., 2010	***	**	***	Peluechi et al., 2014	****	**	**
Duffy et al., 2009	****	**	***	Playdon et al., 2017	***	**	***
Dray et al., 2003	***	**	***	Sandoval et al., 2009	****	**	***
Feronha et al., 2012	****	**	**	Skuladottir et al., 2006	***	**	***
Gould Rothberga et al., 2014	****	**	***	Shen et al., 2012	****	**	***
Han et al., 2010	****	**	***	Shi et al., 2018	***	**	***
Huang et al., 2000	***	**	***	Taborelli et al., 2016	****	**	***
Jochems et al., 2018	***	**	***	Tamakoshi et al., 2017	****	**	**
Kuzmann et al., 2016	***	**	***	Tang et al., 2010	***	**	***
Lang et al., 2019	****	**	***	Thomson et al., 2014	****	**	***
Leo et al., 2015	****	**	***	Wu et al., 2015	***	**	***

Table S6. Quality assessment of studies included in this systematic review and meta-analysis according to the STROBE-nut Statement.

Section	Topic	Checklist item	% of studies met the criteria
Title and abstract		Nut-1. State the dietary/nutritional assessment method(s) used in the title, abstract, or keywords.	35.7
		Provide in the abstract an informative and balanced summary of what was done and what was found	100
Introduction	Background/rationale	Explain the scientific background and rationale for the investigation being reported	100
	Objectives	State specific objectives, including any pre-specified hypotheses	100
Methods	Study design	Present key elements of study design early in the paper	75
	Setting	Nut-5. Describe any characteristics of the study settings that might affect the dietary intake or nutritional status of the participants, if applicable.	100
	Participants	Nut-6. Report particular dietary, physiological, or nutritional characteristics that were considered when selecting the target population.	100
	Variables	Nut-7.1. Clearly define foods, food groups, nutrients, or other food components.	96.4
	Data sources/ measurement	Nut-8.1. Describe the dietary assessment method(s), e.g., portion size estimation, number of days and items recorded, how it was developed and administered, and how quality was assured. Report if and how supplement intake was assessed.	100
		Nut-8.3. Describe the nutrient requirements, recommendations, or dietary guidelines and the evaluation approach used to compare intake with the dietary reference values, if applicable.	17.9
		Nut-8.5. Describe the assessment of nondietary data (e.g., nutritional status and influencing factors) and timing of the assessment of these variables in relation to dietary assessment.	100
		Nut-8.6. Report on the validity of the dietary or nutritional assessment methods and any internal or external validation used in the study, if applicable.	67.9
	Bias	Nut-9. Report how bias in dietary or nutritional assessment was addressed, e.g., misreporting, changes in habits as a result of being measured, or data imputation from other sources.	21.4
	Study size	Explain how the study size was arrived at	3.6
	Quantitative variables	Nut-11. Explain the categorization of dietary/nutritional data (e.g., use of N-tiles and handling of non-consumers) and the choice of reference category, if applicable.	100
	Statistical methods	Nut-12.1. Describe any statistical method used to combine dietary or nutritional data, if applicable.	100
		Nut-12.2. Describe and justify the method for energy adjustments, intake modeling, and use of weighting factors, if applicable.	50

Results	Participants	Nut-13. Report the number of individuals excluded based on missing, incomplete, or implausible dietary/nutritional data.	82.1
	Descriptive data	Nut-14. Give the distribution of participant characteristics across the exposure variables if applicable. Specify if food consumption of total population or consumers only were used to obtain results.	96.4
	Outcome data	Report numbers of outcome events or summary measures over time	96.4
	Main results	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	57.1
		Report category boundaries when continuous variables were categorized	96.4
	Other analyses	Report other analyses done--e.g. analyses of subgroups and interactions, and sensitivity analyses	100
Discussion	Key results	Summarize key results with reference to study objectives	100
	Limitations	Nut-19. Describe the main limitations of the data sources and assessment methods used and implications for the interpretation of the findings.	82.1
	Interpretation	Nut-20. Report the nutritional relevance of the findings, given the complexity of diet or nutrition as an exposure.	100
	Generalizability	Discuss the generalizability (external validity) of the study results	100
Other information	Funding	Give the source of funding and the role of the funders for the present study, and if applicable, for the original study on which the present article is based	89.3
	Ethics	Nut-22.1. Describe the procedure for consent and study approval from ethics committee(s).	82.1
	Supplementary material	Nut-22.2. Provide data collection tools and data as online material or explain how they can be accessed.	35.7

DISCUSIÓN GLOBAL



5. DISCUSIÓN GLOBAL

Las hortalizas y frutas suponen el principal aporte dietético en compuestos bioactivos y su consumo ha sido ampliamente relacionado con la prevención de ENT. En general, estos compuestos bioactivos se conocen comúnmente como fitoquímicos, debido a que se producen a través del metabolismo de las plantas con el fin de desempeñar funciones fisiológicas (fotosintéticas y no fotosintéticas) y de defensa. Estos compuestos, a pesar de no ser esenciales, son muy importantes para el ser humano porque se obtienen exclusivamente a partir de la dieta y presentan propiedades biológicas beneficiosas (antioxidantes, antiinflamatorias, etc), favoreciendo el mantenimiento de la homeostasis. Además, en los últimos años el papel de la microbiota intestinal y su relación con la salud ha adquirido una gran relevancia, demostrándose que los productos metabólicos de la microbiota (ácidos grasos volátiles, ácidos fenólicos, etc) son activos biológicamente y desempeñan funciones vitales. También ha sido fuertemente evidenciada la relación entre la dieta y la composición microbiana, especulándose que una dieta saludable puede tener efectos directos e indirectos sobre la salud.

Por otro lado, el número de casos de ENT aumenta cada año debido al envejecimiento de la población y a otros factores de riesgo (sedentarismo, dieta no saludable, tabaquismo y otros hábitos tóxicos, etc). Las ENT que provocan mayor número de muertes al año son las ECV y el cáncer, siendo las responsables de casi la mitad de la mortalidad en el mundo [14]. Entre los factores de riesgo de estas ENT se incluye el consumo de una dieta pobre en hortalizas y frutas. Numerosos autores han destacado el papel cardioprotector de una dieta rica en compuestos bioactivos [42,67,68]. Sin embargo, la mayoría de los autores apuestan por estudiar los efectos de la dieta en poblaciones de edad avanzada o con alteración de algunos parámetros biológicos (colesterol, presión arterial, etc) que ya tienen una mayor predisposición a sufrir ciertas enfermedades. En cuanto al efecto de la dieta sobre el cáncer, a pesar de que el nivel de evidencia es menor, también se especula que los compuestos bioactivos tienen propiedades quimiopreventivas y pueden disminuir el riesgo de algunos tipos de cáncer [42,68,69]. Sin embargo, las recomendaciones dietéticas reportadas por el WCRF/AICR se enfocan en la prevención primaria, pero son insuficientes las investigaciones sobre las consecuencias de la dieta en los pacientes para mejorar su prognosis [44]. A excepción del cáncer de mama, no existen recomendaciones específicas para el resto de supervivientes de cáncer [214].

Por lo tanto, la hipótesis que plantea la tesis es que una dieta rica en hortalizas y frutas, como la dieta mediterránea, puede tener efectos cardioprotectores mejorando los

biomarcadores vasculares e inflamatorios, incluso en sujetos adultos y sanos que no presentan ninguna alteración aparente en los parámetros bioquímicos (colesterol, triglicéridos, presión arterial, etc). Además, se plantea que el consumo de hortalizas y frutas puede aumentar la supervivencia en pacientes con cáncer. A partir de esta hipótesis se propuso un objetivo general: verificar si el consumo de una dieta rica en hortalizas y frutas puede afectar positivamente a la homeostasis vascular e inflamatoria, incluso en sujetos con un riesgo aparentemente bajo, gracias a su composición en compuestos bioactivos; y corroborar si este beneficio es también extrapolable a los pacientes de cáncer, evitando así la recurrencia de la enfermedad y disminuyendo la mortalidad por cáncer y global.

Primero se abordó la relación entre la dieta, los compuestos bioactivos y los parámetros vasculares en sujetos sanos. Para ello, fue llevado a cabo un primer estudio de intervención cruzado para evaluar el efecto a corto plazo de restringir los alimentos ricos en antioxidantes de la dieta (sección *4.1*. Publicación 2). La intervención consistió en el consumo de una dieta baja en antioxidantes (LAD) con adherencia baja a la dieta mediterránea durante dos semanas, en comparación con su dieta habitual (UD, con una adherencia media a la dieta mediterránea de 8,5 puntos en una escala de 14). La TPE en orina, utilizada como marcador de la adherencia a la intervención, disminuyó ~35% después de la LAD con respecto a la UD. Para evaluar los cambios a nivel vascular se determinaron los siguientes parámetros: NO, ICAM-1, VCAM-1, TXI_2 y PGI_2. Curiosamente, el NO plasmático disminuyó también significativamente y en un porcentaje muy similar al observado en la TPE (~35%) después de la LAD. Además, ambos marcadores estaban directamente relacionados con la adherencia a la dieta mediterránea. El NO es un potente vasodilatador y se ha descrito previamente su asociación con un alto consumo de polifenoles [215]. Esta disminución de NO podría estar relacionada con la menor producción de iNOS debido a la expresión de NF-κB [84,85]. Por otra parte, recientemente se ha demostrado que los fitoquímicos pueden reducir el nitrato ingerido a partir de las hortalizas a NO, especulándose también una posible interacción entre la vía L-arginina-NOS y la nitrato-nitrito-NO que podría explicar parcialmente el efecto beneficioso del consumo de hortalizas sobre las ECV [192]. No se observaron diferencias significativas en las moléculas de adhesión solubles en plasma (ICAM-1 y VCAM-1), ni en los eicosanoides cuantificados en orina (TXI_2 y PGI_2). Sin embargo, si se vio una tendencia favorable en la concentración de estos últimos siendo significativo el aumento del cociente TXI_2/PGI_2 después de la LAD. Es importante destacar que estos valores se encontraban dentro de un rango normal, debido a que se trataba de pacientes sanos. Tras solo dos semanas de seguimiento, la intervención con una dieta baja en antioxidantes indicó los efectos

negativos, incluso sin alteraciones previas de los marcadores fisiológicos. Esto resultados corroboran el papel crucial de los fitoquímicos, y en particular de los polifenoles, en la regulación de los procesos biológicos relacionados con la salud cardiovascular. Se ha demostrado previamente que un consumo elevado de estos compuestos reduce el riesgo de ciertas ENT y, además, el presente estudio sugiere que un bajo consumo de fitoquímicos podría tener consecuencias perjudiciales sobre la función endotelial incluso a corto plazo y con un buen estado de salud.

Los fitoquímicos son ingeridos a través de la dieta, pero solo una proporción de estos compuestos es liberada desde la matriz del alimento para ser metabolizada, siendo en general el grado de absorción de estos compuestos muy bajo [193]. Sin embargo, se conocen algunos factores que modifican la bioaccesibilidad y la biodisponibilidad de los fitoquímicos (ver más detalles en las secciones *2.3.1.2.* y *2.3.2.2.*). En concreto, en esta tesis se estudió i) el efecto de consumir *sofrito,* una salsa procesada que contiene tomate, cebolla y aceite de oliva; y ii) la concentración de compuestos bioactivos en el organismo según el método de cultivo de los alimentos: ecológico *versus* convencional.

A raíz de los efectos beneficiosos del tomate en la prevención de ENT [194,195], algunos autores han demostrado que el procesado podría aportar una mayor concentración de algunos de los fitoquímicos predominantes en el tomate como los carotenoides y los polifenoles y además parecen mantenerse más estables durante el almacenamiento [196]. El procesado térmico de los vegetales puede favorecer la biodisponibilidad de los carotenoides, debido a que favorece la isomerización de la forma *trans* a *cis*. En particular, este efecto ha sido demostrado en el caso del licopeno, el principal fitoquímico de los tomates y sus derivados. [138]. Además, se ha observado que la bioaccesibilidad y biodisponibilidad de los polifenoles y carotenoides de los tomates aumenta con la adicción de una matriz lipídica como el aceite de oliva [53,55,56] y que la mejora en algunos marcadores de inflamación relacionados con la función vascular es mayor con respecto al tomate crudo [197]. Los estudios llevados a cabo hasta la fecha sobre los efectos del tomate cocinado se han centrado principalmente en el estudio de estos dos factores. Sin embargo, el *sofrito* presenta otra ventaja con respecto al tomate cocinado con aceite debido a la interacción con otros vegetales como la cebolla, que favorece la isomerización del licopeno [59]. Por lo tanto, en esta tesis nos propusimos evaluar el efecto de la ingesta de *sofrito* sobre algunos marcadores de inflamación sistémica de bajo grado en sujetos sanos y adultos. En la publicación 3 (sección *4.2.*) se describen la investigación llevada a cabo en 22 individuos que consumieron una sola dosis de *sofrito* (240 g/ 70 kg de peso corporal). Tras la ingesta, se detectaron diferencias significativas en la concentración de todos los

compuestos bioactivos cuantificados y disminuyeron los marcadores pro-inflamatorios CRP y TNF-α. Los carotenoides fueron analizados en plasma y en todos los casos aumentaron 24 horas después del consumo de *sofrito*. Además, se evaluó la TPE en distintos intervalos de tiempo y se observó que la excreción aumentaba desde las 5 horas, siendo su máximo incremento entre las 12 y las 24 horas. Los biomarcadores pro-inflamatorios se analizaron en plasma y en particular la CRP y el TNF-α disminuyeron después de la intervención, pero no se encontraron diferencias significativas en las concentraciones de citoquinas, IL-6 e IL-1β (esta última no se detectó en ningún caso). A pesar de que los beneficios del tomate y sus derivados son principalmente atribuidos al licopeno [198,199], en el presente estudio se observó una disminución del TNF-α con el incremento de la TPE y de β-caroteno, sugiriendo la importancia de otros compuestos bioactivos.

En cuanto al método de cultivo, los alimentos obtenidos mediante agricultura ecológica parecen contener una mayor cantidad de polifenoles [118,200] y probablemente de carotenoides [136]. Como ya se ha explicado anteriormente, las plantas producen fitoquímicos durante su metabolismo, necesarios para llevar a cabo sus funciones vitales y defensivas. Por lo tanto, una mayor exposición a estímulos estresantes puede aumentar la producción de estos compuestos. Los cultivos ecológicos limitan mucho el uso de productos sintéticos, tales como pesticidas y fertilizantes, lo cual favorece el estrés de las plantas y con ello la síntesis de fitoquímicos. Sin embargo, los efectos en el ser humano de llevar a cabo una dieta ecológica no han sido esclarecidos hasta la fecha. Hay pocos estudios y la mayoría son observacionales, lo cual indica posibles sesgos en las muestras poblacionales. En general, se ha mostrado que los consumidores de alimentos ecológicos están más concienciados con la salud y el medioambiente y tienen estilos de vida más saludables [201]. Por lo tanto, la publicación 4 (sección *4.3.*) resume el trabajo llevado a cabo en este ámbito y los resultados obtenidos. Fue realizado un estudio piloto abierto, cruzado, aleatorizado y controlado en 19 adultos sanos (10 mujeres y 9 hombres). Cada sujeto llevo a cabo una dieta de tipo saludable con alimentos ecológicos (≥80% de la dieta) y otra similar con alimentos cultivados mediante métodos convencionales. Cada intervención tuvo una duración de un mes y entre ambas hubo un período de descanso de dos meses. El análisis de ácidos fenólicos en orina mostró un aumento significativo del metabolito 4-HBA, aunque no se encontraron diferencias en las concentraciones plasmáticas de carotenos, minerales o metales pesados. El 4-HBA es un fenol clasificado dentro del grupo de los ácidos benzoicos, que es sintetizado a partir del metabolismo de las antocianinas [202–204], y además se ha mostrado que este compuesto puede generarse desde la microbiota intestinal [205]. Algunos autores han

especulado sus beneficios, directos o indirectos, en la prevención de enfermedades cardiovasculares y neurológicas y de algunos tipos de cáncer [205–210].

También ha sido previamente demostrado el impacto de la dieta sobre la microbiota [152–154]. En particular, el consumo de fibra dietética favorece la producción de VFA, debido a que es su sustrato principal. Numerosos autores han investigado los efectos beneficiosos de los VFA en el mantenimiento del metabolismo energético y la prevención de enfermedades crónicas [180,182,183,211,212]. Sin embargo, hay muy poca evidencia sobre la interacción de los fitoquímicos ingeridos en la dieta y la liberación de VFA desde la microbiota. Se ha sugerido una interacción bidireccional entre los polifenoles y la microbiota, debido a que una gran proporción de estos compuestos no se absorbe y llegan hasta el colon. Una vez allí, los polifenoles pueden modificar la composición microbiana, lo cual influye en la liberación de VFA y a su vez en la producción de ácidos fenólicos a partir de la microbiota [29,187,213]. En cambio, el impacto de otros compuestos bioactivos sobre la microbiota resulta completamente desconocido hasta la fecha. En la publicación 5 (sección *4.4.*) se expone un estudio que forma parte de la presente tesis, en el cual se evalúa la asociación de los carotenos en plasma y la concentración fecal de VFA, así como la influencia de esto compuestos sobre los marcadores lipídicos relacionados con la salud cardiovascular. Los carotenos son compuestos liposolubles que se relacionan con el consumo de hortalizas y frutas [214]. Tanto los carotenos como los VFA están implicados en la regulación de la síntesis del colesterol, sin embargo, la interacción de ambos compuestos no ha sido previamente estudiada. Los resultados obtenidos en este trabajo indican algunas asociaciones entre los carotenos y la liberación de VFA. Principalmente, se observó una relación directa de los carotenos y la concentración fecal de propionato, encontrándose que elevadas concentraciones de estos compuestos aumentaban significativamente el colesterol HDL. Lo cual indica que los carotenos podrían regular la síntesis del colesterol, en parte, a través de la modulación de la síntesis de VFA desde la microbiota. Sin embargo, faltan estudios que establezcan una relación de causalidad y evalúen los mecanismos biológicos implicados. Además, el total de carotenos y el *E*-licopeno se relacionaron directamente con la liberación de acetato y se identificaron otras asociaciones entre carotenos y VFA minoritarios. Por otra parte, la liberación de butirato se relacionó con una disminución del colesterol total y del LDL y con un incremento del colesterol HDL, en consonancia con investigaciones previas [185]. Los resultados sugieren que los carotenos consumidos mediante la dieta podrían favorecer la liberación de VFA desde la microbiota y modular la síntesis del colesterol, pero sería necesario realizar más estudios con un

tamaño muestral mayor para verificar estas observaciones y los mecanismos implicados.

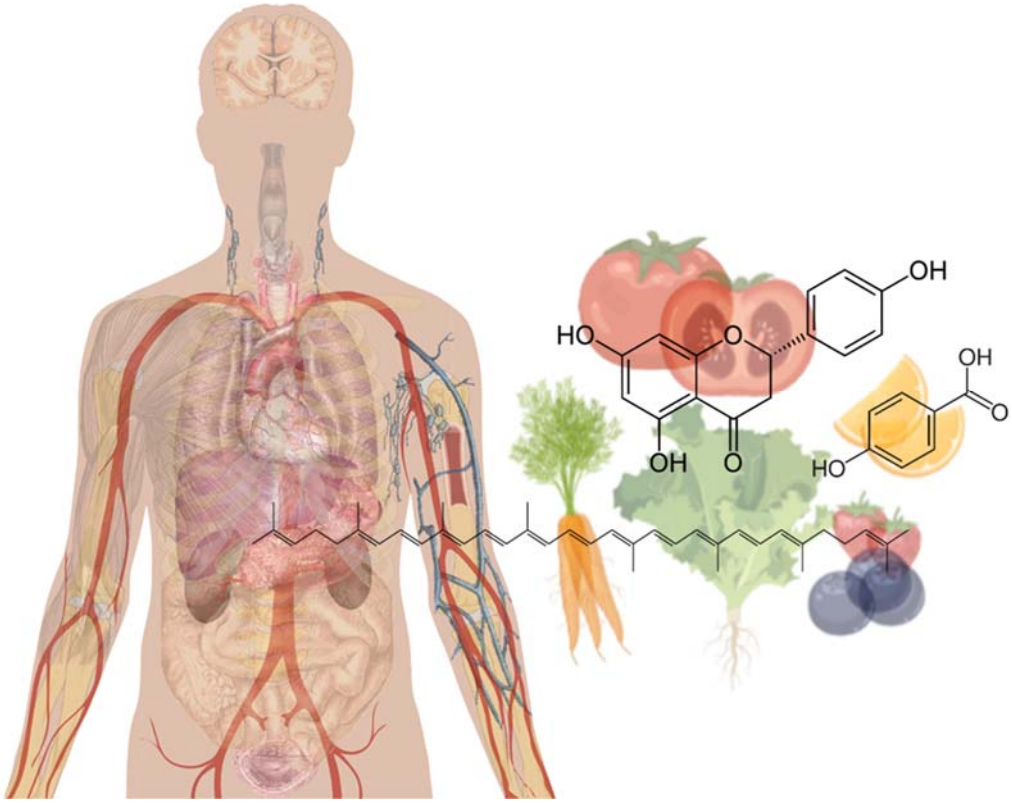
En resumen, observamos que una dieta saludable puede tener efectos beneficiosos y prevenir la inflamación sistémica de bajo grado previa a las enfermedades crónicas por su aporte en compuestos bioactivos. En concreto, encontramos que los polifenoles tienen propiedades cardioprotectoras en individuos sanos, en condiciones homeostáticas, y que los carotenos se asocian directamente con la producción del propionato y otros VFA. El butirato, el propionato y los carotenos parecen estar implicados en la regulación del colesterol. Además, el consumo de *sofrito* parece tener un efecto destacable en el control de la inflamación, gracias a su contenido en carotenos y polifenoles, debido a que con una sola dosis ingerida se observan cambios significativos en la CRP y el TNF-α. Por otra parte, el seguimiento de una dieta con alimentos ecológicos puede incrementar la presencia de compuestos bioactivos, en concreto de ácido fenólico 4-hidroxibenzoico, del que se han descritos efectos beneficiosos para la salud. Sin embargo, más estudios son necesarios para corroborar el valor nutritivo superior de estos alimentos y sus efectos sobre la salud.

En esta tesis también nos planteamos si una dieta rica en hortalizas y frutas podría tener efectos beneficiosos en pacientes de cáncer. Para ello se realizó un meta-análisis de estudios de cohorte a partir de una búsqueda bibliográfica exhaustiva en PubMed y Scopus, evaluando la relación entre la prognosis (recurrencia, mortalidad por cáncer y mortalidad global) y la ingesta de hortalizas y frutas (publicación 6, sección *4.5.*). Fueron analizados un total de 28 artículos y los resultados indicaron que el consumo de hortalizas podría disminuir un 25% y un 22% la mortalidad por todas las causas en los pacientes de cabeza y cuello y de ovario, respectivamente. El consumo de frutas también se asoció con una reducción del 18% de la mortalidad total en las pacientes de cáncer de ovario. No se extrajeron resultados claros sobre el efecto de las hortalizas y frutas en los supervivientes de linfoma no Hodgkin, debido a que los estudios analizados eran moderadamente heterogéneos. Tampoco se observaron resultados concluyentes en cuanto a los subgrupos de hortalizas y frutas. Por lo tanto, un elevado consumo de hortalizas y frutas podría favorecer a los supervivientes de cáncer de cabeza y cuello y de ovario, pero se necesitan más estudios para dilucidar la asociación con la prognosis de los demás tipos de cáncer y los mecanismos implicados.

Acorde con las recomendaciones de la OMS y las indicaciones sobre la dieta mediterránea, se sugiere que un consumo mínimo de 400 g/ día (equivalente a 5 raciones/ día) de hortalizas y frutas combinadas es necesario para prevenir las ENT [47]. Sin embargo, el meta-análisis de dosis-respuesta llevado a cabo por Aune *et al*.,

mostró que el menor riesgo de ECV y de mortalidad por todas las causas se alcanzó con un consumo de 800 g/día (10 raciones al día) y de cáncer total con 550-600 g/día (7-7,5 raciones al día). El riesgo de ECV y mortalidad por todas las causas disminuyeron un 28% y un 31%, respectivamente, con la ingesta de 800 g/día de hortalizas y frutas combinadas y fue más destacable el efecto de algunos subgrupos específicos como las manzanas y peras, frutas cítricas, crucíferas y vegetales de hoja verde/ensaladas. Además, el consumo de hortalizas y frutas combinadas se asoció a una reducción del 14% en el riesgo de cáncer total, siendo más significativo el consumo de crucíferas y vegetales amarillos y verdes [42]. Resultados similares fueron obtenidos a partir del meta-análisis sobre el consumo de hortalizas y frutas y la prognosis del cáncer, sugiriendo una mejora de la supervivencia de cáncer de cabeza y cuello cuando la ingesta de hortalizas es igual o superior a 8 raciones diarias. En el caso del cáncer de ovario, acorde con las recomendaciones de la OMS, el consumo de 5 raciones de hortalizas o frutas podría disminuir la tasa de mortalidad.

CONCLUSIONES



6. CONCLUSIONES

A continuación, se exponen las conclusiones extraídas a partir de los objetivos planteados y los resultados obtenidos en los estudios que forman parte de esta tesis.

Conclusión general:

- El consumo de una dieta de tipo mediterránea, rica en hortalizas y frutas, aporta compuestos bioactivos que son necesarios para mantener un buen estado de salud y prevenir la mortalidad en pacientes diagnosticados algunos cánceres con el de cabeza y cuello y el de ovario.

Conclusiones específicas:

Sobre el efecto del consumo de una dieta baja en antioxidantes en biomarcadores de la función endotelial:

- La TPE se correlaciona directamente con la adherencia a la dieta mediterránea.

- En tan solo dos semanas de adherencia a una LAD se observó una disminución en la concentración de NO en voluntarios sanos.

- La disminución de NO en plasma se asoció con la menor adherencia a la dieta mediterránea.

- El cociente TXA_2/PGI_2 aumentó significativamente después de la LAD, sugiriendo la implicación activa de la dieta en la homeostasis de los eicosanoides.

- El consumo de alimentos ricos en antioxidantes es necesario para el mantenimiento de la homeostasis vascular a través de la regulación de moléculas implicadas en la función endotelial.

Sobre la ingesta de una única dosis de *sofrito* (240 g/ 70 kg de peso corporal) y marcadores relacionados con la inflamación sistémica de bajo grado:

- Tras el consumo de *sofrito* se observó un aumento en la excreción de TPE, alcanzando su máximo entre las 12 y las 24 horas.

- A las 24 horas de la ingesta, se observó un aumento en las concentraciones plasmáticas de carotenos y xantofilas.

- Los niveles de CRP y TNF-α cuantificados en el plasma de los individuos sanos disminuyeron tras el consumo de *sofrito*.

- La reducción del TNF-α se relacionó inversamente con la TPE y con la concentración de β-caroteno.

- Los polifenoles y el β-caroteno presentes en el *sofrito* podrían favorecer la prevención de ENT evitando la inflamación sistémica de bajo grado.

Sobre los compuestos bioactivos y los elementos inorgánicos tras el consumo de una dieta ecológica:

- Tras un mes siguiendo una dieta basada en alimentos saludables y de origen ecológico se observó un aumento en la excreción urinaria del metabolito fenólico 4-HBA.

- No se observaron cambios significativos en la concentración de otros compuestos bioactivos ni de elementos inorgánicos (minerales y metales pesados).

- Una dieta ecológica podría conllevar un mayor aporte de compuestos bioactivos, en particular, de aquellos que se originan a partir del metabolismo.

Sobre los carotenos de la dieta, los ácidos grasos volátiles producidos por la microbiota y el perfil lipídico:

- Un incremento en la concentración plasmática de carotenos se asoció directamente con los niveles fecales de VFA, particularmente con propionato.

- Tanto el aumento de los carotenos totales en plasma (y particularmente del α-caroteno) como del propionato en heces se relacionaron directamente con el incremento del colesterol HDL plasmático.

- El efecto del butirato sobre el perfil lipídico fue aún más evidente, debido a que se asoció con una disminución del colesterol total y del LDL y con un aumento del HDL.

- Los carotenos podrían regular la síntesis del colesterol en plasma mediante la producción de VFA a partir de la microbiota.

Sobre la asociación entre el consumo de hortalizas y frutas y la prognosis de cáncer:

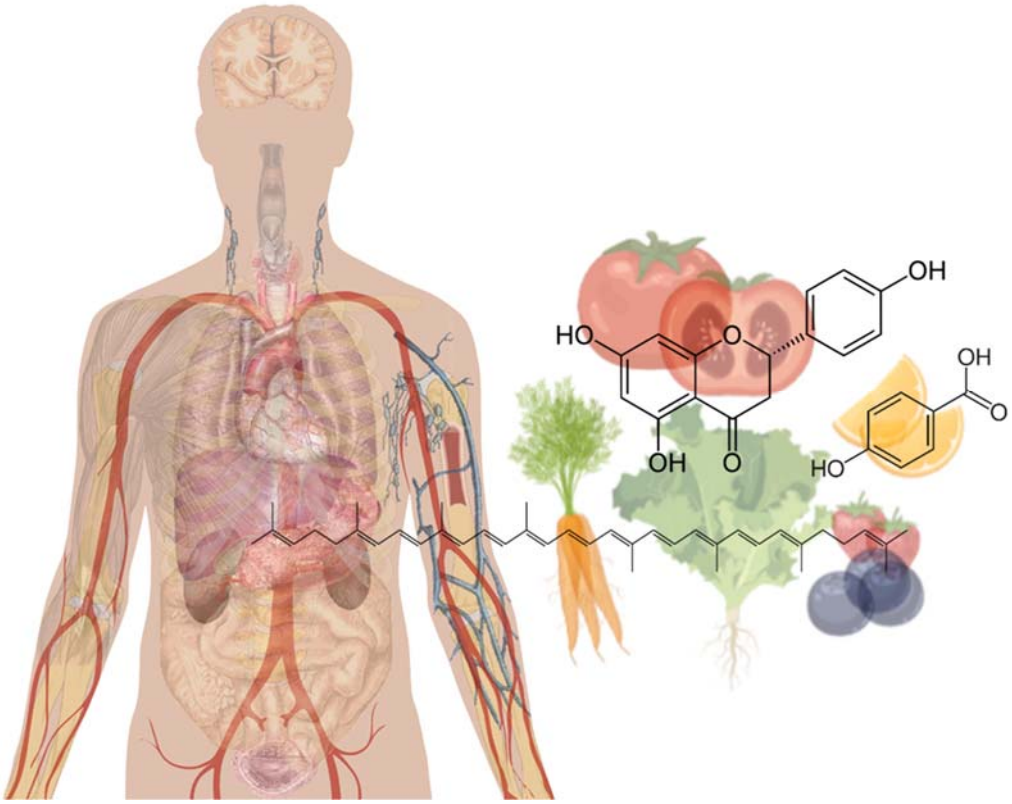
- El consumo de hortalizas podría disminuir la mortalidad global en pacientes de cabeza y cuello y de ovario un 25% y un 22%, respectivamente.

- El consumo de frutas disminuye un 18% la mortalidad en pacientes de cáncer de ovario.

- Las pacientes que consumían al menos 5 raciones al día de hortalizas o frutas tuvieron una mejor tasa de supervivencia. Efectos similares fueron observados en el caso del cáncer de cabeza y cuello con un consumo superior de hortalizas (≥8 raciones al día).

- Los resultados sobre la asociación de hortalizas y frutas y la prognosis fueron no concluyentes o insuficientes para el resto de cánceres.

BIBLIOGRAFÍA



7. BIBLIOGRAFÍA

1. Organización Mundial de la Salud. Temas de salud. Epidemiología Available online: https://www.who.int/topics/epidemiology/es/ (accessed on Jun 3, 2019).
2. Satija, A.; Yu, E.; Willett, W. C.; Hu, F. B. Understanding Nutritional Epidemiology and Its Role in Policy. *Adv. Nutr.* **2015**, *6*, 5–18, doi:10.3945/an.114.007492.
3. Richens, A. *Proof of efficacy trials: cross-over versus parallel-group*; 2001; Vol. 45;.
4. Evans, S. R. Clinical trial structures. *J. Exp. Stroke Transl. Med.* **2010**, *3*, 8–18.
5. Evans, S. R. Fundamentals of clinical trial design. *J. Exp. Stroke Transl. Med.* **2010**, *3*, 19–27.
6. Munnangi, S.; Boktor, S. W. *Epidemiology Of Study Design*; StatPearls Publishing, 2019;
7. Belbasis, L.; Bellou, V. Introduction to Epidemiological Studies. In; Humana Press, New York, NY, 2018; pp. 1–6.
8. Haneuse, S. Distinguishing Selection Bias and Confounding Bias in Comparative Effectiveness Research. *Med. Care* **2016**, *54*, e23–e29, doi:10.1097/MLR.0000000000000011.
9. Berlin, J. A.; Golub, R. M. Meta-analysis as Evidence. *JAMA* **2014**, *312*, 603, doi:10.1001/jama.2014.8167.
10. Boeing, H. Nutritional epidemiology: New perspectives for understanding the diet-disease relationship? *Eur. J. Clin. Nutr.* **2013**, *67*, 424–429, doi:10.1038/ejcn.2013.47.
11. Alissa, E. M.; Ferns, G. A. Dietary Fruits and Vegetables and Cardiovascular Diseases Risk. *Crit. Rev. Food Sci. Nutr.* **2015**, *57*, 00–00, doi:10.1080/10408398.2015.1040487.
12. Milenkovic, D.; Morand, C.; Cassidy, A.; Konic-Ristic, A.; Tomás-Barberán, F.; Ordovas, J. M.; Kroon, P.; De Caterina, R.; Rodriguez-Mateos, A. Interindividual Variability in Biomarkers of Cardiometabolic Health after Consumption of Major Plant-Food Bioactive Compounds and the Determinants Involved. *Adv. Nutr.* **2017**, *8*, 558–570, doi:10.3945/an.116.013623.
13. Boeing, H. Nutritional epidemiology at a crossroad: how to link observations with interventions and why? *Eur. J. Clin. Nutr.* **2018**, *72*, 1287–1290, doi:10.1038/s41430-018-0241-8.
14. (OMS), O. M. de la S. Enfermedades no transmisibles Available online:

https://www.who.int/es/news-room/fact-sheets/detail/noncommunicable-diseases (accessed on Aug 27, 2019).

15. Hannah Ritchie and Max Roser "Causes of Death". Published online at OurWorldInData.org. Available online: https://ourworldindata.org/causes-of-death (accessed on Aug 27, 2019).

16. Global Burden of Disease Collaborative Network Global Burden of Disease Study 2017 (GBD 2017) All-cause Mortality and Life Expectancy 1950-2017 | GHDx Available online: http://ghdx.healthdata.org/record/ihme-data/gbd-2017-all-cause-mortality-and-life-expectancy-1950-2017 (accessed on Aug 27, 2019).

17. (OMS), O. M. de la S. Enfermedades cardiovasculares Available online: https://www.who.int/es/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed on Aug 27, 2019).

18. Andersson, C.; Vasan, R. S. Epidemiology of cardiovascular disease in young individuals. *Nat. Rev. Cardiol.* 2018, *15*, 230–240.

19. Micha, R.; Peñalvo, J. L.; Cudhea, F.; Imamura, F.; Rehm, C. D.; Mozaffarian, D. Association Between Dietary Factors and Mortality From Heart Disease, Stroke, and Type 2 Diabetes in the United States. *JAMA* **2017**, *317*, 912–924, doi:10.1001/jama.2017.0947.

20. (OMS), O. M. de la S. Cancer Available online: https://www.who.int/news-room/fact-sheets/detail/cancer (accessed on Aug 27, 2019).

21. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, B. F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int. J. Cancer* **2019**, *144*, 1941–1953, doi:10.1002/ijc.31937.

22. Wiseman, M. J. Nutrition and cancer: prevention and survival. *Br. J. Nutr.* **2018**, 1–7, doi:10.1017/S0007114518002222.

23. Renzella, J.; Townsend, N.; Jewell, J.; Breda, J.; Roberts, N.; Rayner, M.; Wickramasinghe, K. *What national and subnational interventions and policies based on Mediterranean and Nordic diets are recommended or implemented in the WHO European Region, and is there evidence of effectiveness in reducing noncommunicable diseases? Copenhagen: WHO Regional Office for Europe; 2018 (Health Evidence Network (HEN) synthesis report 58).*;

24. Guasch-Ferré, M.; Salas-Salvadó, J.; Ros, E.; Estruch, R.; Corella, D.; Fitó, M.; Martínez-González, M. A.; PREDIMED Investigators The PREDIMED trial, Mediterranean diet and health outcomes: How strong is the evidence? *Nutr. Metab. Cardiovasc. Dis.* **2017**, *27*, 624–632, doi:10.1016/j.numecd.2017.05.004.

25. Galbete, C.; Schwingshackl, L.; Schwedhelm, C.; Boeing, H.; Schulze, M. B. Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses. *Eur. J. Epidemiol.* **2018**, *33*, 909–931, doi:10.1007/s10654-018-0427-3.

26. Dinu, M.; Pagliai, G.; Casini, A.; Sofi, F. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. *Eur. J. Clin. Nutr.* **2018**, *72*, 30–43, doi:10.1038/ejcn.2017.58.

27. Di Daniele, N.; Noce, A.; Vidiri, M. F.; Moriconi, E.; Marrone, G.; Annicchiarico-Petruzzelli, M.; D'Urso, G.; Tesauro, M.; Rovella, V.; De Lorenzo, A. Impact of Mediterranean diet on metabolic syndrome, cancer and longevity. *Oncotarget* **2017**, *8*, 8947–8979, doi:10.18632/oncotarget.13553.

28. Widmer, R. J.; Flammer, A. J.; Lerman, L. O.; Lerman, A. The Mediterranean Diet, its Components, and Cardiovascular Disease. *Am. J. Med.* **2015**, *128*, 229–238, doi:10.1016/j.amjmed.2014.10.014.

29. Tomás-Barberán, F. A.; Selma, M. V.; Espín, J. C. Interactions of gut microbiota with dietary polyphenols and consequences to human health. *Curr. Opin. Clin. Nutr. Metab. Care* **2016**, *19*, 471–476, doi:10.1097/MCO.0000000000000314.

30. Edwards, C. A.; Havlik, J.; Cong, W.; Mullen, W.; Preston, T.; Morrison, D. J.; Combet, E. Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota. *Nutr. Bull.* **2017**, *42*, 356–360, doi:10.1111/nbu.12296.

31. Trichopoulou, A.; Lagiou, P. Healthy Traditional Mediterranean Diet: An Expression of Culture, History, and Lifestyle. *Nutr. Rev.* **1997**, *55*, 383–389, doi:10.1111/j.1753-4887.1997.tb01578.x.

32. Allbaugh LG. *Crete: A case study of an underdeveloped area. Princeton University Press, Princeton, NJ. Food and nutrition (chapter 6).*; 1953;

33. Keys, A.; Menotti, A.; Karvonen, M. J.; Aravanis, C.; Blackburn, H.; Buzina, R.; Djordjevic, B. S.; Dontas, A. S.; Fidanza, F.; Keys, M. H.; Kromhout, D.; Nedeljkovic, S.; Punsar, S.; Seccareccia, F.; Toshima, H. The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* **1986**, *124*, 903–915, doi:10.1093/oxfordjournals.aje.a114480.

34. Keys, A. Mediterranean diet and public health: personal reflections. *Am. J. Clin. Nutr.* **1995**, *61*, 1321S–1323S, doi:10.1093/ajcn/61.6.1321S.

35. US News & World Report. Best Diet Rankings. 2019 Available online: https://health.usnews.com/best-diet (accessed on Jun 4, 2019).

36. European Commission. EU Science HUB. Health Promotion & Disease Prevention. Nutrition. Fruis and Vegetables. Available online:

https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/nutrition/fruit-vegetables (accessed on Jun 4, 2019).

37. Oz, A. T.; Kafkas, E. Phytochemicals in Fruits and Vegetables. In *Superfood and Functional Food - An Overview of Their Processing and Utilization*; InTech, 2017.

38. Craig, W. J. Phytochemicals: guardians of our health. *J. Am. Diet. Assoc.* **1997**, *97*, S199-204.

39. Fulton, S. L.; McKinley, M. C.; Young, I. S.; Cardwell, C. R.; Woodside, J. V. The Effect of Increasing Fruit and Vegetable Consumption on Overall Diet: A Systematic Review and Meta-analysis. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 802–816, doi:10.1080/10408398.2012.727917.

40. Boeing, H.; Bechthold, A.; Bub, A.; Ellinger, S.; Haller, D.; Kroke, A.; Leschik-Bonnet, E.; Müller, M. J.; Oberritter, H.; Schulze, M.; Stehle, P.; Watzl, B. Critical review: vegetables and fruit in the prevention of chronic diseases. *Eur. J. Nutr.* **2012**, *51*, 637–63, doi:10.1007/s00394-012-0380-y.

41. Angelino, D.; Godos, J.; Ghelfi, F.; Tieri, M.; Titta, L.; Lafranconi, A.; Marventano, S.; Alonzo, E.; Gambera, A.; Sciacca, S.; Buscemi, S.; Ray, S.; Galvano, F.; Del Rio, D.; Grosso, G. Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies. *Int. J. Food Sci. Nutr.* **2019**, *70*, 652–667, doi:10.1080/09637486.2019.1571021.

42. Aune, D.; Giovannucci, E.; Boffetta, P.; Fadnes, L. T.; Keum, N.; Norat, T.; Greenwood, D. C.; Riboli, E.; Vatten, L. J.; Tonstad, S. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int. J. Epidemiol.* **2017**, *46*, 1029–1056, doi:10.1093/ije/dyw319.

43. Yip, C. S. C.; Chan, W.; Fielding, R. The Associations of Fruit and Vegetable Intakes with Burden of Diseases: A Systematic Review of Meta-Analyses. *J. Acad. Nutr. Diet.* **2019**, *119*, 464–481, doi:10.1016/j.jand.2018.11.007.

44. Aicr; WCRF *World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Wholegrains, vegetables and fruit and the risk of cancer.*;

45. Schwingshackl, L.; Schwedhelm, C.; Hoffmann, G.; Lampousi, A.-M.; Knüppel, S.; Iqbal, K.; Bechthold, A.; Schlesinger, S.; Boeing, H. Food groups and risk of all-cause mortality: a systematic review and meta-analysis of prospective studies. *Am. J. Clin. Nutr.* **2017**, ajcn153148, doi:10.3945/ajcn.117.153148.

46. Global Burden of Disease Collaborative Network Global Burden of Disease Study 2017 (GBD 2017) Burden by Risk 1990-2017. Seattle, United States: Institute for Health Metrics and Evaluation (IHME). Available online: http://ghdx.healthdata.org/record/ihme-data/gbd-2017-burden-risk-1990-2017

(accessed on Aug 27, 2019).

47. World Health Organization (WHO)/Food and Agriculture Organization (FAO) of the United Nations *Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series. Vol. 916*; 2003;

48. Eurostat, European Union Statistics. Available online: https://ec.europa.eu/eurostat/web/microdata/european-health-interview-survey (accessed on Jun 4, 2019).

49. Frank, S. M.; Webster, J.; McKenzie, B.; Geldsetzer, P.; Manne-Goehler, J.; Andall-Brereton, G.; Houehanou, C.; Houinato, D.; Gurung, M. S.; Bicaba, B. W.; McClure, R. W.; Supiyev, A.; Zhumadilov, Z.; Stokes, A.; Labadarios, D.; Sibai, A. M.; Norov, B.; Aryal, K. K.; Karki, K. B.; Kagaruki, G. B.; Mayige, M. T.; Martins, J. S.; Atun, R.; Bärnighausen, T.; Vollmer, S.; Jaacks, L. M. Consumption of Fruits and Vegetables Among Individuals 15 Years and Older in 28 Low- and Middle-Income Countries. *J. Nutr.* **2019**, doi:10.1093/jn/nxz040.

50. Schröder, H.; Fitó, M.; Estruch, R.; Martínez-González, M. A.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M.; Lapetra, J.; Vinyoles, E.; Gómez-Gracia, E.; Lahoz, C.; Serra-Majem, L.; Pintó, X.; Ruiz-Gutierrez, V.; Covas, M. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older Spanish Men and Women. *J. Nutr.* **2011**, *141*, 1140–1145, doi:10.3945/jn.110.135566.

51. Martínez-González, M. A.; García-Arellano, A.; Toledo, E.; Salas-Salvadó, J.; Buil-Cosiales, P.; Corella, D.; Covas, M. I.; Schröder, H.; Arós, F.; Gómez-Gracia, E.; Fiol, M.; Ruiz-Gutiérrez, V.; Lapetra, J.; Lamuela-Raventos, R. M.; Serra-Majem, L.; Pintó, X.; Muñoz, M. A.; Wärnberg, J.; Ros, E.; Estruch, R.; PREDIMED Study Investigators, for the P. S. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS One* **2012**, *7*, e43134, doi:10.1371/journal.pone.0043134.

52. Vallverdú-Queralt, A.; de Alvarenga, J. F. R.; Estruch, R.; Lamuela-Raventos, R. M. Bioactive compounds present in the Mediterranean sofrito. *Food Chem.* **2013**, *141*, 3365–3372, doi:10.1016/j.foodchem.2013.06.032.

53. Rinaldi de Alvarenga, J. F.; Quifer-Rada, P.; Francetto Juliano, F.; Hurtado-Barroso, S.; Illan, M.; Torrado-Prat, X.; Lamuela-Raventós, R. M. Using Extra Virgin Olive Oil to Cook Vegetables Enhances Polyphenol and Carotenoid Extractability: A Study Applying the sofrito Technique. *Molecules* **2019**, *24*, 1555, doi:10.3390/molecules24081555.

54. Arranz, S.; Martínez-Huélamo, M.; Vallverdu-Queralt, A.; Valderas-Martinez, P.; Illán, M.; Sacanella, E.; Escribano, E.; Estruch, R.; Lamuela-Raventos, R. M. Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. *Food Chem.* **2015**, *168*, 203–210,

doi:10.1016/j.foodchem.2014.07.053.

55. Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Di Lecce, G.; Valderas-Martínez, P.; Tulipani, S.; Jáuregui, O.; Escribano-Ferrer, E.; Estruch, R.; Illan, M.; Lamuela-Raventós, R. M. Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. *Mol. Nutr. Food Res.* **2016**, *60*, 1578–1589, doi:10.1002/mnfr.201500820.

56. Tulipani, S.; Martinez Huelamo, M.; Rotches Ribalta, M.; Estruch, R.; Ferrer, E. E.; Andres-Lacueva, C.; Illan, M.; Lamuela-Raventós, R. M. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chem.* **2012**, *130*, 581–590, doi:10.1016/J.FOODCHEM.2011.07.078.

57. Martínez-Huélamo, M.; Tulipani, S.; Estruch, R.; Escribano, E.; Illán, M.; Corella, D.; Lamuela-Raventós, R. M. The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food Chem.* **2015**, *173*, 864–872, doi:10.1016/J.FOODCHEM.2014.09.156.

58. Rinaldi de Alvarenga, J. F.; Quifer-Rada, P.; Westrin, V.; Hurtado-Barroso, S.; Torrado-Prat, X.; Lamuela-Raventós, R. M. Mediterranean sofrito home-cooking technique enhances polyphenol content in tomato sauce. *J. Sci. Food Agric.* **2019**, jsfa.9934, doi:10.1002/jsfa.9934.

59. Rinaldi de Alvarenga, J. F.; Tran, C.; Hurtado-Barroso, S.; Martinez-Huélamo, M.; Illan, M.; Lamuela-Raventos, R. M. Home cooking and ingredient synergism improve lycopene isomer production in Sofrito. *Food Res. Int.* **2017**, *99*, 851–861, doi:10.1016/j.foodres.2017.01.009.

60. Vilahur, G.; Cubedo, J.; Padró, T.; Casaní, L.; Mendieta, G.; González, A.; Badimon, L. Intake of cooked tomato sauce preserves coronary endothelial function and improves apolipoprotein A-I and apolipoprotein J protein profile in high-density lipoproteins. *Transl. Res.* **2015**, *166*, 44–56, doi:10.1016/j.trsl.2014.11.004.

61. Rodriguez-Rodriguez, R.; Jiménez-Altayó, F.; Alsina, L.; Onetti, Y.; Rinaldi de Alvarenga, J. F.; Claro, C.; Ogalla, E.; Casals, N.; Lamuela-Raventos, R. M. Mediterranean tomato-based sofrito protects against vascular alterations in obese Zucker rats by preserving NO bioavailability. *Mol. Nutr. Food Res.* **2017**, *61*, 1601010, doi:10.1002/mnfr.201601010.

62. Sandoval, V.; Rodríguez-Rodríguez, R.; Martínez-Garza, Ú.; Rosell-Cardona, C.; Lamuela-Raventós, R. M.; Marrero, P. F.; Haro, D.; Relat, J. Mediterranean Tomato-Based Sofrito Sauce Improves Fibroblast Growth Factor 21 (FGF21) Signaling in White Adipose Tissue of Obese ZUCKER Rats. *Mol. Nutr. Food Res.* **2018**, *62*, 1700606, doi:10.1002/mnfr.201700606.

63. Hurtado-Barroso, S.; Martínez-Huélamo, M.; Rinaldi de Alvarenga, J. F.; Quifer-Rada, P.; Vallverdú-Queralt, A.; Pérez-Fernández, S.; Lamuela-Raventós, R. M. Acute Effect of a Single Dose of Tomato Sofrito on Plasmatic Inflammatory Biomarkers in Healthy Men. *Nutrients* **2019**, *11*, 851, doi:10.3390/nu11040851.

64. Urquiza-Salvat, N.; Pascual-Geler, M.; Lopez-Guarnido, O.; Rodrigo, L.; Martinez-Burgos, A.; Cozar, J. M.; Ocaña-Peinado, F. M.; Álvarez-Cubero, M. J.; Rivas, A. Adherence to Mediterranean diet and risk of prostate cancer. *Aging Male* **2019**, *22*, 102–108, doi:10.1080/13685538.2018.1450854.

65. Biesalski, H.-K.; Dragsted, L. O.; Elmadfa, I.; Grossklaus, R.; Müller, M.; Schrenk, D.; Walter, P.; Weber, P. Bioactive compounds: Definition and assessment of activity. *Nutrition* **2009**, *25*, 1202–1205, doi:10.1016/j.nut.2009.04.023.

66. Rodriguez-Casado, A. The Health Potential of Fruits and Vegetables Phytochemicals: Notable Examples. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1097–1107, doi:10.1080/10408398.2012.755149.

67. Sanchez, M.; Romero, M.; Gomez-Guzman, M.; Tamargo, J.; Perez-Vizcaino, F.; Duarte, J. Cardiovascular effects of flavonoids. *Curr. Med. Chem.* **2018**, *26*, doi:10.2174/0929867326666181220094721.

68. Vanduchova, A.; Anzenbacher, P.; Anzenbacherova, E. Isothiocyanate from Broccoli, Sulforaphane, and Its Properties. *J. Med. Food* **2019**, *22*, 121–126, doi:10.1089/jmf.2018.0024.

69. Baena Ruiz, R.; Salinas Hernández, P. Cancer chemoprevention by dietary phytochemicals: Epidemiological evidence. *Maturitas* **2016**, *94*, 13–19, doi:10.1016/j.maturitas.2016.08.004.

70. Lin, D.; Xiao, M.; Zhao, J.; Li, Z.; Xing, B.; Li, X.; Kong, M.; Li, L.; Zhang, Q.; Liu, Y.; Chen, H.; Qin, W.; Wu, H.; Chen, S. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules* **2016**, *21*, 1374, doi:10.3390/molecules21101374.

71. Fernando, W. M. A. D. B.; Somaratne, G.; Goozee, K. G.; Williams, S.; Singh, H.; Martins, R. N. Diabetes and Alzheimer's Disease: Can Tea Phytochemicals Play a Role in Prevention? *J. Alzheimer's Dis.* **2017**, *59*, 481–501, doi:10.3233/JAD-161200.

72. Mopuri, R.; Islam, M. S. Medicinal plants and phytochemicals with anti-obesogenic potentials: A review. *Biomed. Pharmacother.* **2017**, *89*, 1442–1452, doi:10.1016/j.biopha.2017.02.108.

73. Ghaedi, E.; Varkaneh, H. K.; Rahmani, J.; Mousavi, S. M.; Mohammadi, H.; Fatahi, S.; Pantovic, A.; Darooghegi Mofrad, M.; Zhang, Y. Possible anti-obesity

effects of phytosterols and phytostanols supplementation in humans: A systematic review and dose–response meta-analysis of randomized controlled trials. *Phyther. Res.* **2019**, *33*, 1246–1257, doi:10.1002/ptr.6319.

74. Cicero, A. F. G.; Colletti, A. Role of phytochemicals in the management of metabolic syndrome. *Phytomedicine* **2016**, *23*, 1134–1144, doi:10.1016/j.phymed.2015.11.009.

75. Akaberi, M.; Hosseinzadeh, H. Grapes (Vitis vinifera) as a Potential Candidate for the Therapy of the Metabolic Syndrome. *Phyther. Res.* **2016**, *30*, 540–556, doi:10.1002/ptr.5570.

76. Davicco, M.-J.; Wittrant, Y.; Coxam, V. Berries, their micronutrients and bone health. *Curr. Opin. Clin. Nutr. Metab. Care* **2016**, *19*, 453–457, doi:10.1097/MCO.0000000000000324.

77. Zhao, L.-G.; Zhang, Q.-L.; Zheng, J.-L.; Li, H.-L.; Zhang, W.; Tang, W.-G.; Xiang, Y.-B. Dietary, circulating beta-carotene and risk of all-cause mortality: a meta-analysis from prospective studies. *Sci. Rep.* **2016**, *6*, 26983, doi:10.1038/srep26983.

78. Rienks, J.; Barbaresko, J.; Nöthlings, U. Association of Polyphenol Biomarkers with Cardiovascular Disease and Mortality Risk: A Systematic Review and Meta-Analysis of Observational Studies. *Nutrients* **2017**, *9*, 415, doi:10.3390/nu9040415.

79. Obermeier, M. T.; White, R. E.; Yang, C. S. Effects of bioflavonoids on hepatic P450 activities. *Xenobiotica* **1995**, *25*, 575–584, doi:10.3109/00498259509061876.

80. Uno, T.; Ogura, C.; Izumi, C.; Nakamura, M.; Yanase, T.; Yamazaki, H.; Ashida, H.; Kanamaru, K.; Yamagata, H.; Imaishi, H. Point mutation of cytochrome P450 2A6 (a polymorphic variant CYP2A6.25) confers new substrate specificity towards flavonoids. *Biopharm. Drug Dispos.* **2015**, *36*, 552–563, doi:10.1002/bdd.1966.

81. Koenig, W. Low-grade inflammation modifies cardiovascular risk even at very low LDL-C levels are we aiming for a dual target concept? *Circulation* 2018, *138*, 150–153.

82. León-Pedroza, J. I.; González-Tapia, L. A.; Del Olmo-Gil, E.; Castellanos-Rodríguez, D.; Escobedo, G.; González-Chávez, A. Inflamación sistémica de grado bajo y su relación con el desarrollo de enfermedades metabólicas: de la evidencia molecular a la aplicación clínica. *Cir. Cir.* **2015**, *83*, 543–551, doi:10.1016/j.circir.2015.05.041.

83. Gewurz H, Mold C, Siegel J, F. B. C-reactive protein and the acute phase response. *Adv. Intern. Med.* **1982**, *27*, 345–72.

84. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1260–1270, doi:10.1080/10408398.2016.1251390.

85. Upadhyay, S.; Dixit, M. Role of Polyphenols and Other Phytochemicals on Molecular Signaling. *Oxid. Med. Cell. Longev.* **2015**, *2015*, 504253, doi:10.1155/2015/504253.

86. Vita, J. A.; Keaney, J. F. Endothelial function: a barometer for cardiovascular risk? *Circulation* **2002**, *106*, 640–2.

87. Herman, A. G.; Moncada, S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. *Eur. Heart J.* **2005**, *26*, 1945–1955, doi:10.1093/eurheartj/ehi333.

88. Arumugam, A.; Abdull Razis, A. F. Apoptosis as a Mechanism of the Cancer Chemopreventive Activity of Glucosinolates: a Review. *Asian Pac. J. Cancer Prev.* **2018**, *19*, 1439–1448, doi:10.22034/APJCP.2018.19.6.1439.

89. Chen, H.; Liu, R. H. Potential Mechanisms of Action of Dietary Phytochemicals for Cancer Prevention by Targeting Cellular Signaling Transduction Pathways. *J. Agric. Food Chem.* **2018**, *66*, 3260–3276, doi:10.1021/acs.jafc.7b04975.

90. Ahmed, K.; Zaidi, S.; Cui, Z.; Zhou, D.; Saeed, S.; Inadera, H. Potential proapoptotic phytochemical agents for the treatment and prevention of colorectal cancer (Review). *Oncol. Lett.* **2019**, doi:10.3892/ol.2019.10349.

91. Hegazy, A. M.; El-Sayed, E. M.; Ibrahim, K. S.; Abdel-Azeem, A. S. Dietary antioxidant for disease prevention corroborated by the Nrf2 pathway. *J. Complement. Integr. Med.* **2019**, *0*, doi:10.1515/jcim-2018-0161.

92. Kaulmann, A.; Bohn, T. Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention. *Nutr. Res.* 2014, *34*, 907–929.

93. Ooi, B. K.; Chan, K. G.; Goh, B. H.; Yap, W. H. The Role of Natural Products in Targeting Cardiovascular Diseases via Nrf2 Pathway: Novel Molecular Mechanisms and Therapeutic Approaches. *Front. Pharmacol.* 2018, *9*.

94. Menegon, S.; Columbano, A.; Giordano, S. The Dual Roles of NRF2 in Cancer. *Trends Mol. Med.* **2016**, *22*, 578–593, doi:10.1016/j.molmed.2016.05.002.

95. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J. P. E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892, doi:10.1089/ars.2012.4581.

96. Francenia Santos-Sánchez, N.; Salas-Coronado, R.; Hernández-Carlos, B.; Villanueva-Cañongo, C. Shikimic Acid Pathway in Biosynthesis of Phenolic

Compounds. In *Plant Physiological Aspects of Phenolic Compounds [Working Title]*; IntechOpen, 2019.

97. Crozier, A.; Yokota, T.; Jaganath, I. B.; Marks, S.; Saltmarsh, M.; Clifford, M. N. Secondary Metabolites in Fruits, Vegetables, Beverages and Other Plant-based Dietary Components. In *Plant Secondary Metabolites*; Blackwell Publishing Ltd: Oxford, UK; pp. 208–302.

98. Tresserra-Rimbau, A.; Medina-Remón, A.; Pérez-Jiménez, J.; Martínez-González, M. A.; Covas, M. I.; Corella, D.; Salas-Salvadó, J.; Gómez-Gracia, E.; Lapetra, J.; Arós, F.; Fiol, M.; Ros, E.; Serra-Majem, L.; Pintó, X.; Muñoz, M. A.; Saez, G. T.; Ruiz-Gutiérrez, V.; Warnberg, J.; Estruch, R.; Lamuela-Raventós, R. M. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study. *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 953–959, doi:10.1016/j.numecd.2012.10.008.

99. D'Archivio, M.; Filesi, C.; Varì, R.; Scazzocchio, B.; Masella, R. Bioavailability of the polyphenols: status and controversies. *Int. J. Mol. Sci.* **2010**, *11*, 1321–42, doi:10.3390/ijms11041321.

100. Cheynier, V. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.* **2005**, *81*, 223S–229S, doi:10.1093/ajcn/81.1.223S.

101. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747, doi:10.1093/ajcn/79.5.727.

102. Parada, J.; Aguilera, J. M. Food Microstructure Affects the Bioavailability of Several Nutrients. *J. Food Sci.* **2007**, *72*, R21–R32, doi:10.1111/j.1750-3841.2007.00274.x.

103. Palafox-Carlos, H.; Ayala-Zavala, J. F.; González-Aguilar, G. A. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *J. Food Sci.* **2011**, *76*, R6–R15, doi:10.1111/j.1750-3841.2010.01957.x.

104. Hedrén, E.; Diaz, V.; Svanberg, U. Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *Eur. J. Clin. Nutr.* **2002**, *56*, 425–430, doi:10.1038/sj.ejcn.1601329.

105. Williamson, G.; Kay, C. D.; Crozier, A. The Bioavailability, Transport, and Bioactivity of Dietary Flavonoids: A Review from a Historical Perspective. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 1054–1112, doi:10.1111/1541-4337.12351.

106. Thilakarathna, S.; Rupasinghe, H. Flavonoid Bioavailability and Attempts for Bioavailability Enhancement. *Nutrients* **2013**, *5*, 3367–3387, doi:10.3390/nu5093367.

107. Marín, L.; Miguélez, E. M.; Villar, C. J.; Lombó, F. Bioavailability of Dietary

Polyphenols and Gut Microbiota Metabolism: Antimicrobial Properties. *Biomed Res. Int.* **2015**, *2015*, 1–18, doi:10.1155/2015/905215.

108. Scalbert, A.; Williamson, G. Dietary Intake and Bioavailability of Polyphenols. *J. Nutr.* **2000**, *130*, 2073S–2085S, doi:10.1093/jn/130.8.2073S.

109. Bang, S.-H.; Hyun, Y.-J.; Shim, J.; Hong, S.-W.; Kim, D.-H. Metabolism of rutin and poncirin by human intestinal microbiota and cloning of their metabolizing α-L-rhamnosidase from Bifidobacterium dentium. *J. Microbiol. Biotechnol.* **2015**, *25*, 18–25.

110. Rechner, A. R.; Smith, M. A.; Kuhnle, G.; Gibson, G. R.; Debnam, E. S.; Srai, S. K. S.; Moore, K. P.; Rice-Evans, C. A. Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. *Free Radic. Biol. Med.* **2004**, *36*, 212–25.

111. Aura, A.-M. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochem. Rev.* **2008**, *7*, 407–429, doi:10.1007/s11101-008-9095-3.

112. Kim, D.-H.; Kobashi, K. The role of intestinal flora in metabolism of phenolic sulfate esters. *Biochem. Pharmacol.* **1986**, *35*, 3507–3510, doi:10.1016/0006-2952(86)90619-2.

113. Kim, D.-H.; Konishi, L.; Kobashi, K. Purification, characterization and reaction mechanism of novel arylsulfotransferase obtained from an anaerobic bacterium of human intestine. *Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol.* **1986**, *872*, 33–41, doi:10.1016/0167-4838(86)90144-5.

114. Gonthier, M.-P.; Cheynier, V.; Donovan, J. L.; Manach, C.; Morand, C.; Mila, I.; Lapierre, C.; Rémésy, C.; Scalbert, A. Microbial Aromatic Acid Metabolites Formed in the Gut Account for a Major Fraction of the Polyphenols Excreted in Urine of Rats Fed Red Wine Polyphenols. *J. Nutr.* **2003**, *133*, 461–467, doi:10.1093/jn/133.2.461.

115. Spencer, J. P. E.; Abd El Mohsen, M. M.; Minihane, A.-M.; Mathers, J. C. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br. J. Nutr.* **2008**, *99*, 12–22, doi:10.1017/S0007114507798938.

116. Vallverdú-Queralt, A.; Lamuela-Raventós, R. M. Foodomics: A new tool to differentiate between organic and conventional foods. *Electrophoresis* **2016**, *37*, 1784–1794, doi:10.1002/elps.201500348.

117. Vallverdú-Queralt, A.; Medina-Remón, A.; Casals-Ribes, I.; Amat, M.; Lamuela-Raventós, R. M. A metabolomic approach differentiates between conventional and organic ketchups. *J. Agric. Food Chem.* **2011**, *59*, 11703–10, doi:10.1021/jf202822s.

118. Vallverdú-Queralt, A.; Medina-Remón, A.; Casals-Ribes, I.; Lamuela-Raventos, R. M. Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chem.* **2012**, *130*, 222–227, doi:10.1016/j.foodchem.2011.07.017.

119. Roussos, P. A.; Gasparatos, D. Apple tree growth and overall fruit quality under organic and conventional orchard management. *Sci. Hortic. (Amsterdam).* **2009**, *123*, 247–252, doi:10.1016/j.scienta.2009.09.011.

120. Rembiałkowska, E. Review Quality of plant products from organic agriculture. *J. Sci. Food Agric. J Sci Food Agric* **2007**, *87*, 2757–2762, doi:10.1002/jsfa.3000.

121. Barański, M.; Średnicka-Tober, D.; Volakakis, N.; Seal, C.; Sanderson, R.; Stewart, G. B.; Benbrook, C.; Biavati, B.; Markellou, E.; Giotis, C.; Gromadzka-Ostrowska, J.; Rembiałkowska, E.; Skwarło-Sońta, K.; Tahvonen, R.; Janovská, D.; Niggli, U.; Nicot, P.; Leifert, C.; Srednicka-Tober, D.; Volakakis, N.; Seal, C.; Sanderson, R.; Stewart, G. B.; Benbrook, C.; Biavati, B.; Markellou, E.; Giotis, C.; Gromadzka-Ostrowska, J.; Rembiałkowska, E.; Skwarło-Sońta, K.; Tahvonen, R.; Janovská, D.; Niggli, U.; Nicot, P.; Leifert, C. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *Br. J. Nutr.* **2014**, *112*, 794–811, doi:10.1017/S0007114514001366.

122. Pérez-Jiménez, J.; Serrano, J.; Tabernero, M.; Arranz, S.; Díaz-Rubio, M. E.; García-Diz, L.; Goñi, I.; Saura-Calixto, F. Bioavailability of Phenolic Antioxidants Associated with Dietary Fiber: Plasma Antioxidant Capacity After Acute and Long-Term Intake in Humans. *Plant Foods Hum. Nutr.* **2009**, *64*, 102–107, doi:10.1007/s11130-009-0110-7.

123. Tamura, M.; Nakagawa, H.; Tsushida, T.; Hirayama, K.; Itoh, K. Effect of Pectin Enhancement on Plasma Quercetin and Fecal Flora in Rutin-Supplemented Mice. *J. Food Sci.* **2007**, *72*, S648–S651, doi:10.1111/j.1750-3841.2007.00557.x.

124. Bohn, T. Dietary factors affecting polyphenol bioavailability. *Nutr. Rev.* **2014**, *72*, 429–452, doi:10.1111/nure.12114.

125. Alan Crozier, *,†; Michael E. J. Lean, ‡; Morag S. McDonald, † and; Black‡, C. Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery. **1997**, doi:10.1021/JF960339Y.

126. Bugianesi, R.; Salucci, M.; Leonardi, C.; Ferracane, R.; Catasta, G.; Azzini, E.; Maiani, G. Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and ?-carotene in cherry tomatoes. *Eur. J. Nutr.* **2004**, *43*, 360–366, doi:10.1007/s00394-004-0483-1.

127. Tagashira, T.; Choshi, T.; Hibino, S.; Kamishikiryou, J.; Sugihara, N. Influence of Gallate and Pyrogallol Moieties on the Intestinal Absorption of (−)-

Epicatechin and (−)-Epicatechin Gallate. *J. Food Sci.* **2012**, *77*, H208–H215, doi:10.1111/j.1750-3841.2012.02902.x.

128. Stange, C. *Carotenoids in Nature : Biosynthesis, Regulation and Function*; Springer, 2016; ISBN 9783319391267.

129. Maiani, G.; Periago Castón, M. J.; Catasta, G.; Toti, E.; Cambrodón, I. G.; Bysted, A.; Granado-Lorencio, F.; Olmedilla-Alonso, B.; Knuthsen, P.; Valoti, M.; Böhm, V.; Mayer-Miebach, E.; Behsnilian, D.; Schlemmer, U. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol. Nutr. Food Res.* **2009**, *53*, S194–S218, doi:10.1002/mnfr.200800053.

130. Chacón-Ordóñez, T.; Carle, R.; Schweiggert, R. Bioaccessibility of carotenoids from plant and animal foods. *J. Sci. Food Agric.* **2019**, *99*, 3220–3239, doi:10.1002/jsfa.9525.

131. Faulks, R. M.; Southon, S. Challenges to understanding and measuring carotenoid bioavailability. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2005**, *1740*, 95–100, doi:10.1016/J.BBADIS.2004.11.012.

132. Reboul, E. Absorption of Vitamin A and Carotenoids by the Enterocyte: Focus on Transport Proteins. *Nutrients* **2013**, *5*, 3563–3581, doi:10.3390/nu5093563.

133. Kopec, R. E.; Failla, M. L. Recent advances in the bioaccessibility and bioavailability of carotenoids and effects of other dietary lipophiles. *J. Food Compos. Anal.* **2018**, *68*, 16–30, doi:10.1016/J.JFCA.2017.06.008.

134. González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P.; Soliva-Fortuny, R. In Vitro Bioaccessibility of Colored Carotenoids in Tomato Derivatives as Affected by Ripeness Stage and the Addition of Different Types of Oil. *J. Food Sci.* **2018**, *83*, 1404–1411, doi:10.1111/1750-3841.14140.

135. Wang, F.; Wang, G.-L.; Hou, X.-L.; Li, M.-Y.; Xu, Z.-S.; Xiong, A.-S. The genome sequence of 'Kurodagosun', a major carrot variety in Japan and China, reveals insights into biological research and carrot breeding. *Mol. Genet. Genomics* **2018**, *293*, 861–871, doi:10.1007/s00438-018-1428-3.

136. Vallverdú-Queralt, A.; Martínez-Huélamo, M.; Casals-Ribes, I.; Lamuela-Raventós, R. M. Differences in the carotenoid profile of commercially available organic and conventional tomato-based products. *J. Berry Res.* **2014**, *4*, 69–77, doi:10.3233/JBR-140069.

137. Cardoso, P. C.; Tomazini, A. P. B.; Stringheta, P. C.; Ribeiro, S. M. R.; Pinheiro-Sant'Ana, H. M. Vitamin C and carotenoids in organic and conventional fruits grown in Brazil. *Food Chem.* **2011**, *126*, 411–416, doi:10.1016/J.FOODCHEM.2010.10.109.

138. Shi, J.; Le Maguer, M. Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 1–42, doi:10.1080/10408690091189275.

139. van het Hof, K. H.; West, C. E.; Weststrate, J. A.; Hautvast, J. G. A. J. Dietary Factors That Affect the Bioavailability of Carotenoids. *J. Nutr.* **2000**, *130*, 503–506, doi:10.1093/jn/130.3.503.

140. Cervantes-Paz, B.; Ornelas-Paz, J. de J.; Ruiz-Cruz, S.; Rios-Velasco, C.; Ibarra-Junquera, V.; Yahia, E. M.; Gardea-Béjar, A. A. Effects of pectin on lipid digestion and possible implications for carotenoid bioavailability during pre-absorptive stages: A review. *Food Res. Int.* **2017**, *99*, 917–927, doi:10.1016/j.foodres.2017.02.012.

141. Boileau, T. W.-M.; Boileau, A. C.; Erdman, J. W. Bioavailability of all-trans and cis–Isomers of Lycopene. *Exp. Biol. Med.* **2002**, *227*, 914–919, doi:10.1177/153537020222701012.

142. Schieber, A.; Carle, R. Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. *Trends Food Sci. Technol.* **2005**, *16*, 416–422, doi:10.1016/J.TIFS.2005.03.018.

143. Priyadarshani, A. M. B. A review on factors influencing bioaccessibility and bioefficacy of carotenoids. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1710–1717, doi:10.1080/10408398.2015.1023431.

144. Fardet, A.; Morise, A.; Kalonji, E.; Margaritis, I.; Mariotti, F. Influence of Phytosterol and Phytostanol Food Supplementation on Plasma Liposoluble Vitamins and Provitamin A Carotenoid Levels in Humans: An Updated Review of the Evidence. *Crit. Rev. Food Sci. Nutr.* **2015**, *57*, 00–00, doi:10.1080/10408398.2015.1033611.

145. Johnson, E. J.; Krasinski, S. D.; Russell, R. M. Sex differences in postabsorptive plasma vitamin A transport. *Am. J. Clin. Nutr.* **1992**, *56*, 911–916, doi:10.1093/ajcn/56.5.911.

146. Russell, R. M. Factors in Aging that Effect the Bioavailability of Nutrients. *J. Nutr.* **2001**, *131*, 1359S–1361S, doi:10.1093/jn/131.4.1359S.

147. Borel, P.; Desmarchelier, C. Bioavailability of Fat-Soluble Vitamins and Phytochemicals in Humans: Effects of Genetic Variation. *Annu. Rev. Nutr.* **2018**, *38*, 69–96, doi:10.1146/annurev-nutr-082117-051628.

148. Cardinault, N.; Tyssandier, V.; Grolier, P.; Winklhofer-Roob, B. M.; Ribalta, J.; Bouteloup-Demange, C.; Rock, E.; Borel, P. Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. *Eur. J. Nutr.* **2003**, *42*, 315–323, doi:10.1007/s00394-003-0426-2.

149. Abdul Rahim, M. B. H.; Chilloux, J.; Martinez-Gili, L.; Neves, A. L.; Myridakis, A.; Gooderham, N.; Dumas, M.-E. Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. *Acta Diabetol.* **2019**, *56*, 493–500, doi:10.1007/s00592-019-01312-x.

150. Santos-Marcos, J. A.; Perez-Jimenez, F.; Camargo, A. The role of diet and intestinal microbiota in the development of metabolic syndrome. *J. Nutr. Biochem.* **2019**, *70*, 1–27, doi:10.1016/j.jnutbio.2019.03.017.

151. Pace, L. A.; Crowe, S. E. Complex Relationships Between Food, Diet, and the Microbiome. *Gastroenterol. Clin. North Am.* **2016**, *45*, 253–265, doi:10.1016/j.gtc.2016.02.004.

152. Singh, R. K.; Chang, H.-W.; Yan, D.; Lee, K. M.; Ucmak, D.; Wong, K.; Abrouk, M.; Farahnik, B.; Nakamura, M.; Zhu, T. H.; Bhutani, T.; Liao, W. Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* **2017**, *15*, 73, doi:10.1186/s12967-017-1175-y.

153. Shen, T.-C. D. Diet and Gut Microbiota in Health and Disease. In *Nestle Nutrition Institute workshop series*; 2017; Vol. 88, pp. 117–126.

154. Chassaing, B.; Vijay-Kumar, M.; Gewirtz, A. T. How diet can impact gut microbiota to promote or endanger health. *Curr. Opin. Gastroenterol.* **2017**, *33*, 417–421, doi:10.1097/MOG.0000000000000401.

155. Brial, F.; Le Lay, A.; Dumas, M.-E.; Gauguier, D. Implication of gut microbiota metabolites in cardiovascular and metabolic diseases. *Cell. Mol. Life Sci.* **2018**, *75*, 3977–3990, doi:10.1007/s00018-018-2901-1.

156. Aguilar-Toalá, J. E.; Garcia-Varela, R.; Garcia, H. S.; Mata-Haro, V.; González-Córdova, A. F.; Vallejo-Cordoba, B.; Hernández-Mendoza, A. Postbiotics: An evolving term within the functional foods field. *Trends Food Sci. Technol.* **2018**, *75*, 105–114, doi:10.1016/J.TIFS.2018.03.009.

157. Malashree, L.; Angadi, V.; Yadav, K. S.; Prabha, R. "Postbiotics" - One Step Ahead of Probiotics. *Int.J.Curr.Microbiol.App.Sci* **2019**, *8*, 2049–2053, doi:10.20546/ijcmas.2019.801.214.

158. Shenderov, B. A. Metabiotics: novel idea or natural development of probiotic conception. *Microb. Ecol. Heal. Dis.* **2013**, *24*, doi:10.3402/mehd.v24i0.20399.

159. Nicholson, J. K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S. Host-gut microbiota metabolic interactions. *Science* **2012**, *336*, 1262–7, doi:10.1126/science.1223813.

160. Chuah, L.-O.; Foo, H. L.; Loh, T. C.; Mohammed Alitheen, N. B.; Yeap, S. K.; Abdul Mutalib, N. E.; Abdul Rahim, R.; Yusoff, K. Postbiotic metabolites produced by Lactobacillus plantarum strains exert selective cytotoxicity effects

on cancer cells. *BMC Complement. Altern. Med.* **2019**, *19*, 114, doi:10.1186/s12906-019-2528-2.

161. Dunand, E.; Burns, P.; Binetti, A.; Bergamini, C.; Peralta, G. H.; Forzani, L.; Reinheimer, J.; Vinderola, G. Postbiotics produced at laboratory and industrial level as potential functional food ingredients with the capacity to protect mice against Salmonella infection. *J. Appl. Microbiol.* **2019**, *127*, 219–229, doi:10.1111/jam.14276.

162. Mosca, F.; Gianni, M. L.; Rescigno, M. Can Postbiotics Represent a New Strategy for NEC? In *Advances in experimental medicine and biology*; 2019; Vol. 1125, pp. 37–45.

163. Tsilingiri, K.; Rescigno, M. Postbiotics: what else? *Benef. Microbes* **2013**, *4*, 101–107, doi:10.3920/BM2012.0046.

164. Wong, A. C.; Levy, M. New Approaches to Microbiome-Based Therapies. *mSystems* **2019**, *4*, doi:10.1128/mSystems.00122-19.

165. Gill, P. A.; van Zelm, M. C.; Muir, J. G.; Gibson, P. R. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment. Pharmacol. Ther.* **2018**, *48*, 15–34, doi:10.1111/apt.14689.

166. Macfarlane, S.; Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **2003**, *62*, 67–72, doi:10.1079/PNS2002207.

167. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345, doi:10.1016/j.cell.2016.05.041.

168. Cummings, J. H. Short chain fatty acids in the human colon. *Gut* **1981**, *22*, 763, doi:10.1136/GUT.22.9.763.

169. Fredstrom, S. B.; Lampe, J. W.; Jung, H.-J. G.; Slavin, J. L. Apparent Fiber Digestibility and Fecal Short-Chain Fatty Acid Concentrations With Ingestion of Two Types of Dietary Fiber. *J. Parenter. Enter. Nutr.* **1994**, *18*, 14–19, doi:10.1177/014860719401800114.

170. den Besten, G.; van Eunen, K.; Groen, A. K.; Venema, K.; Reijngoud, D.-J.; Bakker, B. M. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **2013**, *54*, 2325–2340, doi:10.1194/jlr.R036012.

171. Reddy, M. V.; Mohan, S. V.; Chang, Y.-C. Sustainable production of medium chain fatty acids (MCFA) with an enriched mixed bacterial culture: microbial characterization using molecular methods. *Sustain. Energy Fuels* **2018**, *2*, 372–380, doi:10.1039/C7SE00467B.

172. Steinbusch, K. J. J.; Hamelers, H. V. M.; Plugge, C. M.; Buisman, C. J. N. Biological formation of caproate and caprylate from acetate: fuel and chemical production from low grade biomass. *Energy Environ. Sci.* **2011**, *4*, 216–224, doi:10.1039/C0EE00282H.

173. Grootscholten, T. I. M.; Kinsky dal Borgo, F.; Hamelers, H. V. M.; Buisman, C. J. N. Promoting chain elongation in mixed culture acidification reactors by addition of ethanol. *Biomass and Bioenergy* **2013**, *48*, 10–16, doi:10.1016/J.BIOMBIOE.2012.11.019.

174. Yao, C. K.; Muir, J. G.; Gibson, P. R. Review article: insights into colonic protein fermentation, its modulation and potential health implications. *Aliment. Pharmacol. Ther.* **2016**, *43*, 181–196, doi:10.1111/apt.13456.

175. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Thomas Brenna, J. Branched-chain fatty acid content of foods and estimated intake in the USA. *Br. J. Nutr.* **2014**, *112*, 565–572, doi:10.1017/S0007114514001081.

176. Ran-Ressler, R. R.; Glahn, R. P.; Bae, S.; Brenna, J. T. Branched-Chain Fatty Acids in the Neonatal Gut and Estimated Dietary Intake in Infancy and Adulthood. In *Nestle Nutrition Institute workshop series*; 2013; Vol. 77, pp. 133–143.

177. Liu, L.; Wang, Z.; Park, H. G.; Xu, C.; Lawrence, P.; Su, X.; Wijendran, V.; Walker, W. A.; Kothapalli, K. S. D.; Brenna, J. T. Human fetal intestinal epithelial cells metabolize and incorporate branched chain fatty acids in a structure specific manner. *Prostaglandins, Leukot. Essent. Fat. Acids* **2017**, *116*, 32–39, doi:10.1016/j.plefa.2016.12.002.

178. Chambers, E. S.; Preston, T.; Frost, G.; Morrison, D. J. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. *Curr. Nutr. Rep.* **2018**, *7*, 198–206, doi:10.1007/s13668-018-0248-8.

179. Kimura, I.; Ozawa, K.; Inoue, D.; Imamura, T.; Kimura, K.; Maeda, T.; Terasawa, K.; Kashihara, D.; Hirano, K.; Tani, T.; Takahashi, T.; Miyauchi, S.; Shioi, G.; Inoue, H.; Tsujimoto, G. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* **2013**, *4*, 1829, doi:10.1038/ncomms2852.

180. Canfora, E. E.; van der Beek, C. M.; Jocken, J. W. E.; Goossens, G. H.; Holst, J. J.; Olde Damink, S. W. M.; Lenaerts, K.; Dejong, C. H. C.; Blaak, E. E. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci. Rep.* **2017**, *7*, 2360, doi:10.1038/s41598-017-02546-x.

181. Su, X.; Magkos, F.; Zhou, D.; Eagon, J. C.; Fabbrini, E.; Okunade, A. L.; Klein, S. Adipose tissue monomethyl branched-chain fatty acids and insulin sensitivity:

Effects of obesity and weight loss. *Obesity* **2015**, *23*, 329–334, doi:10.1002/oby.20923.

182. Encarnação, J. C.; Abrantes, A. M.; Pires, A. S.; Botelho, M. F. Revisit dietary fiber on colorectal cancer: butyrate and its role on prevention and treatment. *Cancer Metastasis Rev.* **2015**, *34*, 465–478, doi:10.1007/s10555-015-9578-9.

183. Chen, J.; Vitetta, L. Inflammation-Modulating Effect of Butyrate in the Prevention of Colon Cancer by Dietary Fiber. *Clin. Colorectal Cancer* **2018**, *17*, e541–e544, doi:10.1016/j.clcc.2018.05.001.

184. Wong, J. M. W.; de Souza, R.; Kendall, C. W. C.; Emam, A.; Jenkins, D. J. A. Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* **2006**, *40*, 235–43.

185. Chen, Y.; Xu, C.; Huang, R.; Song, J.; Li, D.; Xia, M. Butyrate from pectin fermentation inhibits intestinal cholesterol absorption and attenuates atherosclerosis in apolipoprotein E-deficient mice. *J. Nutr. Biochem.* **2018**, *56*, 175–182, doi:10.1016/j.jnutbio.2018.02.011.

186. Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* **2005**, *81*, 243S–255S, doi:10.1093/ajcn/81.1.243S.

187. Ozdal, T.; Sela, D. A.; Xiao, J.; Boyacioglu, D.; Chen, F.; Capanoglu, E. The Reciprocal Interactions between Polyphenols and Gut Microbiota and Effects on Bioaccessibility. *Nutrients* **2016**, *8*, 78, doi:10.3390/nu8020078.

188. Kawabata, K.; Yoshioka, Y.; Terao, J. Role of Intestinal Microbiota in the Bioavailability and Physiological Functions of Dietary Polyphenols. *Molecules* **2019**, *24*, 370, doi:10.3390/molecules24020370.

189. Parkar, S. G.; Trower, T. M.; Stevenson, D. E. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe* **2013**, *23*, 12–19, doi:10.1016/j.anaerobe.2013.07.009.

190. *World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Survivors of breast and other cancers. Available at dietandcancerreport.org*;

191. Vanhoutte, P. M.; Shimokawa, H.; Feletou, M.; Tang, E. H. C. Endothelial dysfunction and vascular disease - a 30th anniversary update. *Acta Physiol.* **2017**, *219*, 22–96, doi:10.1111/apha.12646.

192. Bondonno, C. P.; Blekkenhorst, L. C.; Liu, A. H.; Bondonno, N. P.; Ward, N. C.; Croft, K. D.; Hodgson, J. M. Vegetable-derived bioactive nitrate and cardiovascular health. *Mol. Aspects Med.* 2018, *61*, 83–91.

193. Epriliati, I.; Ginjom, I. R. *Bioavailability of Phytochemicals. Phytochemicals – A*

Global Perspective of Their Role in Nutrition and Health;

194. Cheng, H. M.; Koutsidis, G.; Lodge, J. K.; Ashor, A.; Siervo, M.; Lara, J. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. *Atherosclerosis* **2017**, *257*, 100–108, doi:10.1016/j.atherosclerosis.2017.01.009.

195. Xu, X.; Li, J.; Wang, X.; Wang, S.; Meng, S.; Zhu, Y.; Liang, Z.; Zheng, X.; Xie, L. Tomato consumption and prostate cancer risk: a systematic review and meta-analysis. *Sci. Rep.* **2016**, *6*, 37091, doi:10.1038/srep37091.

196. Sikorska-Zimny, K.; Badełek, E.; Grzegorzewska, M.; Ciecierska, A.; Kowalski, A.; Kosson, R.; Tuccio, L.; Mencaglia, A. A.; Ciaccheri, L.; Mignani, A. G.; Kaniszewski, S.; Agati, G. Comparison of lycopene changes between open-field processing and fresh market tomatoes during ripening and post-harvest storage by using a non-destructive reflectance sensor. *J. Sci. Food Agric.* **2019**, *99*, 2763–2774, doi:10.1002/jsfa.9484.

197. Valderas-Martinez, P.; Chiva-Blanch, G.; Casas, R.; Arranz, S.; Martínez-Huélamo, M.; Urpi-Sarda, M.; Torrado, X.; Corella, D.; Lamuela-Raventós, R.; Estruch, R. Tomato Sauce Enriched with Olive Oil Exerts Greater Effects on Cardiovascular Disease Risk Factors than Raw Tomato and Tomato Sauce: A Randomized Trial. *Nutrients* **2016**, *8*, 170, doi:10.3390/nu8030170.

198. Agarwal, S.; Rao, A. V. Tomato lycopene and its role in human health and chronic diseases. *Can. Med. Assoc. J. J.* **2000**, *163*, 739–744.

199. Cheng, H. M.; Koutsidis, G.; Lodge, J. K.; Ashor, A. W.; Siervo, M.; Lara, J. Lycopene and tomato and risk of cardiovascular diseases: A systematic review and meta-analysis of epidemiological evidence. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 141–158, doi:10.1080/10408398.2017.1362630.

200. Györe-Kis, G.; Deák, K.; Lugasi, A.; Csúr-Vargaa, A.; Helyes, L. Comparison of conventional and organic tomato yield from a three-year-term experiment. *Acta Aliment.* **2012**, *41*, 486–493, doi:10.1556/AAlim.41.2012.4.10.

201. Hurtado-Barroso, S.; Tresserra-Rimbau, A.; Vallverdú-Queralt, A.; Lamuela-Raventós, R. M. Organic food and the impact on human health. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 704–714, doi:10.1080/10408398.2017.1394815.

202. de Ferrars, R. M.; Czank, C.; Zhang, Q.; Botting, N. P.; Kroon, P. A.; Cassidy, A.; Kay, C. D. The pharmacokinetics of anthocyanins and their metabolites in humans. *Br. J. Pharmacol.* **2014**, *171*, 3268–3282, doi:10.1111/bph.12676.

203. Amini, A. M.; Muzs, K.; Spencer, J. P.; Yaqoob, P. Pelargonidin-3- O -glucoside and its metabolites have modest anti-inflammatory effects in human whole blood cultures. *Nutr. Res.* **2017**, *46*, 88–95, doi:10.1016/j.nutres.2017.09.006.

204. El Mohsen, M. A.; Marks, J.; Kuhnle, G.; Moore, K.; Debnam, E.; Kaila Srai, S.; Rice-Evans, C.; Spencer, J. P. E. Absorption, tissue distribution and excretion of pelargonidin and its metabolites following oral administration to rats. *Br. J. Nutr.* **2006**, *95*, 51–8, doi:10.1079/bjn20051596.

205. Sannino, F.; Sansone, C.; Galasso, C.; Kildgaard, S.; Tedesco, P.; Fani, R.; Marino, G.; de Pascale, D.; Ianora, A.; Parrilli, E.; Larsen, T. O.; Romano, G.; Tutino, M. L. Pseudoalteromonas haloplanktis TAC125 produces 4-hydroxybenzoic acid that induces pyroptosis in human A459 lung adenocarcinoma cells. *Sci. Rep.* **2018**, *8*, 1190, doi:10.1038/s41598-018-19536-2.

206. Distelmaier, F. 4-Hydroxybenzoic acid for multiple system atrophy? *Parkinsonism Relat. Disord.* **2018**, *50*, 119–120, doi:10.1016/j.parkreldis.2018.01.019.

207. Winter, A. N.; Brenner, M. C.; Punessen, N.; Snodgrass, M.; Byars, C.; Arora, Y.; Linseman, D. A. Comparison of the Neuroprotective and Anti-Inflammatory Effects of the Anthocyanin Metabolites, Protocatechuic Acid and 4-Hydroxybenzoic Acid. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 1–13, doi:10.1155/2017/6297080.

208. Wang, X.-N.; Wang, K.-Y.; Zhang, X.-S.; Yang, C.; Li, X.-Y. 4-Hydroxybenzoic acid (4-HBA) enhances the sensitivity of human breast cancer cells to adriamycin as a specific HDAC6 inhibitor by promoting HIPK2/p53 pathway. *Biochem. Biophys. Res. Commun.* **2018**, *504*, 812–819, doi:10.1016/j.bbrc.2018.08.043.

209. Timoshchuk, S. V; Vavilova, H. L.; Strutyns'ka, N. A.; Talanov, S. A.; Petukhov, D. M.; Kuchmenko, O. B.; Donchenko, H. V; Sahach, V. F. [Cardioprotective action of coenzyme Q in conditions of its endogenous synthesis activation in cardiac ischemia-reperfusion in old rats]. *Fiziol. Zh.* **2009**, *55*, 58–63.

210. Kumchenko, E. B.; Petukhov, D. N.; Donchenko, G. V; Mkhitarian, L. S.; Timoshchuk, S. V; Strutinskaia, N. A.; Vavilova, G. L.; Sagach, V. F. [Effect of precursors and modulators of coenzyme Q biosynthesis on the heart mitochondria function in aged rats]. *Biomed. Khim. 56*, 244–56.

211. Weitkunat, K.; Schumann, S.; Nickel, D.; Kappo, K. A.; Petzke, K. J.; Kipp, A. P.; Blaut, M.; Klaus, S. Importance of propionate for the repression of hepatic lipogenesis and improvement of insulin sensitivity in high-fat diet-induced obesity. *Mol. Nutr. Food Res.* **2016**, *60*, 2611–2621, doi:10.1002/mnfr.201600305.

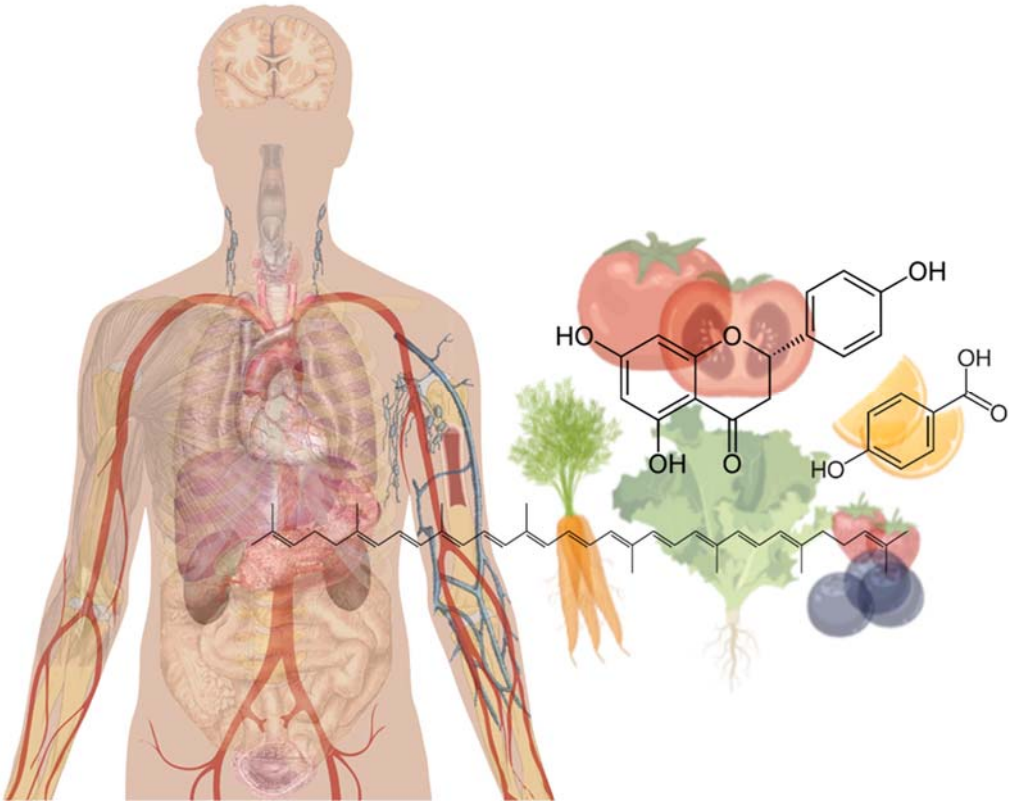
212. Chambers, E. S.; Viardot, A.; Psichas, A.; Morrison, D. J.; Murphy, K. G.; Zac-Varghese, S. E. K.; MacDougall, K.; Preston, T.; Tedford, C.; Finlayson, G. S.; Blundell, J. E.; Bell, J. D.; Thomas, E. L.; Mt-Isa, S.; Ashby, D.; Gibson, G. R.; Kolida, S.; Dhillo, W. S.; Bloom, S. R.; Morley, W.; Clegg, S.; Frost, G. Effects of targeted delivery of propionate to the human colon on appetite regulation,

body weight maintenance and adiposity in overweight adults. *Gut* **2015**, *64*, 1744–1754, doi:10.1136/gutjnl-2014-307913.

213. Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* **2005**, *81*, 243S–255S, doi:10.1093/ajcn/81.1.243S.

214. Woodside, J. V.; Draper, J.; Lloyd, A.; McKinley, M. C. Use of biomarkers to assess fruit and vegetable intake. *Proc. Nutr. Soc.* **2017**, *76*, 308–315, doi:10.1017/S0029665117000325.

ANEXO



8. ANEXO

8.1. Otras publicaciones en revistas

En esta sección se presentan otras publicaciones en las cuales he colaborado, pero no forman parte de esta tesis doctoral.

Publicación 7: Hrvolová B, Martínez-Huélamo M, Colmán-Martínez M, Hurtado-Barroso S, Lamuela-Raventós RM, Kalina J. Development of an Advanced HPLC-MS/MS Method for the Determination of Carotenoids and Fat-Soluble Vitamins in Human Plasma. Int J Mol Sci. 2016 Oct 14;17(10).

Publicación 8: Rinaldi de Alvarenga JF, Tran C, Hurtado-Barroso S, Martinez-Huélamo M, Illan M, Lamuela-Raventos RM. Home cooking and ingredient synergism improve lycopene isomer production in *Sofrito*. Food Res Int. 2017 Sep;99(Pt 2):851-861.

Publicación 9: Rinaldi de Alvarenga JF, Quifer-Rada P, Francetto Juliano F, Hurtado-Barroso S, Illan M, Torrado-Prat X, Lamuela-Raventós RM. Using Extra Virgin Olive Oil to Cook Vegetables Enhances Polyphenol and Carotenoid Extractability: A Study Applying the *sofrito* Technique. Molecules. 2019 Apr 19;24(8).

Publicación 10: Marhuenda-Muñoz M, Hurtado-Barroso S, Tresserra-Rimbau A, Lamuela-Raventós RM. A review of factors that affect carotenoid concentrations in human plasma: differences between Mediterranean and Northern diets. Eur J Clin Nutr. 2019 Jul;72(Suppl 1):18-25.

Publicación 11: Rinaldi de Alvarenga JF, Quifer-Rada P, Westrin V, Hurtado-Barroso S, Torrado-Prat X, Lamuela-Raventós RM. Mediterranean *sofrito* home-cooking technique enhances polyphenol content in tomato sauce. J Sci Food Agric. 2019 Jul 19.

8.2. Comunicaciones en congresos

En esta sección se presentan las comunicaciones presentadas en congresos nacionales e internacionales.

8.2.1.Comunicación 1. Póster

Título: Diferencias nutricionales entre una dieta rica en antioxidantes y una dieta baja en antioxidants.
Autores: Sara Hurtado-Barroso, Jose Fernando Rinaldi Alvarenga, Paola Quifer-Rada, Anna Creus-Cuadros, Rosa María Lamuela-Raventós.
Congreso: XI Congreso Internacional sobre la Dieta Mediterránea.
Barcelona (España).
27-28 de abril de 2016.

Diferencias nutricionales entre una dieta rica en antioxidantes y una dieta baja en antioxidantes

Sara Hurtado-Barroso[1,2], Jose Fernando Rinaldi Alvarenga[1], Paola Quifer-Rada[1,2], Anna Creus-Cuadros[1], Rosa María Lamuela-Raventós[1,2]

[1]Department of Nutrition, Food Science, and Gastronomy, School of Pharmacy, INSA-University of Barcelona, Barcelona, Spain

[2]CIBER Physiopathology of obesity and nutrition (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain

INTRODUCCIÓN

La dieta mediterránea, rica en hortalizas y frutas, previene enfermedades crónicas tales como las enfermedades cardiovasculares debido en parte al alto contenido en compuestos con propiedades antioxidantes para la salud.

OBJETIVO

Valorar si con el seguimiento de una dieta típicamente mediterránea, rica en antioxidantes, respecto a una dieta con un consumo de 2 piezas de fruta u hortalizas (pobre en antioxidantes), mejoraba el aporte de antioxidantes.

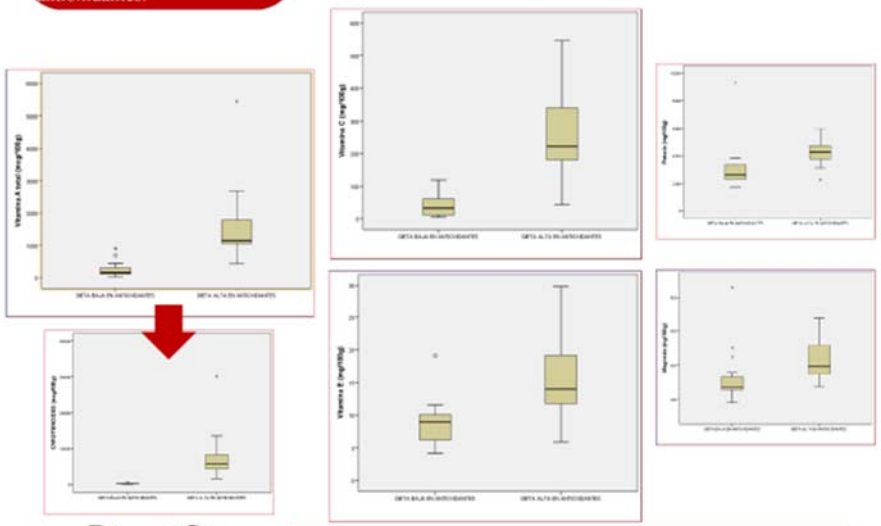
DISEÑO DEL ESTUDIO

- Randomizado
- Cruzado
- Aleatorizado

18 varones
18-32 años
No fumadores
Sanos

REGISTROS DIETÉTICOS DE LOS 3 ULTIMOS DÍAS

RESULTADOS



CONCLUSIÓN

Las recomendaciones nutricionales van dirigidas a aumentar el consumo de alimentos ricos en antioxidantes (frutas y hortalizas), incrementar la ingesta de las vitaminas antioxidantes (A, E y C), de los carotenoides; así como de algunos minerales tales como el potasio y magnesio.

8.2.2.Comunicación 2. Póster

Título: Biodisponibilidad de los carotenoides del *sofrito* en varones tras una dieta rica en alimentos con componentes antioxidantes.
Autores: Sara Hurtado-Barroso, Miriam Martínez-Huélamo, Mariel Colmán-Martínez, Jose Fernando Rinaldi Alvarenga, Rosa María Lamuela-Raventós.
Congreso: VIII Seminario sobre Alimentación y Estilos de Vida Saludable. Mallorca (España).
21-22 de julio de 2016.

Biodisponibilidad de los carotenoides del sofrito en varones tras una dieta rica en alimentos con componentes antioxidantes

Sara Hurtado-Barroso[1,2], Miriam Martínez-Huélamo[1,2], Mariel Colmán-Martínez[1], Jose Fernando Rinaldi Alvarenga[1], Rosa María Lamuela-Raventós[1,2]

[1]Departamento de Nutrición, Ciencias de la Alimentación y Gastronomía, Facultad de Farmacia, INSA-Universidad de Barcelona, Barcelona, España
[2]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, España

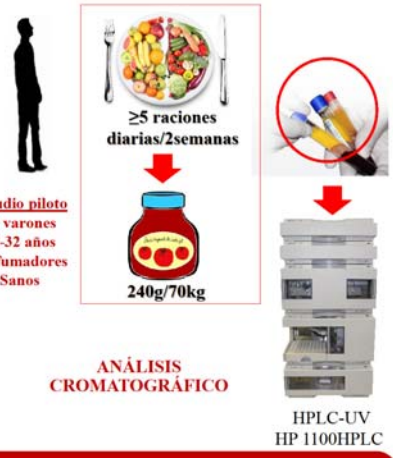
INTRODUCCIÓN

El sofrito es una salsa típica de la dieta mediterránea que contiene tomate como componente principal y otros ingredientes de origen vegetal (aceite de oliva, cebolla y ajo) ricos en componentes bioactivos. Entre ellos cabe destacar los carotenoides, que están presentes principalmente en el tomate. Sin embargo, la biodisponibilidad de los mismos es baja y varios factores como el procesamiento y la matriz del alimento pueden interferir positiva y/o negativamente en su absorción.

OBJETIVO

Evaluar la biodisponibilidad de los carotenoides del sofrito tras una dieta rica en antioxidantes

DISEÑO DEL ESTUDIO

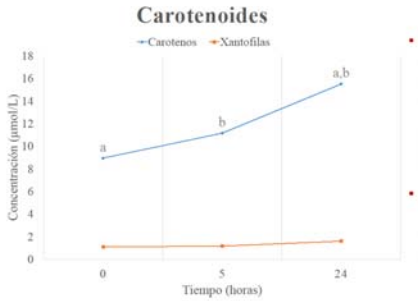


Estudio piloto
22 varones
18-32 años
No fumadores
Sanos

ANÁLISIS CROMATOGRÁFICO

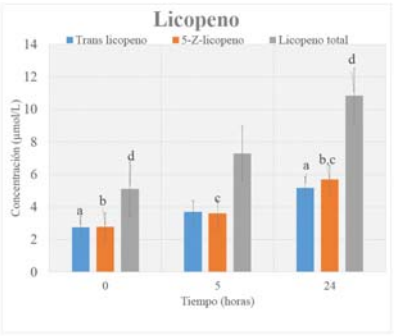
HPLC-UV
HP 1100HPLC

RESULTADOS



- El licopeno es el carotenoide mayoritario en el plasma antes y después de la ingesta de sofrito, alcanzando su máximo a las 5 horas después de la ingesta.
- A las 5 horas, el licopeno representa el 75% de los carotenoides presentes en el plasma.

- En todos los casos, el licopeno aumenta de las 0 a las 24 horas. Además el aumento de 5 a 24 horas del isómero *cis* también es significativo.
- El 5-*cis*-licopeno se encuentra en la misma proporción que el *trans*-licopeno a las 0 y 5 horas, pero a las 24 horas el isómero *cis* aumenta con respecto al *trans* un 4,7%, aunque sin diferencias significativas.



(a-d): diferencias significativas con un p-valor <0,05

CONCLUSIONES

El contenido de carotenoides aumenta significativamente, tras la ingesta de sofrito, debido en gran parte al aumento de licopeno a las 24 horas de su administración.

FINANCIACIÓN

Este estudio ha sido financiado por el Ministerio de Economía y Competitividad (AGL2013-49083-C3-1-R) y por el Centro de Investigación Biomédica en Red-Fisiopatología de la Obesidad y la Nutrición (CIBEROBN).

8.2.3.Comunicación 3. Póster

Título: Bioavailability of carotenoids *sofrito* in healthy and young men following a diet rich in foods with antioxidant compounds.
Autores: Hurtado-Barroso, S, Martínez-Huélamo, M, Colmán-Martínez, M, Alvarenga, JFR, Lamuela-Raventós, RM.
Congreso: II Workshop Anual sobre 'Cacao y chocolate; ciencia y gastronomía'. Santa Coloma de Gramenet, Barcelona (España).
09 de noviembre de 2016.

BIOAVAILABILITY OF CAROTENOIDS SOFRITO IN HEALTHY AND YOUNG MEN FOLLOWING A DIET RICH IN FOODS WITH ANTIOXIDANT COMPOUNDS

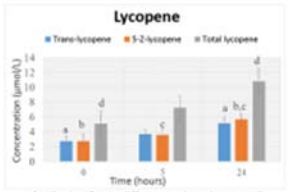
Hurtado-Barroso, S.[1,2*], Martínez-Huélamo, M.[1,2], Colmán-Martínez, M.[1], Alvarenga, J.F.R[1], Lamuela-Raventós, R.M. [1,2]

[1]Department of Nutrition, Food Science, and Gastronomy, School of Pharmacy and Food Science, INSA-University of Barcelona, Barcelona, Spain; [2]CIBER Physiopathology of obesity and nutrition (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain

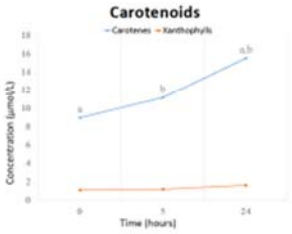
Background and objectives:

The *sofrito* is a typical sauce of Mediterranean diet containing tomato as the main component and other plant-based ingredients (olive oil, onion and garlic) rich in bioactive compounds such as carotenoids. However, the bioavailability is low and various factors such as processing and food matrix may interfere positively and/or negatively. The aim of this study was to evaluate the bioavailability of carotenoids of *sofrito* after a diet rich in antioxidants

Methodology:



(a-d): significant differences (p-value <0.05)



(a,b): significant differences (p-value <0.05)

Results:

LYCOPENE: MAIN CAROTENOID

5 hours after the sofrito intake:

- **maximum concentration**
- **75% carotenoids**

Carotenes increase with time, mainly lycopene

- **0 hour and 5 hours after the sofrito intake:**

5-*cis*-lycopene=trans-lycopene

- **24 hours after the sofrito intake:**

5-*cis*-lycopene= 4.7 % more than trans-lycopene (not significant difference)

Xanthophylls don´t increase with time

HPLC-UV
HP 1100HPLC

- **Column: YMC Carotenoid**
- **Injection volume: 20 µL**
- **Flow: 0.6 mL/min**

Conclusions:

The carotenoids in plasma increase significantly after the *sofrito* intake, mainly lycopene after 24 hours of *sofrito* intervention.

Acknowledge:

This study was funded by the Ministry of Economy and Competitiveness (AGL2013-49083-C3-1-R) and the Biomedical Research Center in Red-Pathophysiology of Obesity and Nutrition (CIBERobn). The *sofrito* was provided by GB Foods.

8.2.4.Comunicación 4. Póster

Título: Effect of composition of tomato-based mediterranean *sofrito* on color changes during accelerated storage.
Autores: Cordero-García, M.; Rinaldi-Alvarenga, J.F.; Hurtado-Barroso, S.; Lamuela-Raventós, R.M.
Congreso: II Workshop Anual sobre 'Cacao y chocolate; ciencia y gastronomía'. Santa Coloma de Gramenet, Barcelona (España).
09 de noviembre de 2016.

EFFECT OF COMPOSITION OF TOMATO-BASED MEDITERRANEAN SOFRITO ON COLOR CHANGES DURING ACCELARATED STORAGE

Marcia Cordeiro-García[1]; José Fernando Rinaldi Alvarenga[2]; Sara Hurtado-Barroso[2,3]; Rosa Maria Lamuela-Raventós[2,3]

[1]National Center of Food Science and Technology, Universidad de Costa Rica, Costa Rica.
[2]Nutrition, Food Science and Gastronomy Department, XaRTA, INSA, Pharmacy and Food Science School. University of Barcelona, Spain
[3]CIBER 06/003 Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Spain

INTRODUCTION

Color in food is a very important quality indicator and marketing factor that affects the buying decision of the consumer, and it is also a good indicator of degradation of components during time. The color of *sofrito*, a tomato-based key component of the Mediterranean diet, is strongly related with the content of carotenoids, bioactive compounds which are also associated with many health benefits.
The objective of these study was to evaluate if different ingredients of *sofrito* formulations might have an effect of the color preservation during accelerated storage.

METHODOLOGY

Sample	Olive oil	Onion	Garlic
1	0%	0%	0%
2	10%	0%	0%
3	0%	20%	0%
4	10%	20%	0%
5	0%	0%	2%
6	10%	0%	2%
7	0%	20%	2%
8	10%	20%	2%

COLOR ANALISYS

Hue

ArcTG (h*/a*)

Total Color Change (ΔE)

$\Delta E^* = [(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{[1/2]}$

KINECTIC EVALUATION

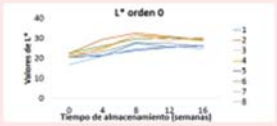
$C = C_0 - kt$ (zero order)

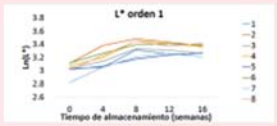
$C = C_0 \exp(kt)$ (first order)

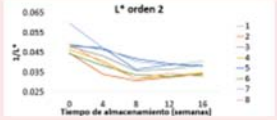
$C = (C_0/1+C_0kt)$ (second order)

RESULTS L*

L*	zero-order model			first-order model			second-order model		
	Co	K(sem-1)	R2	Co	K(sem-1)	R2	Co	K(sem-1)	R2
1	19.666	1.3229	0.6142	2.9511	0.067	0.9663	0.0502	-0.0028	0.6531
2	24.305	1.5217	0.3844	3.1794	0.0585	0.4062	0.042	-0.0023	0.4248
3	20.689	1.4563	0.5832	3.0318	0.0611	0.6051	0.0481	-0.0026	0.6242
4	21.254	2.0223	0.5838	3.0608	0.079	0.6134	0.0467	-0.0031	0.6385
5	18.822	1.558	0.9714	2.9511	0.067	0.9663	0.0516	-0.0029	0.96
6	22.124	1.8315	0.7755	3.104	0.0695	0.7684	0.0446	-0.0027	0.7594
7	16.423	2.0801	0.7598	2.8129	0.0985	0.7496	0.0595	-0.0047	0.7344
8	20.115	2.3977	0.6935	3.0112	0.0946	0.699	0.0489	-0.0038	0.6976

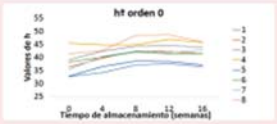


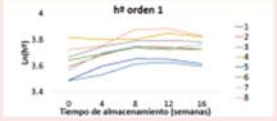




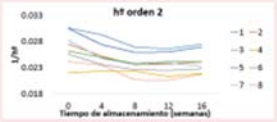
Zero-order model kinetics better describe the luminosity (L*) behavior during accelerated storage, showing an increase of luminosity values in time. Samples containing garlic suffer a bigger change in this parameter, probably due to the loss of carotenoids.

RESULTS h*





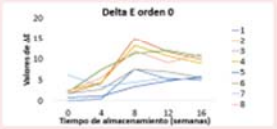
Formulations containing onion and oil help with red color retention of *sofrito* samples, better than those containing garlic. Increase in hue (h°) values were observed as a consequence of storage, showing loss of red color with time. This occurrence is better described using zero-order model kinetics.

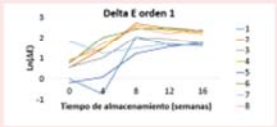


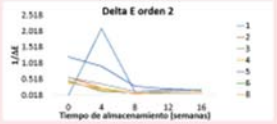
h*	zero-order model			first-order model			second-order model		
	Co	K(sem-1)	R2	Co	K(sem-1)	R2	Co	K(sem-1)	R2
1	33.455	1.0708	0.5128	3.476	0.0315	0.6884	0.0309	-0.0009	0.6945
2	36.546	1.2932	0.6412	3.5989	0.033	0.6427	0.0273	-0.0008	0.6435
3	39.252	1.1867	0.68994	3.6705	0.0284	0.6859	0.0255	-0.0007	0.682
4	44.718	0.2668	0.2309	3.8005	0.0058	0.2314	0.0224	-0.0001	0.2318
5	33.455	1.0708	0.5128	3.5092	0.0303	0.5183	0.0299	-0.0009	0.523
6	38.254	0.7922	0.6521	3.6445	0.0198	0.6543	0.0261	-0.0005	0.6561
7	35.585	1.6669	0.7359	3.5735	0.0425	0.7225	0.028	-0.0011	0.7091
8	36.546	1.2932	0.6412	3.71	0.0343	0.5447	0.0245	-0.0008	0.5598

RESULTS ΔE*

Delta E	zero-order model			first-order model			second-order model		
	Co	K(sem-1)	R2	Co	K(sem-1)	R2	Co	K(sem-1)	R2
1	-0.8773	1.5299	0.5305	-0.7958	0.572	0.5529	0.9799	-0.1526	0.0746
2	1.4094	2.3752	0.4743	0.5458	0.4492	0.6402	0.5431	-0.1101	0.6405
3	1.3787	1.1782	0.5304	0.4829	0.3225	0.6453	0.5764	-0.0996	0.7069
4	2.1703	1.935	0.4693	0.8699	0.3464	0.6194	0.3968	-0.0716	0.6861
5	-0.894	1.3537	0.9587	-0.7227	0.5361	0.9207	1.3971	-0.2785	0.8656
6	2.2383	2.1596	0.6977	0.8955	0.3707	0.6481	0.4171	-0.0809	0.5786
7	5.0824	-0.0624	0.0088	1.5741	-0.0018	0.0002	0.2187	-0.002	0.004
8	1.6102	2.1403	0.6987	0.8409	0.3642	0.7224	0.4026	-0.0748	0.6706







Increase of ΔE values was percieved for all formulations, except for sample containing garlic and onion, which suffered degradation of color during heating. Color change of the sample containing only garlic (5) showed a good correlation with zero-order model kinetics during accelerated storage.

CONCLUSION

The presence of onion and extra virgin olive oil allows a better preservation of the color, showed by the Hue values and total color change kinetic model. Formulations containing garlic accelerate the degradation of color, indicated by good models of L* and suffering the most expressive total color change during storage. The presence of onion and extra virgin olive oil must be encouraged in the *sofrito* formulation to preserve the color and carotenoid content.

8.2.5.Comunicación 5. Póster

Título: Development of an advanced HPLC-MS/MS method for the determination of carotenoids and fat-soluble vitamins in human plasma.
Autores: Martínez-Huélamo, M.; Hrvolová, B.; Colmán-Martínez, M.; Hurtado-Barroso, S.; Kalina, J.; Lamuela-Raventós R.M.
Congreso: II Workshop Anual sobre 'Cacao y chocolate; ciencia y gastronomía'. Santa Coloma de Gramenet, Barcelona (España).
09 de noviembre de 2016.

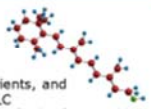
Development of an advanced HPLC-MS/MS method for the determination of carotenoids and fat-soluble vitamins in human plasma

Martínez-Huélamo, M.[1,2*], Hrvolová, B.[3], Colmán-Martínez, M.[1], Hurtado-Barroso, S.[1,2], Kalina, J.[3], Lamuela-Raventós R.M.[2,3]

[1] Department of Nutrition, Food Science and Gastronomy-XARTA-INSA, School of Pharmacy and Food Science, University of Barcelona, Barcelona 08028, Spain. [2] CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, CIBEROBN, Madrid, Spain. [3] Faculty of Science, University of Ostrava, 701 03 Ostrava, Czech Republic.

BACKGROUND AND OBJECTIVES

Carotenoids, xanthophylls and carotenes, are natural fat-soluble, red, yellow and orange pigments characterized by a wide distribution, structural diversity and numerous physio-chemical and biological properties. Another group of interesting and useful compounds are fat-soluble vitamins and their metabolites such as retinol, retinol acetate, cholecalciferol, and α-tocotrienol. These compounds have free radical scavenging properties that allow them to function as antioxidants.

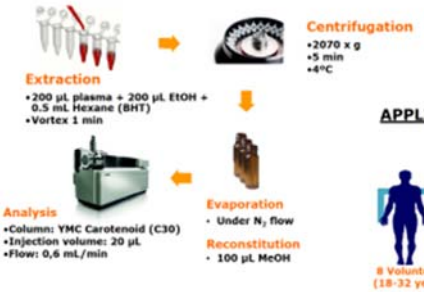


Available methods can determine only a few representatives of the aforementioned fat-soluble micronutrients, and few of them can be applied for the simultaneous analysis of compounds in biological samples. Most use HPLC separation coupled to UV–VIS or DAD detection, but with these methods it is extremely challenging to obtain the sensitivity required for the analysis of human fluids, in which the concentration of fat-soluble micronutrients is very low. The problem of sensitivity can be solved by usage of tandem mass spectrometry detection, although finding general ionization conditions suitable for all targeted analytes is very difficult.

The **aim** of this research was to develop and validate a new HPLC-MS/MS method for the quantification of selected carotenoids and fat-soluble vitamins in human plasma.

METHODOLOGY

EXTRACTION OF CAROTENOIDS AND VITAMINS



APPLICATION METHOD

VALIDATION METHOD PARAMETERS

RESULTS

Table 1. Limit of detection (LOD), limit of quantification (LOQ), recovery, matrix effect, linearity range, interday and intraday precision, interday and intraday accuracy and concentration obtained in the study with high antioxidant diet by the HPLC-MS/MS method.

Analyte	Transition MRM	LOD (nM)	LOQ (nM)	Recovery (%)	Matrix effect (%)	Linearity Range (µg/mL)	Interday Precision (% RSD)	Intraday Precision (% RSD)	Interday Accuracy (%)	Intraday Accuracy (%)	Concentration plasma (nM)
retinol	269 → 181	7.0	17.5	103 ± 9	109 ± 9	17.5-34909	6.7	11.5	101 ± 4	105 ± 4	115 ± 11
25-hydroxycholecalciferol	383 → 365	7.5	27.5	92 ± 3	87 ± 1	27.5-12480	3.3	11.5	102 ± 3	105 ± 6	190 ± 33
retinol acetate	329 → 269	6.1	24.4	103 ± 2	95 ± 2	24.4-30442	1.3	11.1	105 ± 1	106 ± 7	<LOQ [a]
α-tocotrienol	411 → 165	266.1	885.4	99 ± 5	90 ± 3	885.4-11774	10.4	9.5	104 ± 4	108 ± 3	n.d. [b]
cholecalciferol	385 → 367	13.0	46.8	103 ± 3	90 ± 1	46.8-25998	2.0	12.7	103 ± 1	104 ± 7	n.d. [b]
astaxanthin	597 → 147	1.7	5.0	102 ± 3	100 ± 3	5.0-1675	8.8	10.3	103 ± 3	105 ± 6	<LOQ [a]
lutein	551 → 429	14.1	49.2	86 ± 1	91 ± 2	49.2-17579	4.1	12.1	106 ± 2	109 ± 6	260 ± 139
zeaxanthin	568 → 476	741.8	2471.5	86 ± 2	87 ± 3	2471.5-17578	5.5	10.8	107 ± 5	113 ± 2	n.d. [b]
cantaxanthin	565 → 363	3.5	10.6	100 ± 3	104 ± 8	10.6-1770	6.2	11.5	104 ± 6	109 ± 11	28 ± 12
E-β-apo-8'-carotenal	417 → 325	7.2	24.0	104 ± 2	100 ± 1	24.0-24002	13.1	11.5	107 ± 8	114 ± 2	<LOQ [a]
cryptoxanthin	553 → 535	441.3	1468.8	105 ± 3	94 ± 2	1468.8-18088	4.4	14.0	101 ± 1	103 ± 8	<LOQ [a]
13-Z-β-carotene	536 → 444	104.3	348.3	101 ± 2	88 ± 1	348.3-18626	9.1	13.6	101 ± 9	109 ± 2	n.d. [b]
α-carotene	536 → 444	41.0	136.0	104 ± 5	103 ± 4	136.0-9313	5.9	12.1	107 ± 4	112 ± 1	101 ± 19
β-carotene	537 → 413	76.4	257.0	101 ± 2	96 ± 5	257.0-9313	3.4	12.2	100 ± 3	103 ± 4	2634 ± 1870
9-Z-β-carotene	537 → 413	545.8	1816.1	97 ± 7	92 ± 2	1816.1-18626	4.9	14.0	101 ± 5	107 ± 4	n.d. [b]
5-Z-lycopene	537 → 413	352.0	1175.3	105 ± 8	97 ± 3	1175.3-18626	5.2	13.4	102 ± 3	107 ± 9	n.d. [b]

[a] under limit of quantification; n.d. is not detected

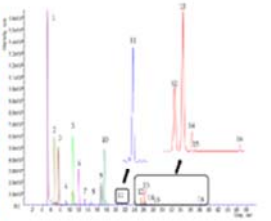


Figure 1. Chromatogram of working standard solutions obtained by HPLC-MS/MS analysis. Peaks: (1) retinol; (2) 25-hydroxycholecalciferol; (3) retinol acetate; (4) α-tocotrienol; (5) cholecalciferol; (6) astaxanthin; (7) lutein; (8) zeaxanthin; (9) cantaxanthin; (10) E-β-apo-8'-carotenal; (11) cryptoxanthin; (12) 13-Z-β-carotene; (13) α-carotene; (14) β-carotene; (15) 9-Z-β-carotene; and (16) 9-Z-lycopene.

CONCLUSIONS

A unique HPLC-MS/MS method for the simultaneous quantification of 16 carotenoids and fat-soluble vitamins in human plasma was designed and fully validated. Good quality values of LOD, LOQ, recovery, linearity, matrix effect, accuracy, and precision were obtained by the proposed method. According to our knowledge, no similar HPLC-MS/MS method for the determination of such a large number of analytes has been previously published. In the future, considering the excellent validation results obtained, this method could be used in various applied clinical studies or investigations.

ACKNOWLEDGMENTS

This work was supported by CICYT (AGL2013-49083-C3-1-R and AGL2016-70113-R), the Instituto de Salud Carlos III, ISCIII (CIBEROBN) from the Spanish Ministry of Economy and Competitivity (MEC), Generalitat de Catalunya (GC) 2014 SGR 773, and by the Project LO1208 (TEWEP) of the National Feasibility Programme I of the Czech Republic. Hrvolová, B. also thanks the student grant n. SGS04/PřF/2016 from University of Ostrava, Czech Republic.

8.2.6.Comunicación 6. Oral

Título: Home cooking and ingredients synergism improve lycopene isomers in *sofrito*.
Autores: Rinaldi-Alvarenga, J.F.; Tran, C.; Hurtado-Barroso, S.; Martínez-Huélamo, M.; Illan, M.; Lamuela- Raventós, R. M.
Congreso: II Workshop Anual sobre 'Cacao y chocolate; ciencia y gastronomía'. Santa Coloma de Gramenet, Barcelona (España).
09 de noviembre de 2016.

HOME COOKING AND INGREDIENTS SYNERGISM IMPROVE LICOPENE ISOMERS IN *SOFRITO*

Alvarenga, J.F.R.[1*], Tran, C.[1], Hurtado-Barroso, S.[1,2], Martínez-Huélamo, M.[1,2], Illan, M.[1], Lamuela-Raventós, R.M.[1,2]

[1] *Nutrition, Food Science and Gastronomy Department, XaRTA, INSA, Pharmacy and Food Science School. University of Barcelona, Spain*

[2] *CIBER 06/003 Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Spain*

Background and objectives:

Tomato products rich in lycopene Z-isomers are of interest since these carotenoids present more bioavailability and antioxidant capacity than the all-E forms. Intrinsic food properties, processing, and the interaction between dietary components are all factors that can influence the content, type and bioavailability of carotenoids. The aim of this study was to evaluate how the content of carotenoids and their isomerization in tomato-based Mediterranean, *sofrito,* might be affected by the process of home cooking as well as by the presence of other ingredients.

Methodology:

A full factorial design 2^4 was applied to clarify the contribution of the ingredients: extra virgin olive oil (5-10%), onion (20-40%) and garlic (2-4%), and cooking duration (30-60 min) on the carotenoid composition of *sofrito*. The identification of the carotenoids was based on retention time; chromatography with standards; UV/VIS absorption spectrum: λmax, %III/II and %Ab/II; and mass spectrum. Quantification was performed by HPLC-DAD, using external calibration curves with standards.

Results and conclusions:

The factors associated with a higher production of 5-Z-lycopene, 9-Z-lycopene and 13-Z-lycopene in *sofrito* were the cooking duration and onion content. Onion proved to be the most interesting ingredient in the *sofrito* formulation due to its enhancing effect on lycopene isomerization. Combined with an adequate processing time, this vegetable could be explored as an ingredient to improve the bioavailability of lycopene in tomato products.

Acknowledge:

CNPq (Brazil); MECD, ME AGL2013-49083_C3-1C, CIBERON and Generalitat de Catalunya (Spain)

☒ **All authors of the present work authorize the Organizing Committee of the II Workshop INSA-UB to include the present abstract as a part of a conference report to be published in a journal of the field.**

8.2.7.Comunicación 7. Póster

Título: Synergism effect of home cooking and ingredients enhance tomato polyphenols in Mediterranean *sofrito*.
Autores: Rinaldi-Alvarenga, J.F.; Weastrin, V.; Quifer-Rada, P.; Hurtado-Barroso, S.; Torrado-Prat, X.; Lamuela- Raventós, R. M.
Congreso: II Workshop Anual sobre 'Cacao y chocolate; ciencia y gastronomía'. Santa Coloma de Gramenet, Barcelona (España).
09 de noviembre de 2016.

SYNERGISM EFFECT OF HOME COOKING AND INGREDIENTS ENHANCE TOMATO POLYPHENOLS IN MEDITERRANEAN SOFRITO

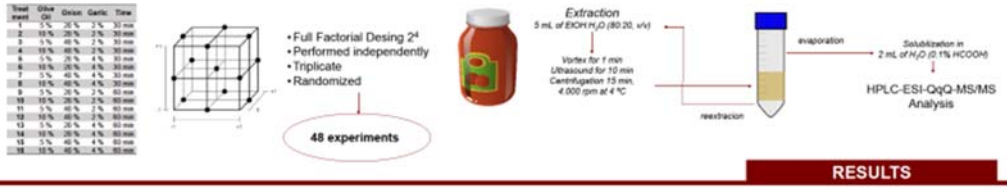
Alvarenga, J.F.R.[1*], Weastrin, V.[1], Quifer-Rada, P.[1,2], Hurtado-Barroso, S.[1,2], Torrado-Prat, X.[1], Lamuela-Raventós, R.M.[1,2]

[1]Department of Nutrition, Food Sciences and Gastronomy, XaRTA, INSA-UB, Faculty of Pharmacy and Food Sciences. University of Barcelona, Spain
[2]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Spain

INTRODUCTION

There has been increasing the interest in the food matrix study since the bioavailability of bioactive compounds, like polyphenols, are affected by intrinsic food proprieties, processing and interaction of these factors. Sofrito, a typical home-made Mediterranean tomato based sauce, present a complex matrix by the addition of ingredients like olive oil, onion and garlic that can influence the content, type and bioavailability of polyphenols. The aim of this study was to evaluate whether home cooking and ingredients addition in Mediterranean sofrito sauce may interact and improve tomato's polyphenols.

METHODOLOGY



RESULTS

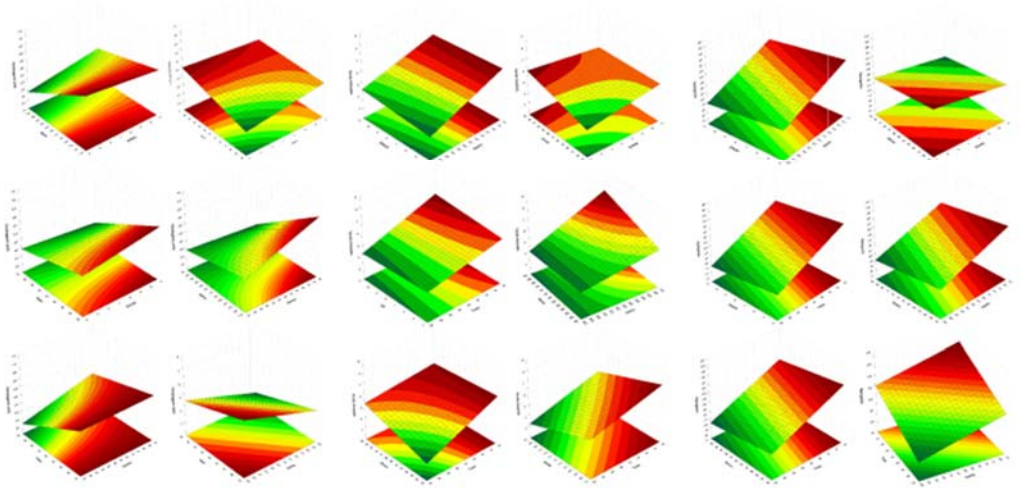


Figure 1. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on 0 level (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) on the content of chlorogenic acid of home cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

Figure 2. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on 0 level (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) on the content of naringenin of home cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

Figure 3. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on 0 level (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) on the content of ferulic acid hexoside of home cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

Extract polyphenols from food matrix
Improve extraction of polyphenols from cell plant fraction

EXTRA VIRGIN OLIVE OIL + **ONION**

Source of quercetin
Stabilizes free radicals in water/oil interface, avoid oxidation of lipids and consequently polyphenols

PRESERVE POLYPHENOLS IN *SOFRITO*

CONCLUSION

Short cooking time was able to increase the content of chlorogenic acid, ferulic acid hexoside and naringenin. The presence of olive oil enhances the extractability of some polyphenols from tomato improving the bioaccessibility. Onion shows to be capable to protect some phenolic compounds from oxidation during cooking process. The use of olive oil and onion with adequate cooking time may improve tomato's polyphenols stability.

8.2.8.Comunicación 8. Póster

Título: Total Polyphenols and antioxidant capacity in organic versus conventional white wine from two Spanish varieties: Chardonnay and Xarel-lo.
Autores: Hurtado-Barroso, S, Alvarenga, JFR, Treserra-Rimbau, A, Francetto Juliano F, Lamuela-Raventós, RM.
Congreso: Wine and Health 2017 Meeting.
Logroño, La Rioja (España).
16-18 de febrero de 2017

Total Polyphenols and antioxidant capacity in organic versus conventional white wines from two spanish varieties: Chardonnay and Xarel·lo

Hurtado-Barroso, S.[1,2], Alvarenga, J.F.R.[1], Tresserra-Rimbau, A.[1,2], Juliano F.F.[3], Lamuela-Raventós, R.M.[1,2]

[1]Department of Nutrition, Food Science, and Gastronomy, School of Pharmacy and Food Science, INSA-University of Barcelona, Barcelona, Spain;
[2]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain;
[3]Department of Agri-food Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil

Background

It is well-known that the amount of polyphenols in wines depends on several factors such us grape variety, growing conditions, and wine making process. However there are not many studies about the differences between organic and conventional wines.

Methodology

White wines:

- Organic Chardonnay (n=6)
- Conventional Chardonnay (n=8)
- Organic Xarel·lo (n=12)
- Conventional Xarel·lo (n=4)

Samples

✓ Total Polyphenols (TP) – Folin Ciocalteau method:
Standard: gallic acid
184 µL Milli-Q water + 24 µL sample + 12 µL Folin-Ciocalteau solution + 30 µL Na_2CO_3 20% + 1h-darkness → 765 nm

✓ Antioxidant Capacity (ABTS and DPPH):
Standard: Trolox
5 µL sample + 250 µL ABTS + 1h-darkness → 734 nm
5 µL sample + 250 µL DPPH + 1/2h-darkness → 515 nm

96-well plates

Results

TP, ABTS and DPPH are significantly higher in Chardonnay than in Xarel·lo wines

All organic wines had higher levels in the antioxidant capacity; however only DPPH was significatively higher in organic Chardonnay versus conventional Chardonnay

	TP		ABTS		DPPH	
	Organic	Conventional	Organic	Conventional	Organic	Conventional
Chardonnay	254,93±36,28	276,33±33,16	2,30±0,43	2,28±0,15	0,79±0,13*	0,62±0,13*
Xarel·lo	214,09±103,53	212,86±4,72	1,79±1,05	1,59±0,71	0,59±0,26	0,36±0,23

*Significative difference ($p<0,05$)

Conclusion

Antioxidant capacity seems higher in organic wine than conventional wine; however more research is needed to corroborate this results.

Acknowledgement

This work was supported in part by:

- CICYT(AGL2013-49083-C3-1-R and AGL2016-79113-R) and the Instituto de Salud Carlos III, ISCIII (CIBEROBN) from the Ministerio de Economía y Competitividad (MEC) (AEI/FEDER, UE) and Generalitat de Catalunya (GC) 2014 SGR 773.
- INSA (Institut de Recerca en Nutrició i Seguretat Alimentària)
- Generalitat de Catalunya. Ajuts per incentivar la recerca aplicada en producció agroalimentària Ecològica 530 50122 016

8.2.9.Comunicación 9. Oral

Título: Cooking effects on the bioavailability and bioactivity of phenolic and carotenoids of Mediterranean *sofrito*.
Autores: Rosa M. Lamuela-Raventos, Jose Fernando-Rinaldi, Sara Hurtado-Barroso, Miriam Martinez-Huelamo, Montse Illan and Xavier Torrado.
Congreso: 253rd National Meeting of the American-Chemical-Society (ACS) on Advanced Materials, Technologies, Systems, and Processes. San Francisco, CA. 02-06 de abril de 2017

AGFD 124 Cooking effects on the bioavailability and bioactivity of phenolic and carotenoids of Mediterranean *sofrito*

Rosa M Lamuela-Raventos[1,2] lamuela@ub.edu, José Fernando-Rinaldi[3], Sara Hurtado-Barroso[1,2], Miriam Martínez-Huélamo[1,2], Montse Illan[1], Xavier Torrado[1].

(1) Univ. of Barcelona, Spain (2) CIBEROBN, Madrid, Spain

Tomato is one of the vegetables most consumed worldwide, mainly in its processed forms, as sauce, canned or juice and it is very rich in bioactive compounds such as vitamin C, carotenoids and polyphenols. The behavior of bioactive compounds during cooking has been extensible studied, however the effect of time the addition of different ingredients and the complexity of these interaction improving the bioavailability of bioactive compounds is a challenging subject for research. The aim of this study was to evaluate the effect of home cooking process and the presence of the ingredients used in the preparation of Mediterranean *sofrito* (extra virgin olive oil, onion and garlic) could interact, modify and improve the content of carotenoid and phenolics from tomato. A Full Factorial Design was applied to clarify how the contribution of each ingredient and time modify the phenolic and carotenoid profile and possible synergism between the ingredients.

8.2.10. Comunicación 10. Oral y póster

Título: Effect of *sofrito* intake on diastolic blood pressure in healthy men after high and low antioxidants diet.
Autores: Hurtado-Barroso, S.; Alvarenga, J.F.R.; Marhuenda-Muñoz, M.; Lamuela-Raventós, R.M.
Congreso: Mediterranean Diet & Health.
Halkidiki (Greece).
19 de octubre de 2017.

Effect of *sofrito* intake on diastolic blood pressure in healthy men after high and low antioxidants diet

Hurtado-Barroso, S.[1,2]; Alvarenga, J.F.R.[1]; Marhuenda-Muñoz, M.[1]; Lamuela-Raventós, R.M.[1,2]

[1]Department of Nutrition, Food Science, and Gastronomy, School of Pharmacy and Food Science, INSA-University of Barcelona, Barcelona, Spain

[2]CIBER Physiopathology of obesity and nutrition (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain

Introduction: *Sofrito* is a typical tomato-based Mediterranean sauce made with olive oil and onions that are rich in bioactive compounds. **Hypothesis:** The hypothesis was that *sofrito* intake could have a beneficial effect on cardiovascular health, particularly in the regulation of blood pressure. **Methodology:** A cross-sectional, controlled and randomized study in which 22 healthy men between 18 and 32 years followed a high antioxidants diet (≥ 5 vegetables and fruits) and a low antioxidants diet (≤2 vegetables and fruits) for 2 weeks and after that intake a single portion of *sofrito* (240g/70kg) was carried. Volunteers were previously informed about the antioxidants level in foods to perform a free diet with high and low content in antioxidants. The blood pressure and heart rate was measured on basal and after the *sofrito* intake by a digital blood pressure monitor. **Results:** Lower diastolic blood pressure was observed after five hours of *sofrito* intake compare to basal measure in both diets (75,89±8,25 vs. 70,86±8,31, p value= 0.006 and 76,14±9,06 vs. 71,87±8,11, p value=0,039). **Conclusion:** According to these preliminary results, *sofrito* intake could improve cardiovascular health, reducing diastolic blood pressure. The mechanisms involved in this fact will be studied soon. **Acknowledgment.** This work was supported by Ministerio de Economía y Competitividad (AGL2013-49083-C3-1-R) and Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN).

Effect of *sofrito* intake on diastolic blood pressure in healthy men after high and low antioxidants diet

Hurtado-Barroso, S.[1,2]; Alvarenga, J.F.R.[1]; Marhuenda-Muñoz, M.[1,2]; Lamuela-Raventós, R.M.[1,2]

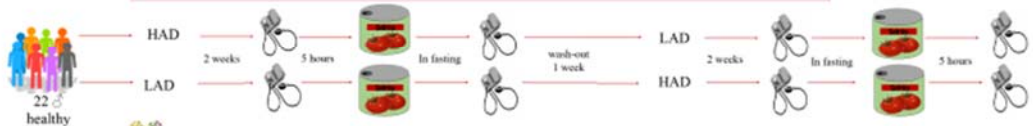
[1]Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, INSA-University of Barcelona, Barcelona, Spain
[2]CIBER Physiopathology of obesity and nutrition (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain, electronic address: sara.hurtado_17@ub.edu

INTRODUCTION

Sofrito is a typical tomato-based Mediterranean sauce made with olive oil and onions that are rich in bioactive compounds. The hypothesis was that *sofrito* intake could have a beneficial effect on cardiovascular health, particularly in the regulation of blood pressure.

METHODOLOGY

Interventional study · Cross-over · Controlled · Randomized · Open · Acute

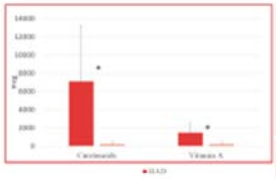


22 ♂
healthy
18-32 years

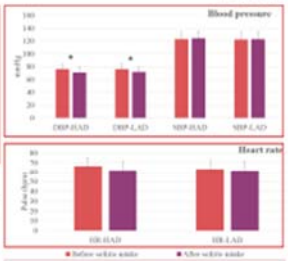
HAD (High Antioxidant Diet): (≥5 vegetables and fruits) || LAD (Low Antioxidant Diet): (≤2 vegetables and fruits)

DBP, SBP, HR

RESULTS



Carotenoids and vitamin A in plasma after a HAD (High Antioxidant Diet) and LAD (Low Antioxidant Diet). p-value <0.001(*). The dietary registers were analysed with the PCN Pro software (Programa de Cálcul Nutricional Professional).



Significantly lower diastolic blood pressure was observed after five hours of *sofrito* intake compared to basal measure in both groups.

	HAD		LAD		p-value
BMI (kg/m²)	24.91±3.73		24.84±3.70		0.476
WHR (cm/cm)	0.839±0.046		0.839±0.047		0.793
		p-value		p-value	
DBP 0H (mmHg)	75.89±8.25	0.006*	76.14±9.06	0.039*	0.897
DBP 5H (mmHg)	70.86±8.31		71.87±8.11		0.426
SBP 0H (mmHg)	122.77±10.81	0.531	122.44±11.66	0.987	0.715
SBP 5H (mmHg)	124.09±11.26		122.63±11.62		0.745
HR 0H (bpm)	66.06±9.49	0.106	63.03±9.85	0.322	0.118
HR 5H (bpm)	61.91±9.31		61.57±10.19		0.782

BMI: Body Mass Index, WHR: waist-hip ratio, DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure, HR: Heart Rate, HAD: High Antioxidant Diet, LAD: Low Antioxidant Diet, 0H: 0 hours (before intake *sofrito*), 5H: 5 hours (after intake *sofrito*). p-value <0.05(*).

CONCLUSION

According to these preliminary results, *sofrito*, reduce diastolic blood pressure. The mechanisms involved in this fact will be evaluated soon.

ACKNOWLEDGMENT

This work was supported by Ministerio de Economía, Industria y Competitividad (AGL2013-49083-C3-1-R and AGL2016-79113-R) and Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN).

8.2.11. Comunicación 11. Póster

Título: Comparison of urinary excretion of total polyphenols after a high antioxidant diet and a low antioxidant diet.
Autores: Hurtado-Barroso, S; Tresserra-Rimbau, A; Alvarenga, JFR; Lamuela-Raventós, RM.
Congreso: III Workshop INSA-UB: «La salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana».
Barcelona (España).
16 de noviembre de 2017

Comparison of Urinary Excretion of Total Polyphenols after a High Antioxidant Diet and a Low Antioxidant Diet

Hurtado-Barroso, S.[1,2*], Tresserra-Rimbau, A.[1,2], Alvarenga, J.F.R.[3], Lamuela-Raventós, R.M.[1,2]

[1] Dept Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació,Universitat de Barcelona (UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA UB), Universitat de Barcelona (UB)

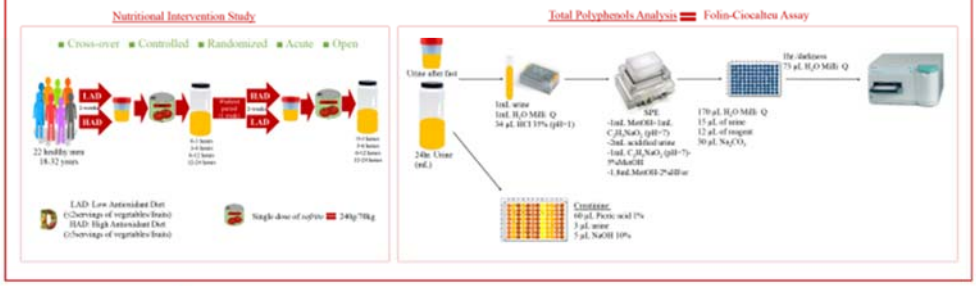
[2]CIBER Physiopathology of Obesity and Nutrition (CIBERobn), Institute of Health Carlos III, ISCIII, Madrid, Spain

Electronic address: sara.hurtado_17@ub.edu

Background and objectives

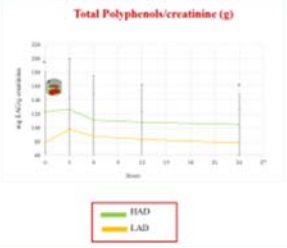
The hypothesis is that the bioavailability of bioactive compounds, such as polyphenols, may be higher when the body is under a stressful situation (such a low antioxidant diet). The aim of this work was to compare the urinary excretion of total polyphenols before and after the intake of a single dose of *sofrito* after following either a high or a low antioxidant diet.

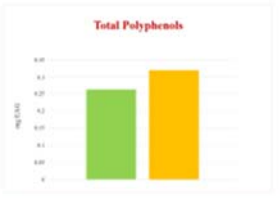
Methodology



Results and Conclusions

Urinary excretion of total polyphenols before and after *sofrito* intake in HAD and LAD. (*): p-value<0.05.





The urinary excretion of total polyphenols after *sofrito* intake was higher after LAD (p-value<0.001). This response may be due to a higher absorption of polyphenols after following a low antioxidant diet.

***Sofrito* intake:**
119.395 mg polyphenols/240g

In conclusión, after the high antioxidant diet, the urinary excretion of phenolic compounds was higher. However, when a rich polyphenols food was administered, the excreted polyphenols increase was higher in the low antioxidant diet.

Acknowledgements

This work was supported by Ministerio de Economía, Industria y Competitividad (AGL2013-49083-C3-1-R and AGL2016-79113-R) and Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERobn).

8.2.12. Comunicación 12. Póster

Título: Following a low-polyphenol diet for a short time cause changes in vascular biomarkers in healthy men

Autores: Sara Hurtado-Barroso, Paola Quifer-Rada, José Fernando Rinaldide Alvarenga, Silvia Pérez-Fernández, Anna Tresserra-Rimbau and Rosa M. Lamuela Raventos**.**

Congreso: XI Jornada de Recerca de la Facultat de Farmàcia i Ciències de l'Alimentació.

Barcelona (España).

17 de octubre de 2018.

FOLLOWING A LOW-POLYPHENOL DIET FOR A SHORT TIME CAUSE CHANGES IN VASCULAR BIOMARKERS IN HEALTHY MEN

Sara Hurtado-Barroso[1,2,3], Paola Quifer-Rada[4], José Fernando Rinaldi de Alvarenga[1,3], Silvia Pérez-Fernández[5,6], , Anna Tresserra-Rimbau[2,7] and Rosa M Lamuela Raventos[1,2,3]

[1]Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain; [2]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Madrid, Spain; [3]INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, Barcelona, Spain; [4]Department of Endocrinology & Nutrition, CIBER of Diabetes and Associated Metabolic Diseases, Biomedical Reseacrh Institute Sant Pau, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; [5]IMIM Hospital del Mar Medical Research Institute, Barcelona, Spain; [6]CIBER Cardiovascular (CIBERCV), Institute of Health Carlos III, Madrid, Spain; [7]Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Faculty of Medicine and Health Sciences, Pere Virgili Health Research Center, University Rovira i Virgili, Reus, Spain.

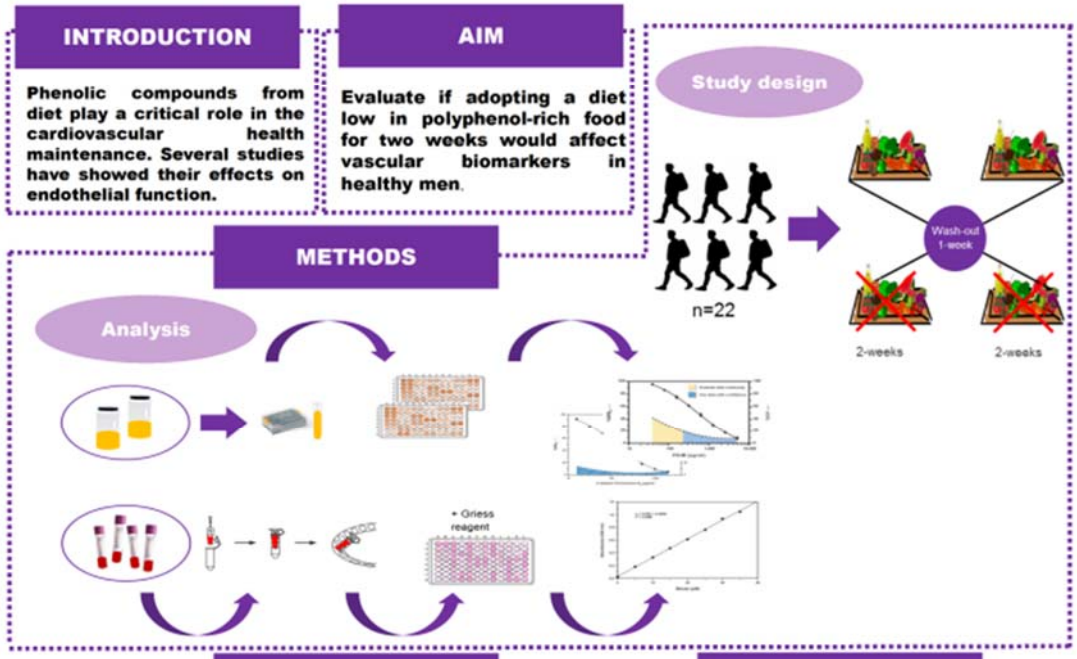
INTRODUCTION

Phenolic compounds from diet play a critical role in the cardiovascular health maintenance. Several studies have showed their effects on endothelial function.

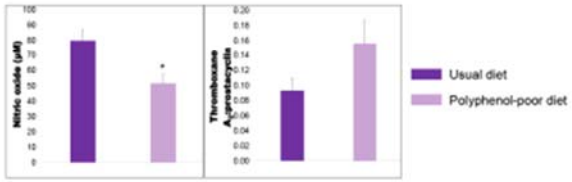
AIM

Evaluate if adopting a diet low in polyphenol-rich food for two weeks would affect vascular biomarkers in healthy men.

METHODS



RESULTS



After a diet low in polyphenols, a reduction of nitric oxide (adjusted p value= 0.006) and an increase of the ratio Thromboxane A_2/prostacyclin (adjusted p value= 0.093) were observed regarding the usual diet.

CONCLUSION

Polyphenols poor diet for a short period may modify the vascular biomarkers, even in healthy population.

ACKNOWLEDGEMENT

This work was supported by CYCIT (AGL2016-79113-R) from Ministerio de Economía, Industria y Competitividad and CIBERobn (Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición).

8.2.13. Comunicación 13. Oral

Título: Total polyphenols and antioxidant capacity are higher in Chardonnay than in Xarel-lo white wines
Autores: Hurtado-Barroso, S, Alvarenga, JFR, Treserra-Rimbau, A, Francetto Juliano F, Lamuela-Raventós, RM.
Congreso: IV Workshop INSA-UB vi i cava.
Santa Coloma de Gramenet, Barcelona (España).
15 de noviembre de 2018.

TOTAL POLYPHENOLS AND ANTIOXIDANT CAPACITY IS HIGHER IN CHARDONNAY THAN IN XAREL·LO WHITE WINE

Hurtado-Barroso, S.[1,2*], Alvarenga, J.F.R.[1], Treserra-Rimbau, A.[2,3], Francetto Juliano F.[4], Lamuela-Raventós, R.M.[1,2]

[1]Department of Nutrition, Food Science, and Gastronomy, School of Pharmacy and Food Science, INSA-University of Barcelona, Barcelona, Spain

[2]CIBER Physiopathology of obesity and nutrition (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain

[3]Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Faculty of Medicine and Health Sciences, Pere Virgili Health Research Center, University Rovira i Virgili. Reus, Spain

[4]Department of Agri-food Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil

Background and objectives:

Several studies in the field of the dietary antioxidants are carried out using the wine as polyphenol-rich product. However, the concentration of these bioactive compounds contained in the wine depends on the factors like the grape variety. We evaluated the total polyphenols (TP) and the antioxidant capacity (AC) in two Spanish varieties of white wine: Chardonnay and Xarel·lo.

Methodology:

TP and AC were analyzed in a total of 30 samples of white wine, of which 14 were of the Chardonnay variety and 16 of Xarel·lo variety. TP was measured by Folin-Ciocalteu method and AC by ABTS and DPPH methods.

Results and conclusions:

Both TP and AC were higher in Chardonnay than in Xarel·lo wines (267.16±34.92 vs. 217.78±88.68 μg GAE/mL, $p=0.001$; 2.28±0.29 vs. 1.75±0.94 mM TE/L, $p<0.001$ and 0.69±0.53 vs. 0.15±0.27 mM TE/L, $p=0.012$). So, it seems that Chardonnay could be better regarding to bioactive compounds content than Xarel·lo variety.

Acknowledgements:

This work was supported by CICYT (AGL2016-79113-R) from Ministerio de Economía y Competitividad (MEC) and Generalitat de Catalunya. In addition, thanks to the collaboration of Physiopathology of obesity and nutrition CIBER (CIBEROBN) and the Institut de Recerca en Nutrició i Seguretat Alimentària (INSA).

8.2.14. Comunicación 14. Póster

Título: Following a low-polyphenol diet for a short time causes changes in vascular biomarkers in healthy men

Autores: Sara Hurtado-Barroso, Paola Quifer-Rada, José Fernando Rinaldide Alvarenga, Silvia Pérez-Fernández, Anna Tresserra-Rimbau and Rosa M. Lamuela Raventos.

Congreso: Simposio Anual CIBEROBN 'Obesity and Nutrition in the 21st Century'.

Madrid (España).

21-22 de noviembre de 2018.

FOLLOWING A LOW-POLYPHENOL DIET FOR A SHORT TIME CAUSES CHANGES IN VASCULAR BIOMARKERS IN HEALTHY MEN

Sara Hurtado-Barroso[1,2,3], Paola Quifer-Rada[4], José Fernando Rinaldi de Alvarenga[1,3], Silvia Pérez-Fernández[5,6], , Anna Tresserra-Rimbau[2,7] and Rosa M Lamuela Raventos[1,2,3]

[1]Department of Nutrition, Food Sciences and Gastronomy, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain; [2]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Madrid, Spain; [3]INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, Barcelona, Spain; [4]Department of Endocrinology & Nutrition, CIBER of Diabetes and Associated Metabolic Diseases, Biomedical Research Institute Sant Pau, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; [5]IMIM Hospital del Mar Medical Research Institute, Barcelona, Spain; [6]CIBER Cardiovascular (CIBERCV), Institute of Health Carlos III, Madrid, Spain; [7]Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Faculty of Medicine and Health Sciences, Pere Virgili Health Research Center, University Rovira i Virgili, Reus, Spain.

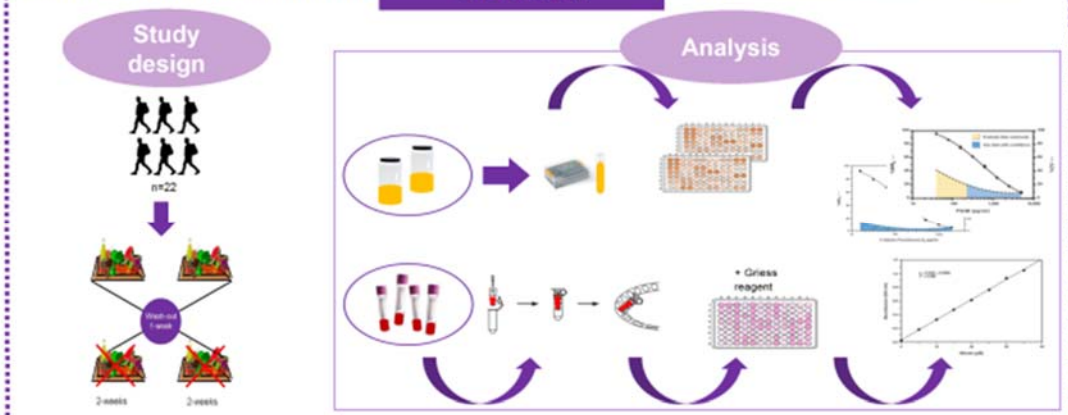
INTRODUCTION

Phenolic compounds from diet play a critical role in the cardiovascular health maintenance. Several studies have showed their effects on endothelial function.

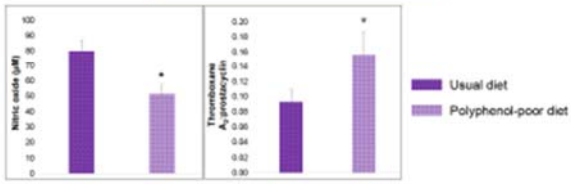
AIM

Evaluate if adopting a diet low in polyphenol-rich food for two weeks would affect vascular biomarkers in healthy men.

METHODS



RESULTS



After a diet low in polyphenols, a reduction of nitric oxide (adjusted p value= 0.002) and an increase of the ratio Thromboxane A_2/prostacyclin (adjusted p value= 0.048) were observed regarding the usual diet.

CONCLUSION

Polyphenols poor diet for a short period may modify the vascular biomarkers, even in healthy population.

ACKNOWLEDGEMENT

This work was supported by CYCIT (AGL2016-79113-R) from Ministerio de Economía, Industria y Competitividad and CIBERobn (Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición).

8.2.15. Comunicación 15. Oral

Título: El efecto de la dieta mediterránea y sus compuestos bioactivos sobre los marcadores vasculares en humanos sanos.
Autores: Sara Hurtado-Barroso and Rosa M. Lamuela Raventos.
Congreso: 2ª Jornada de Recerca. Departament de Nutrició, Ciències de l'Alimentació i Gastronomia.
Santa Coloma de Gramenet, Barcelona (España).
16 de julio de 2019.

UNIVERSITAT DE BARCELONA

F. Javier Luque Garriga
Departament de Nutrició, Ciències de l'Alimentació i Gastronomia

Campus de l'Alimentació de Torribera
Facultat de Farmàcia

Avda. Prat de la Riba 171
08921 Santa Coloma de Gramenet

Tel. +34 93 4033788
e-mail: fjluque@ub.edu
http://www.ub.edu/cbdd

Barcelona, Julio 17, 2019

A quien pueda interesar.

Mediante el presente escrito, como responsable de la organización de la II Jornada de Investigación del Departamento de Nutrición, Ciencias de la Alimentación y Gastronomía, celebrada el 16 de Julio de 2019 en el Campus de Torribera, hago constar que:

Sara Hurtado Barroso participó en dicha Jornada y presentó una comunicación que lleva por título

El efecto de la dieta mediterránea y sus compuestos bioactivos sobe los marcadores vasculares en humanos sanos

Atentamente.

UNIVERSITAT DE BARCELONA
Administració de Campus de l'Alimentació Torribera

Prof. F. Javier Luque