VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

Universidad de Salamanca

Programa de Doctorado en Farmacia y Salud

TESIS DOCTORAL

EFECTO DEL CHOCOLATE CON ALTO PORCENTAJE DE CACAO SOBRE LA SALUD EN MUJERES POSMENOPÁUSICAS. ENSAYO CLÍNICO ALEATORIZADO

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2021

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La Tesis Doctoral titulada **«Efecto del chocolate con alto porcentaje de cacao sobre la salud en mujeres posmenopáusicas. Ensayo clínico aleatorizado»**, realizada por Dña. Irene Ailing García Yu bajo la dirección del Dr. José Ángel Maderuelo Fernández y el Dr. José Ignacio Recio Rodríguez, corresponde a un compendio de artículos publicados cuyas referencias se detallan a continuación:

1. Garcia-Yu IA, Garcia-Ortiz L, Gómez-Marcos MA, Alonso-Dominguez R, Gonzalez-Sanchez J, Mora-Simon S, González-Manzano S, Rodriguez-Sanchez E, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial. BMJ Open. 2018 Dec 14;8(12):e024095. doi: 10.1136/bmjopen-2018-024095.

2. Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Agudo-Conde C, Gonzalez-Sanchez J, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Effects of Cocoa-Rich Chocolate on Blood Pressure, Cardiovascular Risk Factors, and Arterial Stiffness in Postmenopausal Women: A Randomized Clinical Trial. Nutrients. 2020 Jun 12;12(6):1758. doi: 10.3390/nu12061758.

3. Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Lugones-Sanchez C, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Cocoa-rich chocolate and body composition in postmenopausal women: a randomised clinical trial. Br J Nutr. 2021 Mar 14;125(5):548-556. doi: 10.1017/S0007114520003086. Epub 2020 Aug 4.

4. Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Mora-Simon S, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial. Nutr Neurosci. 2020 Nov 15:1-12. doi: 10.1080/1028415X.2020.1840119. Epub ahead of print.

5. Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Tamayo-Morales O, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Cocoa-Rich Chocolate and Quality of Life in Postmenopausal Women: A Randomized Clinical Trial. Nutrients. 2020 Sep 10;12(9):2754. doi: 10.3390/nu12092754.

Los directores de la Tesis Doctoral titulada **«Efecto del chocolate con alto porcentaje de cacao sobre la salud en mujeres posmenopáusicas. Ensayo clínico aleatorizado»** presentada por la doctoranda Dña. Irene Ailing García Yu, autorizan la presentación de esta Tesis en la modalidad de compendio de artículos.

Fdo. D. José Ignacio Recio Rodríguez

Fdo. D. José Ángel Maderuelo Fernández

En Salamanca, a 21 de marzo de 2021

Los siguientes coautores declaran que la doctoranda Dña. Irene Ailing García Yu, es la principal autora de la investigación recogida en los artículos que conforman la Tesis Doctoral titulada **«Efecto del chocolate con alto porcentaje de cacao sobre la salud en mujeres posmenopáusicas. Ensayo clínico aleatorizado»** y así lo aceptan por escrito y con su firma.

1. Garcia-Yu IA, Garcia-Ortiz L, Gómez-Marcos MA, Alonso-Dominguez R, Gonzalez-Sanchez J, Mora-Simon S, González-Manzano S, Rodriguez-Sanchez E, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial. BMJ Open. 2018 Dec 14;8(12):e024095. doi: 10.1136/bmjopen-2018-024095.

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Cristina Lugones Sánchez

Cristina Agudo Conde

D. **José Ignacio Recio Rodríguez**, Doctor en Biociencias: Biología y Clínica del Cáncer y Medicina Traslacional, Profesor Contratado Doctor del Departamento de Enfermería y Fisioterapia de la Universidad de Salamanca, y D. **José Ángel Maderuelo Fernández**, Doctor en Medicina y Cirugía, médico especialista en Medicina Preventiva y Salud Pública de la Gerencia de Atención Primaria de Salamanca.

Certifican:

Que el trabajo titulado **«*Efecto del chocolate con alto porcentaje de cacao sobre la salud en mujeres posmenopáusicas. Ensayo clínico aleatorizado»,*** realizado bajo su dirección por Dña. Irene Ailing García Yu, reúne las condiciones de calidad y originalidad requeridas para optar al grado de Doctor.

Para que así conste, y a efectos oportunos, firman el presente certificado en Salamanca, a veintiuno de marzo del año dos mil veintiuno.

Fdo. José Ignacio Recio Rodríguez Fdo. José Ángel Maderuelo Fernández

Agradecimientos

A José Ignacio Recio Rodríguez y José Ángel Maderuelo Fernández, por su profesionalidad, dedicación y ayuda en todo momento. Gracias por enseñarme tanto y confiar en mí.

A todo el equipo apisaliano de la Unidad de Investigación de Atención Primaria de Salamanca, porque su apoyo y esfuerzo han sido imprescindibles para llevar a cabo este proyecto.

A todas las participantes en este estudio, sin su colaboración este proyecto no hubiera sido posible.

A la Junta de Castilla y León (GRS 1583/B/17), por su apoyo con la concesión de fondos para esta investigación.

A Lindt & Sprüngli por proporcionar el chocolate necesario para la realización de este estudio.

A todos los profesionales y compañeros que me han enseñado, y de los que sigo aprendiendo, la bonita especialidad de Medicina Preventiva y Salud Pública.

A mis amigos y a mis frikitos.

Por último, pero más importante, a mis padres y mi hermano, por estar a mi lado siempre y apoyarme en cada paso de la vida. A mi familia, gracias.

A mi Pequeñuela Mei Lin, por ser la más grande. A Nacho, por todo.

A todos vosotros, muchas gracias.

Abreviaturas

ANCOVA: análisis de la covarianza

ANOVA: análisis de la varianza

APISAL: Unidad de Investigación de Atención Primaria de Salamanca

cAIx: índice de aumento central

cAIx75: índice de aumento central corregido a 75 latidos por minuto

CAVI: cardio-ankle vascular index (índice vascular cardio-tobillo)

CdV: calidad de vida

cHDL: colesterol de las HDL

cLDL: colesterol de las LDL

CT: colesterol total

DE: desviación estándar

FC: frecuencia cardíaca

FSH: hormona foliculoestimulante

GC: grupo control

GI: grupo de intervención

HDL: lipoproteínas de alta densidad

HOMA-IR: índice de resistencia a la insulina mediante evaluación del modelo homeostático

IBSAL: Instituto de Investigación Biomédica de Salamanca

IC: intervalo de confianza

IPAQ: Cuestionario Internacional de Actividad Física

ITB: índice tobillo-brazo

LDL: lipoproteínas de baja densidad

l.p.m.: latidos por minuto

MET: equivalente metabólico

PAD: presión arterial diastólica

pAIx: índice de aumento periférico

PAM: presión arterial media

PAS: presión arterial sistólica

PP: presión de pulso

redIAPP: Red de Investigación en Actividades Preventivas y Promoción de la Salud en Atención Primaria

ROS: especies reactivas del oxígeno

TMT-A: Trail Making Test A

TMT-B: Trail Making Test B

VOP: velocidad de la onda del pulso

Índice

Introducción

1. La dieta y su relación con la salud

La dieta es un importante determinante de la salud y se ha demostrado que es el factor de riesgo más significativo para la discapacidad y la muerte prematura (1).

Desde hace unas décadas se está estudiando la relación con la salud de patrones dietéticos concretos, definidos por la cantidad, la proporción, la variedad o la combinación de diferentes alimentos y bebidas, así como la frecuencia con la que son consumidos (2). En este sentido, la dieta mediterránea ha sido uno de los patrones dietéticos más estudiados. Los resultados de diversos estudios indican que la adherencia a la dieta mediterránea no solo es útil en el tratamiento de la obesidad (3), sino que también previene la diabetes de tipo 2 (4), disminuye la incidencia y la mortalidad por cáncer (5), previene el deterioro cognitivo relacionado con la edad (6), disminuye la incidencia y la mortalidad por enfermedades cardiovasculares (7) y disminuye la mortalidad global (8). Otros patrones dietéticos que parecen producir efectos beneficiosos en la salud son la dieta atlántica (9), que se ha asociado con un menor riesgo cardiovascular, y la dieta DASH (Enfoques Dietéticos para Detener la Hipertensión), que también parece mejorar los factores de riesgo cardiovascular y disminuir la presión arterial, entre otros (10,11).

Asimismo, el consumo de alimentos o nutrientes aislados y sus posibles efectos sobre la salud también son objeto de numerosas investigaciones. Recientemente, ha aumentado el interés hacia los alimentos y nutrientes ricos en antioxidantes, ya que estos se han relacionado con resultados positivos en salud, como la disminución del riesgo de enfermedades cardiovasculares (12). Entre estas sustancias se encuentran los polifenoles, que son un amplio grupo de antioxidantes naturales que se encuentran presentes en numerosos alimentos. El propóleo (13), la grosella negra (14), el té verde (15), el aceite de oliva (16), el vino tinto (17), o el chocolate (18) son algunos de los alimentos que han mostrado ciertos beneficios en distintos aspectos de la salud debido a su alto contenido en polifenoles.

2. El chocolate y el cacao

2.1. Historia del chocolate

Los orígenes del chocolate se remontan a la cultura maya, quienes probablemente fueron los primeros en cultivar la planta del cacao, hacia el año 400 d.C. (19).

Antiguamente el cacao se consumía de forma diferente a como se hace hoy en día: se molían los granos de cacao y se disolvían en agua junto con canela y pimienta para realzar

el sabor, que era muy amargo y fuerte. Esta bebida se denominó *xocolatl*. Hacia el año 1200 d.C., tras la invasión de los aztecas sobre los mayas, el emperador Montezuma mostró un gran aprecio hacia esta bebida. Los granos de cacao eran tan valiosos que se utilizaban como moneda de cambio y se mantenía bajo seguridad junto con el oro y piedras preciosas.

En el año 1502 tuvo lugar el primer contacto de la civilización occidental con el cacao, tras la llegada de Cristóbal Colón a la isla de Guanaja (Honduras), donde recibió como regalo una taza de chocolate. Posteriormente, Hernán Cortes llevó las semillas a Europa. Con el intercambio con las colonias españolas en América, el chocolate comenzó a introducirse en el viejo continente. Los europeos lo consumían junto con azúcar, canela y vainilla, lo que aportó un sabor dulce y suave al chocolate. En aquella época este producto se utilizaba como medicina y estimulante.

A pesar de que las características del chocolate se ignoraron durante mucho tiempo en Europa debido a que las desfavorables condiciones ambientales dificultaban su crecimiento, su consumo se fue ampliando poco a poco por el continente. Fue a partir de 1660 cuando esta bebida dulce y caliente se extendió por Europa y años más tarde, en 1753, un naturalista sueco llamado Carl von Linné, debido a las propiedades atribuidas a este producto, denominó al árbol del cacao *Theobroma cacao* el «alimento de los dioses».

A partir de entonces, se han investigado y utilizado distintas técnicas para el procesado del cacao y su transformación en diversas elaboraciones derivadas de este. Actualmente el chocolate, y otros productos derivados del cacao, son apreciados y consumidos ampliamente en todo el mundo.

2.2. Proceso de fabricación del cacao y el chocolate

El chocolate y el cacao son dos términos diferentes. Los granos de cacao son las semillas grasas del *Theobroma cacao L.*, el árbol del cacao. A través de distintos métodos de procesado al que son sometidos los granos de cacao se obtiene el licor de cacao, que contiene aproximadamente 55% de manteca de cacao. El cacao es el componente no graso del licor de cacao, el extracto puro de las semillas de cacao. El chocolate, en cambio, consiste en un producto manufacturado sólido que está formado por porcentajes variables de licor de cacao, manteca de cacao, azúcar y leche; aunque también se puede tomar como una bebida, una opción muy popular en algunos países como España (20). En la Figura 1 se muestra el procesado para la obtención de estos productos a partir de los granos de cacao.

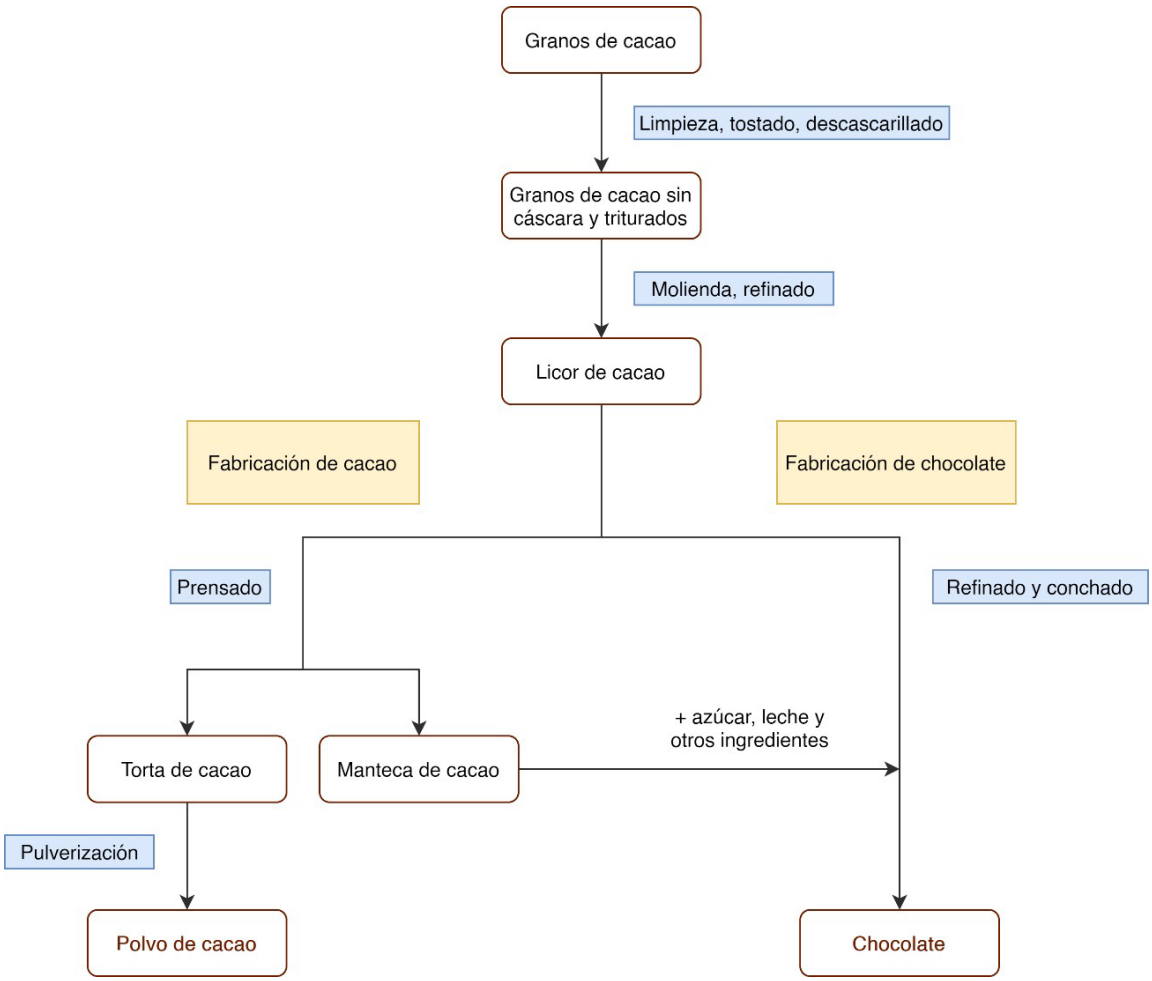

Figura 1. El procesamiento del chocolate a partir de los granos de cacao.
Adaptación de «Chocolate, 'Food of the Gods': History, Science, and Human Health» de M. Montagna, 2019, *Int. J. Environ. Res. Public Health, 16, 4960.*

El proceso de producción de chocolate comprende varias etapas: la fermentación, el secado, el tostado, el molido de granos de cacao, la mezcla de todos los ingredientes (masa de cacao, azúcar, manteca de cacao, emulsionantes, aroma y componentes de la leche si es necesario), el conchado y el templado. Durante la fermentación, el secado, el tostado de los granos de cacao y el conchado de la masa de chocolate se producen reacciones químicas importantes que son fundamentales para el desarrollo del sabor y el aroma (21).

La fermentación de los granos de cacao es un proceso en el que se produce el crecimiento de levaduras y bacterias en la pulpa y que se realiza en plantaciones de cacao como parte de la producción de los granos de cacao. En esta etapa, se produce la descomposición de azúcares y mucílago. Este proceso consta de tres fases:

- En la primera fase predominan las levaduras anaeróbicas y dura 24-36 h. En esta etapa hay bajo contenido en oxígeno y bajo pH (<4).

- En la segunda fase predominan las bacterias del ácido láctico, que están presentes desde el comienzo, pero se activan entre 48-96 h después.
- En la tercera fase predominan las bacterias del ácido acético cuando aumenta la aireación. En esta fase, tiene lugar una reacción exotérmica (conversión de alcohol en ácido acético), que hace que la temperatura aumente 50 °C o más.

La fermentación es la etapa clave en la que se producen los precursores del desarrollo del aroma del chocolate adecuado (22).

Después de la fermentación se lleva a cabo el secado de los granos de cacao para disminuir el contenido de humedad y completar los procesos oxidativos inducidos durante la fermentación de los granos de cacao. Tras el secado, los granos de cacao tienen 6-8% de humedad. El bajo contenido en humedad previene el crecimiento de moho y hace que los granos sean más estables para el transporte y almacenamiento (21,23).

Posteriormente, el tostado del grano de cacao generalmente se realiza en una fábrica de chocolate como una primera etapa de la producción de chocolate. Es un proceso que tiene lugar a altas temperaturas, generalmente entre 120 y 140 °C, lo que resulta importante para que se produzcan las reacciones de Maillard. El tostado reduce el contenido de componentes no deseados, produce el sabor y aroma específicos del chocolate y descontamina los granos de cacao. En esta fase, todos los precursores formados en las fases previas reaccionan y forman numerosos compuestos (21–23).

A continuación, se realiza el molido y la mezcla de todos los ingredientes del chocolate, que resultan muy importantes para lograr el tamaño de partícula adecuado de todos los ingredientes. Los principales ingredientes utilizados en la producción de chocolate son el licor de cacao (obtenido al moler granos de cacao), la manteca de cacao (obtenida al prensar el licor de cacao), el azúcar y la leche (en el caso del chocolate con leche) (23).

El conchado es un tratamiento de mezclado y calentamiento que se realiza para producir chocolate líquido (todas las partículas sólidas están recubiertas de grasa), evaporar los ácidos volátiles, conseguir una viscosidad apropiada, eliminar el exceso de humedad y desarrollar el color deseado (22,24,25).

Por último, el templado es un proceso por el cual se obtiene un producto sólido. Este se realiza térmicamente y da como resultado cristales de manteca de cacao sólidos/estables y de tamaño constante que luego influyen en el crecimiento de una red cristalina estable, equilibrada y fuerte durante el enfriamiento (23).

2.3. Composición química y nutricional del cacao y el chocolate. Diferencia entre cacao y chocolate

El cacao es un producto complejo formado por más de 300 constituyentes (26). Sus componentes principales son la manteca de cacao (ácidos grasos oleico, esteárico y palmítico), los minerales (magnesio, potasio, hierro y zinc), las metilxanitinas (teobromina y cafeína), los polifenoles, además de otros compuestos como la tiramina, el triptófano y la serotonina.

Los granos de cacao se encuentran entre los productos que proporcionan una gran cantidad de polifenoles al cuerpo humano y, tanto en su forma fresca como en las formas procesadas contienen significativamente más polifenoles que el té negro, el té verde o el vino tinto (27).

Entre los compuestos polifenólicos que se encuentran en los granos de cacao, predominan los flavan-3-oles, también conocidos como flavanoles (catequina, epicatequina y proantocianidina). El cacao es conocido por ser una de las fuentes más ricas en flavanoles (28). En menor cantidad, las antocianinas, derivadas de las cianidinas, los flavonoles (quercetina y sus derivados), flavonas, y ácidos fenólicos también se encuentran presentes. En una revisión realizada por Oracz et al. (29), se indica que la composición tanto cualitativa como cuantitativa relativa a los compuestos polifenólicos presentes en los granos de cacao difiere significativamente dependiendo de la variedad y la región de origen de estas materias. La concentración de los compuestos polifenólicos en los granos de cacao es muy variable y depende de múltiples factores, principalmente de las características genéticas, la región geográfica de origen, su madurez, las condiciones climáticas durante su crecimiento y cosecha, los métodos de secado y el almacenamiento tras la recolección. Excepto por los cambios fisicoquímicos, microbiológicos y organolépticos favorables, el proceso termal al que son sometidos los granos de cacao y sus productos derivados de su procesamiento a temperaturas relativamente altas puede reducir la concentración de polifenoles. La concentración de polifenoles también puede disminuir de forma considerable durante el almacenamiento y las operaciones de procesado de los granos de cacao, como la fermentación, el secado y el tostado. La temperatura y el tiempo de los procesos realizados tienen un gran impacto en los cambios en el contenido de antocianinas y flavanoles, incluyendo los polímeros de procianidina. En definitiva, los numerosos cambios químicos y bioquímicos a los que están sujetos los polifenoles de los granos de cacao durante los procesos tecnológicos pueden dar lugar a su descomposición, disminuyendo notablemente su concentración. De este modo, la concentración de todos los fitoquímicos, y la proporción y el tipo de polifenoles en concreto, puede variar enormemente entre las semillas de cacao

y los alimentos que contienen cacao, dependiendo del origen de las semillas y de las condiciones de procesado (30,31).

Existen ciertas evidencias de que es posible aumentar el contenido de los componentes bioactivos beneficiosos para la salud, como los polifenoles, y mejorar la capacidad antioxidante de radicales de oxígeno de los chocolates mediante el incremento del contenido de licor de cacao obtenido de granos sin tostar (32), aunque sería necesario realizar más investigaciones a este respecto.

El chocolate es especialmente rico en catequina, epicatequina y procianidina, conteniendo una media de 41,50 mg/100 g de epicatequina y 11,99 mg/100 g de catequina (33). No obstante, debido a que los procesos modernos de fabricación de chocolate hacen que se pierda hasta más del 80% de los flavonoides originales de los granos de cacao (34), el contenido en flavonoides de los productos de chocolate puede variar según la proporción de sólidos de cacao. El contenido total de catequina del chocolate varía de 46,0 a 61,0 mg/100 g en el chocolate negro y de 15,3 a 16,3 mg/100 g en el chocolate con leche (35). El contenido en procianidina de los chocolates disponibles comercialmente varía entre 9 mg/100 g y 400 mg/100 g (36). Además, una porción de 50 g de chocolate negro también aporta 250 kcal, lo que supone una preocupación por la posible ganancia de peso (33). Los numerosos procesos a los que son sometidos estos productos hacen que la composición química y nutricional del cacao sea diferente en comparación con distintos tipos de chocolate, como el chocolate negro y el chocolate con leche (Tabla 1).

2.4. Tipos de chocolate

Los granos de cacao, que son las semillas del árbol *Theobroma cacao L.*, son la materia base para la producción del chocolate. Existen tres variedades básicas de granos de cacao: Criollo, Forastero y Trinitario, que se caracterizan por poseer composición química, textura y propiedades organolépticas diferentes (32).

Como se ha comentado, a partir de los granos de cacao, y tras varios procesos de transformación, se pueden fabricar distintos tipos de chocolate que presentan ingredientes y características determinadas y diferentes entre ellos (19).

- El chocolate negro contiene granos de cacao (hasta el 80% del peso total) y manteca de cacao. Es suave al tacto y tiene el aroma intenso y persistente del cacao, se derrite en la boca, dejando un sabor amargo y agradable. El porcentaje de cacao es una de las principales características que determinan su calidad. El mejor contiene al

menos el 70% de cacao. La mayoría de los efectos beneficiosos sobre la salud atribuidos al chocolate se asocian con el consumo de chocolate negro.

- El chocolate gianduja consiste en una mezcla de avellanas, cacao y azúcar. A veces se le añaden leche, almendras o nueces. Es de color marrón.
- El chocolate con leche contiene al menos 20-25% de cacao junto con manteca de cacao, azúcar, leche en polvo, lecitina. Tiene un aspecto claro, y un aroma intenso y persistente, así como un sabor dulce con un ligero toque amargo de cacao.
- El chocolate blanco contiene manteca de cacao, leche y azúcar. Tiene un sabor dulce y agradable.

Tabla 1. Composición química y nutricional de 100 g de cacao y de dos tipos de chocolate

Composición química	Cacao	Chocolate negro	Chocolate con leche
Agua (g)	2,5	0,5	0,8
Proteínas (g)	20,4	6,6	7,3
Lípidos (g)	25,6	33,6	36,3
Colesterol (mg)	0	0	10
Carbohidratos (g)	11,5	49,7	50,5
Azúcar (g)	trazas	49,7	50,5
Fibra total (g)	-	8	3,2
Sodio (mg)	-	11	120
Potasio (mg)	-	300	420
Hierro (mg)	14,3	5,0	3,0
Calcio (mg)	51	51	262
Fósforo (mg)	685	186	207
Tiamina (mg)	0,08	0,07	0,09
Riboflavina (mg)	0,30	0,07	0,39
Niacina (mg)	1,7	0,6	0,6
Vitamina A (μg)	7	9	25
Compuestos polifenólicos (mg)	996-3781	579	160
Flavonoides (mg)	-	28	13
Teobromina (mg)	-	802	125
Energía (kcal)	355	515	545

Adaptación de «Chocolate, 'Food of the Gods': History, Science, and Human Health» de M. Montagna, 2019, *Int. J. Environ. Res. Public Health, 16, 4960.*

En nuestro ámbito, como marco normativo y regulador de las características que deben presentar estos productos, cabe mencionar la Directiva 2000/36/CE del Parlamento Europeo y del Consejo de 23 de junio de 2000 relativa a los productos de cacao y de chocolate destinados a la alimentación humana (37) y el Real Decreto 1055/2003, de 1 de agosto, por el que se aprueba la Reglamentación técnico-sanitaria sobre los productos de

cacao y chocolate destinados a la alimentación humana (38), en la que se establecen los requisitos esenciales que deben cumplir los productos de cacao y de chocolate destinados a la alimentación humana. De estos documentos podemos extraer las características que deben presentar los principales tipos de chocolate comercializados:

- Chocolate. Es el producto obtenido a partir de productos de cacao y azúcares que contenga un 35 por ciento, como mínimo, de materia seca total de cacao, del cual un 18 por ciento como mínimo será manteca de cacao y un 14 por ciento como mínimo materia seca y desgrasada de cacao.

- Chocolate blanco. Es el producto obtenido a partir de manteca de cacao, leche o productos lácteos y azúcares y que contenga, como mínimo, un 20 por ciento de manteca de cacao y, al menos, un 14 por ciento de extracto seco de la leche procedente de la deshidratación parcial o total de leche entera, semidesnatada o desnatada, de nata, nata parcial o totalmente deshidratada, de mantequilla o de materia grasa láctea, del que un 3,5 por ciento como mínimo corresponderá a materia grasa láctea.

- Chocolate con leche. Es el producto obtenido a partir de productos de cacao, azúcares y leche o productos lácteos, y que contenga:

    1. Como mínimo, un 25 por ciento de materia seca total de cacao.

    2. Como mínimo, un 14 por ciento de extracto seco de la leche procedente de la deshidratación parcial o total de leche entera, semidesnatada o desnatada, de nata, nata parcial o totalmente deshidratada, de mantequilla o de materia grasa láctea.

    3. Como mínimo, un 2,5 por ciento de materia seca y desgrasada de cacao.

    4. Como mínimo, un 3,5 por ciento de materia grasa láctea.

    5. Como mínimo, un 25 por ciento de materia grasa total (manteca de cacao y materia grasa láctea).

2.5. Consumo de chocolate en población española y recomendaciones de consumo

La producción total global de granos de cacao en 2018-2019 se estima que fue de 4 780 000 toneladas, según los últimos datos publicados por la Organización Internacional del Cacao (ICCO, por sus siglas en inglés) (39). Esta tiene lugar principalmente en África, donde se concentra aproximadamente el 75% de la producción mundial.

El chocolate y los productos de cacao se encuentran ampliamente aceptados en todo el mundo, habiendo un 90% de la población mundial que afirma que le gusta el chocolate (40). El cacao y sus derivados, especialmente el chocolate, son ampliamente consumidos en todo el mundo, debido a sus características organolépticas altamente atractivas. De hecho, los productos de cacao están presentes en la dieta de muchas personas en mayor proporción que el té verde, el vino o las semillas de soja (41).

Se ha estimado que los productos de cacao representan el 10% de la capacidad antioxidante total de la ingesta dietética de la población española (41). En España, atendiendo a los datos publicados por el Ministerio de Agricultura, Pesca y Alimentación, el consumo medio de chocolate, cacao o sucedáneos fue de 3,5 kg por persona en el año 2019 (Tabla 2). Las cifras de consumo en España son inferiores en comparación con otros países como Alemania, Suiza, Bélgica, EE.UU. y Japón donde el consumo se encuentra entre 8 y 13 kg por persona al año (40).

Respecto a las recomendaciones de consumo de estos productos, según la Sociedad Española de Arteriosclerosis (SEA) y la Sociedad Española de Médicos de Atención Primaria (SEMERGEN), en su «Documento de consenso SEA/SEMERGEN 2019. Recomendaciones dietéticas en la prevención cardiovascular» (42), en el contexto de una dieta saludable puede consumirse chocolate negro con cacao ≥70% a diario. Además, indican que es aconsejable tomarlo durante el día, pero no por la noche después de cenar cuando el efecto saciante no se puede compensar ingiriendo menos alimentos en la siguiente comida. No obstante, esta recomendación no incluye la cantidad aconsejable de consumo diario.

Por otro lado, la Autoridad Europea de Seguridad Alimentaria (EFSA, por sus siglas en inglés), establece que para obtener el efecto de los flavanoles del cacao que ayudan a mantener la vasodilatación dependiente del endotelio se deberían consumir 200 mg de flavanoles del cacao a diario (43). Esta cantidad puede obtenerse a través de 2,5 g de polvo de cacao con alto contenido en flavanoles o de 10 g de chocolate negro con alto contenido en flavanoles. Esta recomendación está dirigida a la población general y estas cantidades de polvo de cacao o de chocolate negro se pueden consumir en el contexto de una dieta equilibrada.

Tabla 2. Datos de consumo en hogares del grupo de productos de chocolates/cacaos/sucedáneos. 2019.

Producto	Volumen (miles de kg)	Valor (miles de €)	Precio medio kg	Consumo per cápita	Gasto per cápita
Chocolates / cacaos / sucedáneos	162.198,56	1.189.549,15	7,33	3,50	25,79
Chocolates	57.524,20	479.754,25	8,34	1,26	10,41
Chocolate tabletas	48.638,75	401.261,15	8,25	1,07	8,71
Chocolate tableta con leche	25.760,22	182.066,90	7,07	0,56	3,94
Chocolate tableta sin leche	22.878,54	219.194,25	9,58	0,50	4,74
Chocolate tableta con almendras	8.645,59	79.168,49	9,16	0,18	1,73
Chocolate tableta otros	39.993,16	322.092,68	8,05	0,87	6,98
Turrón de chocolate	8.885,46	78.493,10	8,83	0,19	1,68
Otros productos de chocolate/ cacao	104.674,36	709.794,90	6,78	2,28	15,38
Bombones	12.331,49	177.909,18	14,43	0,28	3,86
Snacks chocolate	13.717,76	163.426,14	11,91	0,31	3,54
Cacao soluble	50.503,87	234.719,04	4,65	1,09	5,08
Normal	47.692,09	206.594,61	4,33	1,03	4,48
Light	2.811,79	28.124,45	10,00	0,06	0,61
Crema de cacao para untar	18.333,52	105.084,34	5,73	0,40	2,27

Fuente: Ministerio de Agricultura, Pesca y Alimentación

3. Los polifenoles del cacao y sus efectos sobre la salud

Los efectos potencialmente beneficiosos del chocolate se atribuyen a los componentes naturales presentes en los granos de cacao, fundamentalmente a los flavonoides, que son polifenoles con potentes capacidades antioxidantes (19).

Como se ha anticipado, el cacao tiene el mayor contenido de flavanoles de todos los alimentos sobre la base del peso, contribuyendo de forma significativa a la ingesta dietética total de flavonoides. El cacao contiene grandes cantidades de flavonoides (−)-epicatequina, (+)-catequina y sus dímeros procianidinas B2 y B1, aunque otros polifenoles como la quercetina, isoquercitrina (quercetina 3-*O*-glucósido), quercetina 3-*O*-arabinosa, hiperósido (quercetina 3-*O*-galactósido), naringenina, luteolina y apigenina se han encontrado en menores cantidades (44).

3.1. Estructura química de los polifenoles

Todos los compuestos fenólicos tienen una característica estructural común que consiste en un anillo aromático que contiene al menos un grupo hidroxilo. Se han descrito miles de compuestos naturales formados a partir de esta estructura básica, desde simples ácidos fenólicos hasta compuestos altamente polimerizados (45).

3.2. Clasificación de los polifenoles

Los polifenoles se pueden clasificar de forma general en flavonoides o no flavonoides teniendo en cuenta su estructura química y complejidad (por ejemplo, los grupos sustituyentes y/o el tipo de unión entre los anillos fenólicos) (46).

Los flavonoides son el grupo de polifenoles más abundante en las plantas. Tienen una estructura C6–C3–C6 y se clasifican en subclases según sus diferencias estructurales (flavanonas, flavonas, dihidroflavonoles, flavonoles, flavan-3-oles o flavanoles, antocianidinas, isoflavonas y proantocianidinas) (47,48). Los subgrupos con mayor importancia dietética son las antocianinas, flavonoles, flavonas, isoflavonas, flavan-3-oles, flavanonas y antocianidinas (49) (Figura 2).

Por otro lado, entre los no flavonoides con mayores implicaciones sobre la dieta se encuentran los ácidos fenólicos C6 - C1, de los cuales el ácido gálico es el más común (50).

Dada la mayor relevancia dietética de los flavonoides, nos centraremos en los aspectos que conciernen a este grupo de polifenoles.

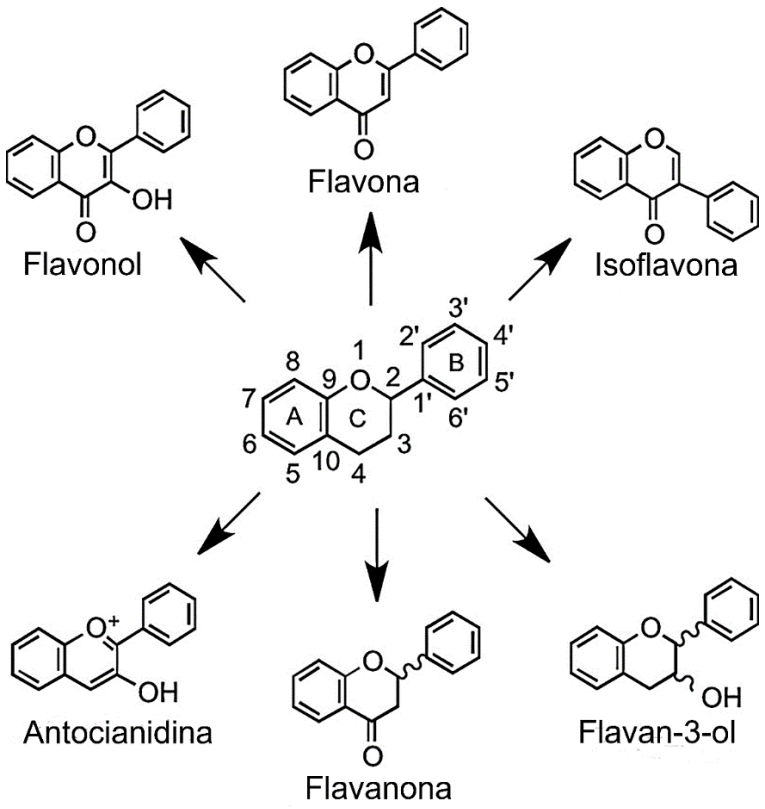

Figura 2. Estructura del esqueleto flavonoide.
Adaptación de «Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases» de D. del Rio, 2013, *Antioxid Redox Signal., 18(14),* 1818–1892.

3.3. Metabolismo y biodisponibilidad de los polifenoles

Los efectos biológicos de los flavonoides dependen de su biodisponibilidad, habiéndose estudiado las distintas formas y los porcentajes de absorción de los flavonoides (51). Los flavan-3-oles no sufren modificaciones tras 40 minutos en el estómago humano, lo que indica que los flavonoles y las procianidinas son estables en un ambiente hostil como el sistema digestivo (52). La absorción intestinal de los flavonoides depende básicamente de su estructura química. Los flavonoides monoméricos y algunas procianidinas diméricas y triméricas se absorben en el intestino delgado y se detectan rápidamente en el plasma (53–55). Además, ciertos monómeros se absorben mejor que otros; en este sentido, la epicatequina fue el flavonoide que principalmente se detectó en el plasma tras la ingesta de una bebida de cacao que contenía cantidades iguales de catequina y epicatequina (55).

Mientras que las procianidinas (dímeros y trímeros) se absorben en el intestino delgado y son detectadas rápidamente en el plasma (56) y la orina (57), las procianidinas largas se absorben menos eficientemente, pero pueden tener una función local importante en el intestino a través de la neutralización de compuestos oxidantes y carcinogénicos. Además, los flavonoides también pueden ser metabolizados por la microflora colónica a ácidos fenólicos, que luego son absorbidos (28,58).

La matriz alimentaria parece influir en la absorción de los flavonoides. A este respecto, cabe destacar la menor absorción de flavonoides del cacao debido a la interacción con las proteínas de la leche, que están presentes en la mayoría de los productos derivados del cacao (59). En contraposición, otros estudios en humanos no han encontrado efectos significativos de la leche sobre la absorción de la epicatequina y la cantidad total de metabolitos excretados tras la ingesta de una bebida de cacao (60,61). Sin embargo, se han reportado diferencias en el perfil de los metabolitos excretados, lo que sugiere que los componentes de la leche ejercen algún efecto sobre el metabolismo del cacao (62). También parece que la presencia de carbohidratos en la matriz alimentaria mejora la absorción de los flavonoides. De hecho, la administración conjunta de flavanoles con alimentos ricos en carbohidratos parece conducir a un aumento significativo en las concentraciones plasmáticas de flavanoles y a una aceleración de su eliminación renal (63). En este sentido, el grado y la tasa de absorción de flavanoles parecen verse afectados por el consumo concurrente de pan, azúcar o zumo de uva, mientras que no se han informado interacciones específicas de los flavanoles con las proteínas o los lípidos con respecto a la absorción y el metabolismo de los flavanoles (63).

Los flavonoides absorbidos se distribuyen ampliamente por el organismo y se pueden detectar en diferentes órganos, como el tejido linfoide (28).

En cuanto a su eliminación, los metabolitos de los polifenoles pueden seguir dos vías de excreción, la vía biliar o la urinaria. Los metabolitos grandes y ampliamente conjugados se eliminan más frecuentemente por la vía biliar, mientras que los conjugados pequeños como los monosulfatos se eliminan preferentemente por la orina. La cantidad total de metabolitos excretados a través de la orina se encuentra altamente correlacionada con las concentraciones máximas en plasma (28).

3.4. Mecanismos de acción de los polifenoles del cacao y sus efectos sobre la salud

El alto contenido de flavonoides que presentan los granos y productos derivados del cacao, los convierte en productos de particular importancia nutricional, farmacéutica e incluso cosmética.

La mayoría de los compuestos polifenólicos, debido a la naturaleza de su estructura y la presencia de grupos hidroxilo, muestran una alta actividad antioxidante y anti radicales libres (45). No obstante, aunque las propiedades favorables a la salud producidas por los flavonoides se han atribuido principalmente a sus funciones antioxidantes (64), se han descrito diversos mecanismos de acción como causantes de numerosos beneficios en la salud.

En la actualidad, se cree que los flavonoides tienen una serie de propiedades farmacológicas y biológicas que incluyen la actividad antiinflamatoria, la antialérgica, la antitrombótica, la antiviral y la antibacteriana (65–69). Cabe señalar la capacidad de los antioxidantes para atrapar radicales libres y especies reactivas del oxígeno que actúan de manera destructiva sobre la estructura celular y tisular, provocando la quelación de iones de metales pesados, los cuales son catalizadores de reacciones de radicales libres (70). Esto es particularmente importante porque la disfunción de las células causadas por los radicales libres son el factor subyacente en el proceso de envejecimiento y el desarrollo del cáncer (71). Además, varios estudios de intervención en humanos han relacionado el cacao y sus polifenoles con cambios favorables en biomarcadores que evalúan el estado antioxidante y antiinflamatorio y que podrían estar implicados en el inicio y la incidencia de la tumorigénesis (72,73).

Diversos estudios epidemiológicos han demostrado que el consumo de alimentos ricos en flavonoides reduce el riesgo de enfermedad cardíaca y cardiovascular (74,75). Los oligómeros de procianidinas presentes en los granos de cacao y el chocolate reducen la

oxidación de las lipoproteínas de baja densidad (LDL) y, por lo tanto, contribuyen a ralentizar el proceso de aterosclerosis (76). También tienen propiedades que aumentan la cantidad de cHDL (77), lo que se traduce en una reducción del riesgo de aterosclerosis (78). La epicatequina también produce efectos beneficiosos sobre el sistema cardiovascular, ya que puede dilatar los vasos sanguíneos al estimular la síntesis de óxido nítrico, el cual tiene un papel importante en este proceso (79). Estudios recientes han demostrado que la epicatequina contenida en el chocolate reduce la actividad plaquetaria, y por lo tanto evita coágulos sanguíneos y disminuye la presión arterial (79–82).

También se ha observado que una dieta rica en antioxidantes procedentes de los granos de cacao facilita la función renal y del sistema inmune, e influye en la regulación de los niveles de insulina en la sangre (29).

Otras propiedades beneficiosas de los granos de cacao, y en concreto de los flavonoides, son la reducción de los síntomas del glaucoma y las cataratas, el retraso de la progresión de la periodontitis, la reducción de la hormona del estrés y la mejora de la flora intestinal (19). También se ha descrito cierto efecto neuroprotector y estimulador de las funciones cognitivas (19).

4. La menopausia

La menopausia es el final del periodo menstrual. La transición a la menopausia es el tiempo desde el inicio de los cambios del ciclo menstrual o los síntomas vasomotores hasta un año tras el final del periodo menstrual (83). Esta transición comienza alrededor de los 47 años de edad y dura unos 5-8 años de media.

Los síntomas que se experimentan durante la transición menopáusica se atribuyen a cambios endocrinos y su presencia e intensidad varían sustancialmente entre mujeres de distintas razas y localizaciones geográficas por motivos aún desconocidos. Los principales síntomas que ocurren en la menopausia son síntomas vasomotores, sequedad vaginal y síntomas urinarios (conocidos como «síndrome genitourinario de la menopausia»), disfunción sexual, y alteraciones del humor y del sueño (84).

Además, entre los síntomas que refieren las mujeres menopáusicas destacan los dolores articulares y musculares, los cambios en el contorno corporal, el aumento de las arrugas de la piel, la incontinencia urinaria y la disfunción sexual (85).

4.1. Síntomas vasomotores

Los síntomas vasomotores afectan a la mayoría de las mujeres posmenopáusicas, aunque de forma variable. Más del 85% de las mujeres posmenopáusicas refieren sufrir sofocos (86). La duración media de los síntomas vasomotores es de alrededor de 7,4 años y aquellas mujeres que comienzan con sofocos antes de que finalice su período menstrual pueden tener los síntomas más persistentes (87). Su causa no está clara, aunque la teoría más accesible apunta a que se produce un restablecimiento y una limitación del sistema termorregulador en asociación con fluctuaciones o pérdida de la producción de estrógenos. Anteriormente, se pensaba que los sofocos se relacionaban únicamente con la retirada de estrógenos; sin embargo, no hay un cambio agudo en el estradiol sérico durante un sofoco. Otros han relacionado los sofocos con la variabilidad tanto en el estradiol como en los niveles de hormona foliculoestimulante (FSH) (88).

4.2. Atrofia vulvovaginal / Síndrome genitourinario

El síndrome genitourinario es un término relativamente nuevo que se utiliza para describir los cambios vulvovaginales y los síntomas urinarios relacionados con la frecuencia, urgencia, nicturia, disuria e infecciones recurrentes del tracto urinario que ocurren en la menopausia (89).

La atrofia vaginal es una afección común en las mujeres posmenopáusicas. Se estima que aproximadamente el 15% de las mujeres premenopáusicas y hasta el 57% de las posmenopáusicas sufren síntomas derivados de la atrofia vaginal (90). Entre los síntomas que causa esta afección se encuentran la sequedad y el prurito vaginal, así como el dolor durante el coito.

Los síntomas de sequedad vaginal pueden tener un impacto negativo considerable en las relaciones interpersonales, la calidad de vida, las actividades de la vida diaria y la función sexual, aunque a menudo se infraestiman (91).

4.3. Sueño

La alteración del sueño puede afectar hasta un tercio de las mujeres durante la transición menopáusica (92). Estas alteraciones incluyen múltiples despertares, dificultad para conciliar el sueño y dificultad para volver a dormirse.

La relación entre los síntomas vasomotores y la alteración del sueño en la menopausia no está bien definida y los problemas del sueño no se deben necesariamente a los síntomas vasomotores (93).

Además, existe una relación bidireccional entre la alteración del sueño y la alteración del estado de ánimo, particularmente el estado de ánimo depresivo (94).

4.4. Disfunción sexual

Los síntomas sexuales más prevalentes relacionados con la menopausia y la transición menopáusica son la disminución del deseo sexual y de la excitación, la sequedad vaginal y la dispareunia, y una alteración de la satisfacción sexual (95). La presencia de estos síntomas se ha relacionado con niveles bajos de estradiol, pero no con los niveles de andrógenos.

Además, la función y la satisfacción sexual están estrechamente relacionadas con el bienestar psicológico general de la mujer. Esta sintomatología debe ser abordada en la práctica clínica habitual para preservar así la calidad de vida de las mujeres que la padecen (96).

4.5. Otras afecciones relacionadas con la menopausia

Depresión

La perimenopausia representa un periodo de vulnerabilidad para la mujer. Diversos estudios han mostrado un riesgo aumentado de padecer un estado de ánimo depresivo durante la transición menopáusica y aproximadamente tres veces más riesgo de desarrollar un episodio depresivo mayor durante la perimenopausia en comparación con la premenopausia (97). Algunos factores de riesgo para el desarrollo de estado de ánimo depresivo durante la menopausia son una edad más joven, la falta de sueño o eventos vitales negativos o estresantes. Puede haber múltiples estresores ambientales en la época en la que la mujer alcanza la menopausia, como cambios laborales o en la estructura familiar, que influyan en su estado de ánimo. Los cambios hormonales que ocurren durante la menopausia también se asocian a un mayor riesgo de depresión, tales como la variabilidad en los niveles de estradiol, niveles de FSH aumentados, la menopausia quirúrgica, la presencia de sofocos y antecedentes de síndrome premenstrual.

Cognición

La función cognitiva también parece verse afectada durante la transición menopáusica. Se han observado déficits en la función cognitiva de estas mujeres, como dificultades de memoria, aunque en un estudio se trataba de un efecto transitorio (96). La interacción entre la serotonina y el estrógeno podría ser un mecanismo subyacente de estas alteraciones (98).

Asimismo, se ha observado una disminución significativa en la atención, velocidad de procesamiento y otras habilidades cognitivas durante la transición menopáusica. Por lo tanto, la evidencia respalda que algunos de los problemas cognitivos que experimentan las mujeres de edad media son atribuibles a la transición a la menopausia (99).

Enfermedades cardiovasculares

Durante la menopausia, la mujer experimenta una serie de cambios fisiológicos que incluyen, entre otros, un incremento de los niveles de colesterol total (CT) y LDL colesterol (cLDL) y, por tanto, un incremento en el riesgo cardiovascular (100). La morbimortalidad cardiovascular es más prevalente en las mujeres posmenopáusicas que en las premenopáusicas, y se ha mencionado una deficiencia de estrógenos como la responsable de un riesgo cardiovascular aumentado (101). Una edad más temprana en la menopausia se ha asociado con un riesgo de morbimortalidad cardiovascular aumentado (96).

Las variaciones en los niveles de estrógenos y andrógenos durante la transición menopáusica se han asociado con un incremento de los factores de riesgo cardiovasculares, como la grasa central y cambios aterogénicos en el perfil lipídico (102,103).

El aumento de la edad se asocia, también, con una redistribución del tejido graso incrementándose el de localización central, lo que favorece una mayor obesidad abdominal y, finalmente, mayor frecuencia de complicaciones metabólicas (104).

A pesar de que las características del perfil lipídico probablemente se ven influenciadas por la transición menopáusica, otros factores de riesgo cardiovascular, como la presión arterial y los niveles de glucosa, parecen estar más influenciados por los efectos del envejecimiento (103).

5. El chocolate y sus efectos sobre la salud

Como se ha expuesto previamente, entre los componentes del chocolate se encuentran los flavonoides. Dichos compuestos confieren una característica especial al chocolate, que ha sido objeto de estudio en las últimas décadas debido a los efectos potencialmente beneficiosos que podría ejercer sobre la salud.

A continuación, se describen los posibles efectos del consumo de chocolate sobre la salud cardiovascular, la composición corporal, el rendimiento cognitivo y la calidad de vida.

5.1. Salud cardiovascular

El riesgo de enfermedad cardiovascular es más bajo en las mujeres que en los hombres (105). Sin embargo, como hemos señalado, con el inicio de la menopausia en las mujeres se observa un incremento del riesgo que se cree que puede ser debido al descenso de los estrógenos que acontece en este periodo (106).

Las intervenciones dirigidas a la prevención de la enfermedad cardiovascular cobran especial importancia en las poblaciones con un riesgo aumentado. En los últimos años se están estudiando tratamientos no farmacológicos y otras estrategias de prevención, como el consejo dietético, dirigidas a mejorar la salud en personas con un riesgo aumentado de padecer enfermedad cardiovascular (107).

A este respecto, el consumo de flavonoides se ha relacionado con efectos beneficiosos en la salud, tal y como se ha mencionado con anterioridad. Estos beneficios incluyen, entre otros, la reducción del riesgo de enfermedad cerebrovascular, de la incidencia de cáncer de pulmón, diabetes tipo 2 o asma (108) y del riesgo de cáncer rectal y de colon, así como la prevención de enfermedad cardíaca isquémica (109). Los efectos beneficiosos de los flavonoides se han atribuido a sus propiedades antioxidantes (110). Aunque estudios recientes proporcionan una explicación adicional del mecanismo de acción de los flavonoides relacionados con la eliminación de especies reactivas del oxígeno (ROS), la inmunomodulación, la regulación del ciclo celular, la modificación epigenética y la regulación genética del metabolismo (111,112).

Diversas revisiones sugieren que las dietas ricas en polifenoles pueden ejercer efectos protectores sobre la salud cardiovascular (113,114). Lockyer et al. observaron que la ingesta de extracto de hoja de olivo rico en polifenoles disminuía la presión arterial y el perfil lipídico en varones con pre-hipertensión (115). Además, Fuchs et al. concluyeron que dosis únicas de teaflavinas y catequinas del té podían tener efectos moderados en la microcirculación periférica en sujetos sanos (116), mientras que otros autores hallaron que un suplemento con extracto de catequinas del té verde reducía los niveles de CT y cLDL en mujeres posmenopáusicas (117). Otros estudios han evaluado los efectos del consumo de chocolate y cacao con una alta concentración de polifenoles. Los resultados de una revisión sistemática mostraron que estos productos mejoraron la dilatación mediada por el flujo y redujeron la resistencia a la insulina, provocando efectos beneficiosos agudos y crónicos en la salud cardiovascular (118).

La evidencia disponible sugiere que el chocolate rico en cacao puede tener beneficios sobre la rigidez arterial, la función vascular y los factores de riesgo cardiovascular en las

mujeres posmenopáusicas. En un ensayo realizado en esta población en el que se evaluó la ingesta diaria o en días alternos de 17 g de un compuesto rico en flavonoles se observó una mejora de la rigidez arterial mediante el descenso de la velocidad de la onda del pulso (VOP) (119). Además, la presión arterial y la presión de pulso (PP) disminuyeron tras la intervención, así como algunos factores de riesgo cardiovascular como la glucosa y los triglicéridos, sin otros cambios en el perfil lipídico. Los resultados de otro estudio mostraron que tras la ingesta de chocolate negro (80% cacao) y chocolate con leche (35% cacao) la velocidad de flujo de la arteria cerebral disminuyó en mujeres posmenopáusicas (120).

5.2. Composición corporal

Los parámetros que habitualmente se han utilizado en el estudio de la composición corporal son el peso, el índice de masa corporal (IMC) y otras medidas indirectas de distribución de grasa corporal y obesidad abdominal como el perímetro de la cintura o los índices cintura-altura y cintura-cadera (121). Más recientemente, la proliferación y validación de diferentes dispositivos de impedanciometría (122) ha permitido evaluar la composición y distribución de grasa corporal y establecer relación entre la masa grasa y la masa magra con la mortalidad. En este sentido, la revisión llevada a cabo por Hoon Lee et al. (123) en 2018, sugiere que un incremento en el contenido de masa grasa y/o un descenso en la masa magra podría asociarse a un incremento en la mortalidad.

Durante la menopausia, las mujeres experimentan una serie de cambios fisiológicos que incluyen un incremento en los niveles de CT y cLDL, con el consecuente incremento en el riesgo cardiovascular (100). El incremento en la edad también se asocia con una redistribución del tejido graso, con un aumento en la localización central, lo que favorece una mayor obesidad abdominal y, a la larga, una mayor frecuencia de complicaciones metabólicas (104).

Entre las intervenciones que evalúan los cambios en la composición corporal, algunas han conseguido resultados beneficiosos sobre la distribución y la composición corporal en mujeres posmenopáusicas utilizando programas de actividad física de manera aislada (124,125). Otras intervenciones, como la diseñada por Seimon et al. (126), combinan actividad física con restricción calórica y un discreto aumento de la ingesta proteica, obteniendo mejoras sobre el peso y la masa grasa. Este incremento moderado de la ingesta de proteínas ha sido señalado también por otros autores como una posible causa en la reducción del porcentaje de grasa corporal (127). La modificación en las cantidades de los macronutrientes de la dieta habitual también fue señalada en el Women's Health Inititative

Dietary Modification Trial (128), donde el grupo de mujeres posmenopáusicas asignadas a una intervención que incluía la reducción de ingesta grasa (<20% energía) obtuvo una reducción en el porcentaje de grasa corporal y en la masa grasa tras un año de seguimiento.

Sin embargo, aún no está claro el efecto de los polifenoles en la modificación de la composición corporal en mujeres posmenopáusicas. En el ensayo de Choquette et al. (129), el suplemento a la dieta habitual de 70 mg/día de isoflavonas consiguió cierta reducción en el porcentaje de masa grasa de las extremidades inferiores, pero no una reducción en otros compartimentos corporales ni en el cuerpo en su conjunto. Un suplemento diario en forma de aperitivo de chocolate (130) fue capaz de reducir tanto la masa grasa como el porcentaje de grasa corporal en mujeres premenopáusicas con sobrepeso u obesidad. Sin embargo, ese estudio combinaba el suplemento con una restricción calórica, por lo que no es posible apreciar el efecto aislado del suplemento de chocolate.

5.3. Rendimiento cognitivo

El consumo de chocolate y de productos ricos en cacao ha mostrado tener múltiples efectos beneficiosos sobre la salud (131–134). La función cognitiva es uno de los aspectos estudiados que podrían mejorar tras la ingesta de este tipo de compuestos. Se cree que los polifenoles actúan, por un lado, como neuroprotectores pudiendo mejorar el rendimiento cognitivo a través de un mecanismo de activación de cascadas de señalización en el cerebro y, por otro lado, mediante su acción sobre el sistema vascular que conduce a cambios beneficiosos en el flujo sanguíneo cerebrovascular (135–137).

La evidencia disponible respecto a los efectos del cacao sobre el rendimiento cognitivo es discrepante. En algunos estudios se han observado cambios en el rendimiento cognitivo reflejados en una mejora en las funciones ejecutivas y la fluidez verbal (138), así como en la memoria de trabajo (139,140), y una disminución de la fatiga mental (141). Además, un mayor consumo de chocolate se ha relacionado con una mejor función cognitiva (142). Asimismo, la ingesta de chocolate se ha asociado a un menor riesgo de demencia (143) y de deterioro cognitivo (144). Por el contrario, los hallazgos de otros estudios no han mostrado una mejora en el rendimiento cognitivo tras el consumo de cacao (145,146).

Los efectos positivos sobre la función cognitiva asociados a la ingesta de cacao se han observado en distintos subgrupos de población, tales como adultos jóvenes (140,147) así como en personas mayores tanto sin alteración cognitiva (138,148) como con deterioro cognitivo leve (149). En las mujeres posmenopáusicas, parece que el cambio en los niveles de estrógenos puede afectar a su estado cognitivo (97). En este grupo de población se ha observado una mejora de la velocidad del flujo sanguíneo cerebral y las respuestas de

conductancia tras el consumo de chocolate con alto contenido en cacao (120). No obstante, la evidencia sobre los efectos del consumo de chocolate sobre la función cognitiva en mujeres posmenopáusicas es escasa.

5.4. Calidad de vida

La menopausia tiene un impacto negativo sobre la calidad de vida (CdV), observándose una disminución gradual desde el periodo premenopáusico al periodo posmenopáusico (150), tanto en lo que se refiere al área de salud física como al de salud mental (151,152). Esta disminución en la calidad de vida en mujeres posmenopáusicas se ha asociado a la aparición de síntomas genitourinarios (95,153,154) y, especialmente, de síntomas vasomotores (150), como los sofocos (155). En muchos casos, además, durante el periodo posmenopáusico concurren otros factores psicológicos, como la depresión, que agravan la percepción sobre la calidad de vida (156).

El consumo de chocolate, y especialmente chocolate negro, se ha asociado con cambios pequeños, pero beneficiosos, en la salud cardiovascular de mujeres posmenopáusicas (157). Sin embargo, muy pocos trabajos han analizado su relación con la salud mental y/o componentes de la calidad de vida. Balboa-Castillo et al. (158) analizó una cohorte de 4599 individuos (edad media 54,1 años, 50,8% mujeres), sin encontrar evidencia de asociación entre la calidad de vida y el consumo mayor o menor de 10 g/día de chocolate, aunque estos autores no incluyeron un análisis por edad y género. En mujeres, el consumo de chocolate podría tener un impacto significativo positivo en la función sexual, especialmente sobre el deseo sexual (159). Un mayor consumo de chocolate se ha relacionado con una puntuación más alta en la escala de depresión CES-D (Center for Epidemiologic Studies Depression Scale) (160), en la que una puntuación de 16 o más alta a menudo refleja un resultado de cribado positivo, aunque sin lograr establecer una relación causa-efecto entre presentar más signos de depresión y un consumo mayor de chocolate (161).

Algunos trabajos (162) han relacionado el consumo de ciertos productos ricos en flavonoles y otros polifenoles, como propóleo (163) o frutas y verduras (164), con la calidad de vida, encontrando resultados poco concluyentes. En mujeres, el consumo habitual de café se relacionó con discretas mejoras en la dimensión mental de la calidad de vida (165). Asimismo, en mujeres posmenopáusicas el consumo de soja fermentada se ha relacionado con una mejora en la calidad de vida (166). Mientras que la dieta mediterránea, que incluye una gran variedad de productos ricos en polifenoles, no ha podido asociarse de manera clara con una mejora de la calidad de vida en personas mayores (167).

En resumen, a pesar del empeoramiento de la calidad de vida que sucede durante la menopausia y de los indicios acerca del posible efecto positivo que el consumo de chocolate puede producir sobre dicho deterioro, son muy pocos los estudios que abordan este tema obteniendo resultados divergentes y poco clarificadores (158,159,161). Más allá de la evaluación de las terapias farmacológicas y/o nutricionales durante la menopausia, es fundamental determinar el impacto real de estas sobre la calidad de vida (168).

Objetivos

Objetivo principal:

- Analizar el efecto de añadir 10 g diarios de chocolate comercial con alto porcentaje de cacao (99%) a la dieta habitual, durante 6 meses, sobre las cifras de presión arterial, factores de riesgo cardiovascular y marcadores de rigidez arterial en una muestra de mujeres posmenopáusicas.

Objetivos secundarios:

- Analizar el efecto de añadir 10 g diarios de chocolate comercial con alto porcentaje de cacao (99%) a la dieta habitual, durante 6 meses, sobre indicadores antropométricos y de composición corporal evaluados mediante impedanciometría en una muestra de mujeres posmenopáusicas.
- Analizar el efecto de añadir 10 g diarios de chocolate comercial con alto porcentaje de cacao (99%) a la dieta habitual, durante 6 meses, sobre indicadores de rendimiento cognitivo en una muestra de mujeres posmenopáusicas.
- Analizar el efecto de añadir 10 g diarios de chocolate comercial con alto porcentaje de cacao (99%) a la dieta habitual, durante 6 meses, sobre la calidad de vida relacionada con la salud en una muestra de mujeres posmenopáusicas.

Metodología

1. Diseño y ámbito de estudio

El diseño de este estudio corresponde a un ensayo clínico controlado y aleatorizado con dos grupos paralelos.

Este estudio se ha llevado a cabo en la Unidad de Investigación de Atención Primaria de Salamanca (APISAL) en Salamanca (España), que forma parte del Instituto de Investigación Biomédica de Salamanca (IBSAL) y de la Red de Investigación en Actividades Preventivas y Promoción de la Salud en Atención Primaria (redIAPP).

El periodo de estudio estuvo comprendido entre junio de 2018 y agosto de 2019. El ensayo clínico se registró en clinicaltrials.gov perteneciente al US National Library of Medicine, con el número de registro NCT03492983.

2. Participantes del estudio

2.1. Generalidades

Participaron las mujeres que cumplían los criterios de selección y firmaron el consentimiento informado tras recibir información sobre los objetivos y procedimientos del estudio.

2.2. Criterios de inclusión

En total, se incluyeron 140 mujeres con una edad comprendida entre 50 y 64 años y que se encontraban en período posmenopáusico definido por amenorrea durante al menos 12 meses consecutivos (Figura 3).

2.3. Criterios de exclusión

No se incluyeron 32 mujeres debido a que presentaban alguno de los siguientes criterios de exclusión: historia personal de enfermedad cardiovascular; antecedentes personales de diabetes mellitus, hipertensión arterial o dislipemia en tratamiento farmacológico; dietas hipocalóricas; enfermedad neurológica y/o neuropsicológica clínicamente demostrable; tratamiento con terapia hormonal sustitutiva; consumo habitual de más de 210 gramos por semana (g/semana) de cacao; intolerancia y/o alergia al cacao o alguno de los componentes del suplemento (Figura 3).

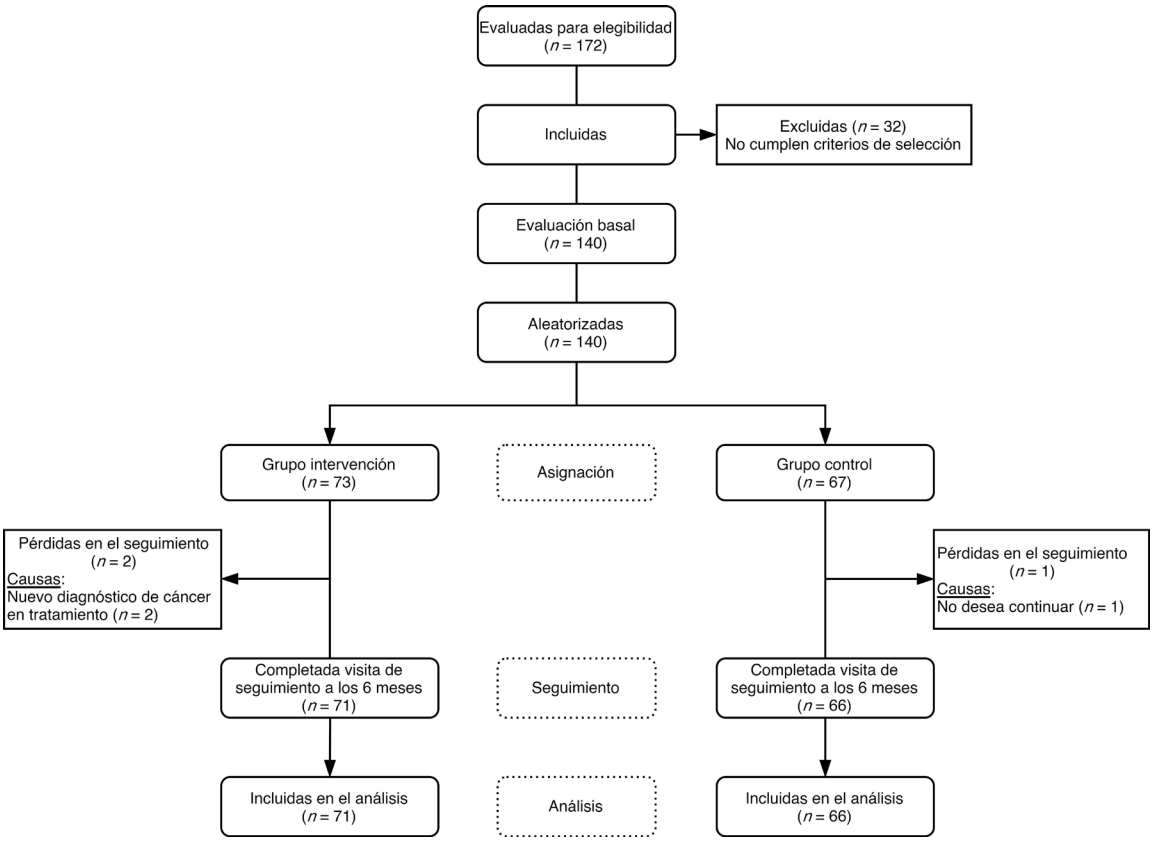

Figura 3. Diagrama de flujo del estudio

2.4. Selección de participantes

El reclutamiento se realizó en las consultas de cuatro centros de atención primaria urbanos de Salamanca, España, que corresponden a las zonas básicas de salud de Alamedilla, Garrido Sur, San Juan y San Bernardo Oeste. Este se llevó a cabo a través de un muestreo consecutivo de las mujeres que cumplían los criterios de selección y firmaron el consentimiento informado para su participación.

3. Tamaño de la muestra

El tamaño de la muestra se estimó en base a la potencial modificación de la variable principal de estudio, la presión arterial sistólica (PAS). Aceptando un riesgo alfa de 0,05, un riesgo beta de 0,20 en un contraste bilateral y una desviación estándar (DE) de 5,8 mmHg, se precisaron 140 participantes (70 por grupo) para detectar una diferencia mínima de 2,9 mmHg en la PAS entre los dos grupos. Esta estimación ha considerado los resultados obtenidos en un estudio de similares características en el que se observó una disminución de la PAS de 6,5 ± 5,8 mmHg (169). Se previó una tasa de pérdidas en el seguimiento del 10%.

4. Procedimientos y aleatorización

Todas las participantes realizaron una visita basal y una visita de seguimiento a los 6 meses de la evaluación basal en las que se realizaron las mediciones de las variables de estudio (Figura 3). El grupo de intervención además realizó 5 visitas de reabastecimiento en los meses 1, 2, 3, 4 y 5, durante las cuales no se llevó a cabo ninguna otra actuación además de la provisión del chocolate necesario hasta la siguiente visita y la recogida de un calendario con el registro de las tomas de chocolate efectuadas (Anexo I).

Los participantes se asignaron de forma aleatoria en dos grupos, un grupo de intervención (GI) compuesto por 73 participantes y un grupo control (GC) en el que se incluyeron 67 participantes. La secuencia de asignación se generó por un investigador independiente mediante el programa informático Epidat 4.2 (170). Los participantes recibieron un número de aleatorización basado en el orden de realización de la evaluación basal que permaneció oculto hasta el momento en que cada participante fue asignada a un grupo. Para garantizar que se mantuviera el enmascaramiento, los pacientes recibieron instrucciones claras de no revelar a qué tratamiento habían sido aleatoriamente asignados mientras los evaluadores ciegos los entrevistaban. La información sobre la asignación del tratamiento se almacenó en un casillero seguro en caso de que se precisara esa información por una emergencia.

Debido a las características de la intervención no fue posible cegar a los participantes. Para minimizar la contaminación entre grupos, el investigador que realizó las evaluaciones fue diferente del investigador que se encargó del reabastecimiento del chocolate en el GI.

5. Intervención

Las participantes del GC no recibieron ningún tipo de intervención. A las participantes del GI se les proporcionó chocolate con una concentración de cacao del 99% y las instrucciones para el consumo diario de 10 g de este compuesto añadidos a su ingesta alimentaria habitual. El periodo de ingesta del chocolate fue de 6 meses. Durante la primera visita de abastecimiento, se facilitaron unas instrucciones sobre su consumo y conservación, recomendándoles que la toma de la dosis diaria de chocolate fuese a la misma hora (Anexo II). Además, se les entregó un calendario para anotar la hora y la toma de cada día, el cual se devolvió a los investigadores en cada visita de reabastecimiento.

Figura 4. Chocolate utilizado en la intervención

El aporte nutricional diario de 10 g de este chocolate es de 59 kcal, 0,8 g de carbohidratos, 1,5 g de proteína y 5,1 g de grasa, de los cuales 3,1 g son grasa saturada. El aporte de polifenoles por cada 10 g de este producto es de 65,4 mg. El perfil polifenólico de este compuesto se puede ver en la Tabla 3. A los participantes de ambos grupos se les indicó que continuasen con el patrón dietético que seguían habitualmente sin modificar sus hábitos alimenticios durante el periodo de estudio.

Tabla 3. Composición polifenólica del compuesto de chocolate con 99% de cacao utilizado en la intervención

Composición	Cantidad (mg/10 g)
Ácido protocatéquico	0,58
Procianidina dímera (B3)	1,76
Catequina	10,35
Procianidina dímera (B2)	14,40
Epicatequina	26,10
Procianidina trímera (C1)	8,53
Procianidina A hexósido	3,54
Quercetina glucósido	0,02
Quercetina arabinósido	0,03

6. Variables de estudio

6.1. Variables clínicas y sociodemográficas

En la visita inicial, se recolectó información sobre variables clínicas y sociodemográficas mediante preguntas sobre la edad, el estado civil y el nivel educativo. Se registraron los antecedentes personales de diabetes gestacional, hipertensión y dislipidemia sin tratamiento farmacológico, y el tratamiento farmacológico prescrito, así como el tiempo transcurrido desde el inicio de la menopausia.

6.2. Evaluación de la presión arterial, parámetros de estructura y función vascular y factores de riesgo cardiovascular

Presión arterial

La presión arterial sistólica (PAS), la presión arterial diastólica (PAD) y la frecuencia cardíaca (FC) fueron medidas con un esfigmomanómetro modelo Omron M10-IT (Omron Healthcare, Kyoto, Japón) validado. Se tomaron 3 mediciones en el brazo dominante en posición sentada después de al menos 5 minutos de reposo, con un brazalete de tamaño adecuado, siguiendo las recomendaciones de la Sociedad Europea de Hipertensión (171). Para los análisis se utilizó la media de las dos últimas mediciones. Se calculó posteriormente la presión de pulso (PP) como la diferencia entre la PAS y la PAD, mientras que la presión arterial media (PAM) se estimó mediante la fórmula PAD + 1/3 (PAS – PAD).

Parámetros de estructura y función vascular

El índice vascular cardio-tobillo (CAVI), el índice tobillo-brazo (ITB) y la velocidad de la onda del pulso brazo-tobillo (VOP) se evaluaron utilizando el dispositivo Vasera VS-2000 (Fukuda Denshi, Tokyo, Japón), siguiendo las instrucciones del fabricante. Las mujeres participantes recibieron instrucciones para evitar fumar o consumir cafeína una hora antes del examen, acudir con ropa cómoda y permanecer en reposo durante al menos 10 minutos antes de la medición. Los manguitos se adaptaron a la circunferencia de los brazos y tobillos. Los electrodos se unieron a los brazos derecho e izquierdo y a los tobillos, y un micrófono de sonido cardíaco se fijó en el segundo espacio intercostal. Los participantes permanecieran quietos y en silencio durante 5 minutos. Solo las mediciones de CAVI obtenidas durante al menos 3 latidos cardíacos consecutivos se consideraron válidas. Los valores de CAVI se calcularon automáticamente mediante la sustitución del parámetro β de rigidez en la siguiente ecuación para detectar la elasticidad vascular y la VOP, parámetro β de rigidez = $2\rho \times 1/(Ps - Pd) \times \ln (Ps / Pd) \times VOP^2$, donde ρ es la densidad de la sangre, Ps y Pd son PAS y PAD en mmHg (172). El ITB se calculó dividiendo la mayor de las presiones medidas en cada uno de los tobillos por la mayor de las presiones medidas en los brazos (173). La VOP se estimó usando la ecuación, VOP = (0,5934 × altura (cm) + 14,4724) / TBA (TBA es el intervalo de tiempo entre las ondas de brazo y tobillo) (172).

La función vascular se evaluó con los parámetros del índice de aumento central (cAIx), índice de aumento central corregido a 75 latidos por minuto (cAIx75) e índice de aumento periférico (pAIx). Estas medidas fueron realizadas con un dispositivo de pulsera, desarrollado por Microsoft Research (Redmond, WA, USA). El dispositivo incluye un tonómetro colocado sobre la arteria radial. Es capaz de realizar mediciones continuas o programadas usando varias configuraciones de estos sensores (174). En este estudio, este

dispositivo se usó para hacer una breve grabación de la onda de pulso radial, de la cual se estimaron el PAIx y el CAIx. A partir de la morfología estimada de la onda aórtica por transformación matemática específica del dispositivo de muñeca, se calculó el CAIx utilizando la siguiente fórmula: presión de aumento central * 100 / presión de pulso. Las estimaciones de la forma de onda de presión aórtica se derivaron de la forma de onda de presión radial utilizando una función de transferencia patentada que es específica del sensor de presión y la geometría de montaje del tonómetro de muñeca. El CAIx se normalizó a una frecuencia cardíaca estándar de 75 latidos por minuto utilizando la siguiente ecuación publicada por los fabricantes del dispositivo Sphygmocor (AtCor Medical Pty Ltd, West Ryde, Australia): CAIx75= [(FC - 75) * (0,48) + (CAIx)] (175). El PAIx fue calculado como (segundo pico PAS - PAD) / (primer pico PAS - PAD) x 100, produciendo un valor expresado en porcentaje (%) (176).

Factores de riesgo cardiovascular

Se midieron la glucosa en plasma (mg/dL), el CT (mg/dL), los triglicéridos totales (mg/dL), el cHDL (mg/dL) y el cLDL (mg/dL). Las variables de laboratorio se evaluaron tras la realización de una extracción de sangre en ayunas de al menos 10-12 horas, entre las 08:00 y las 10:00 de la mañana. Se dieron instrucciones para evitar en las 24 horas previas el consumo de chocolate, y otros productos ricos en polifenoles.

El peso corporal fue medido dos veces con una balanza electrónica validada (Scale 7830, Soehnle Professional, Backnang, Alemania) tras una adecuada (exactitud ± 0,1 kg). La altura fue registrada como la media de dos lecturas redondeadas al centímetro más próximo utilizando un tallímetro validado (Seca 222, Medical Scale and Measurement System, Birmingham, Reino Unido). Ambas medidas fueron realizadas con la mujer descalza y con ropa ligera. El IMC se calculó dividiendo el peso (kg) por la talla al cuadrado (m^2).

6.3. Análisis de composición corporal

Las mediciones de composición corporal mediante bioimpedancia se realizaron con el dispositivo de multifrecuencia Inbody 230 (Biospace Ltd., Seoul, Corea del Sur) (122). Este es un dispositivo de impedancia segmentaria con el que se realizaron diez mediciones utilizando dos frecuencias diferentes (20 y 100 kHz) en cada segmento (brazo derecho, brazo izquierdo, tronco, pierna derecha y pierna izquierda). Los datos son calculados por el algoritmo del fabricante e incluyen masa grasa, masa libre de grasa, masa musculo-esquelética, agua corporal total, proteínas y minerales. Además, el dispositivo calcula el peso corporal (kg) total. Para la determinación de las medidas de composición corporal se

siguieron las recomendaciones del fabricante que se describen a continuación: realizar el test antes de una comida o dos horas después; haber ido al baño antes de iniciar el análisis, ya que el peso de orina y heces estaría incluido y pueden inducir a errores biológicos; no haber realizado ejercicio físico antes del análisis; el paciente debe haber estado de pie durante unos 5 minutos antes del análisis, evitando haber estado tumbado o sentado durante un periodo largo de tiempo, ya que puede inducir a cambios en los resultados debido a que el agua tiende a moverse a las extremidades inferiores cuando la persona se levanta; no realizar el análisis después de una ducha o sauna ni durante la menstruación; mantener una temperatura estable en la sala de exploración de entre 20-25 °C, manteniéndose las mismas condiciones en las evaluaciones posteriores.

6.4. Evaluación del rendimiento cognitivo

El rendimiento cognitivo se evaluó a través de una breve batería neuropsicológica.

Atención y funciones ejecutivas

La atención se midió con el Trail Making Test A (TMT-A), y la velocidad de procesamiento y las funciones ejecutivas se midieron con el Trail Making Test B (TMT-B) (177) (Anexo III). Además, esta prueba permite evaluar la velocidad visomotriz, rastreo visual, función motora y memoria de trabajo (178,179). El TMT-A consiste en unir una serie de números en orden ascendente. El TMT-B consiste en unir una serie de números y letras de forma alterna, los números siguiendo el orden ascendente y las letras según el orden del abecedario. En las dos partes la evaluación se realiza mediante la cuantificación en segundos del tiempo requerido para completar la tarea.

Memoria verbal

La memoria verbal se evaluó con la versión abreviada del Test de Aprendizaje Auditivo Verbal de Rey (180). La memoria verbal inmediata se midió mediante el recuerdo inmediato de una lista de 15 palabras en 3 intentos. La variable resultado fue el número de palabras recordadas en el tercer intento. Después de 10 minutos, se midió la memoria verbal diferida a través del recuerdo libre de las palabras aprendidas en la primera parte de la evaluación.

Memoria de trabajo

La memoria de trabajo se valoró con la tarea de dígitos inversos de la escala de inteligencia de Wechsler para adultos (WAIS, por sus siglas en inglés) (181). Esta prueba está conformada por siete categorías con dos series de números cada una. Las series de cada categoría contienen un número más que en la categoría anterior, comenzando por dos

números. El participante tiene que repetir de forma inmediata e inversa cada serie. El test finaliza cuando se cometen errores en las dos series de una misma categoría. La puntuación equivale a la última categoría de la que se ha acertado al menos una de las series de números.

Fluidez fonológica

La fluidez fonológica se evaluó con el test FAS, que consiste en nombrar el mayor número de palabras posible que comiencen por las letras F, A y S durante un periodo de 1 minuto (182). La puntuación se obtiene otorgando un punto por cada palabra dicha correctamente, sin contar repeticiones, palabras derivadas o nombres propios.

Fluidez categorial

La fluidez categorial mide la fluidez verbal semántica de asociación controlada. Esta prueba consiste en enumerar el mayor número posible de animales en un minuto (183). La puntuación se obtiene del sumatorio de palabras dichas correctamente sin contar repeticiones ni palabras derivadas.

6.5. Evaluación de la calidad de vida

La evaluación de la calidad de vida se realizó utilizando dos herramientas, el cuestionario EuroQol-5D (EuroQoL-5D-3L) y la escala Cervantes.

EuroQol

El EuroQol (184,185) es una medida del estado de salud que incluye dos herramientas: el EuroQol-5D (EQ-5D) y el EuroQol Visual Analogue Scale (EQ-VAS). En este estudio se utilizó la versión de tres niveles del cuestionario EQ-5D (EQ-5D-3L) que analiza de forma descriptiva cinco dimensiones (movilidad, autocuidado, actividades habituales, dolor e incomodidad, ansiedad y depresión) en una escala de tres posibles respuestas categorizadas del 1 al 3 (1 «No tengo problemas», 2 «Tengo algunos problemas», 3 «Tengo problemas extremos»). Los resultados se presentan mediante un análisis descriptivo de cada una de las cinco dimensiones y mediante la estimación de un índice del estado general de salud cuyo rango oscila entre 0 (peor estado de salud) y 1 (mejor estado de salud) (186). El EQ-VAS consiste en una escala visual analógica que evalúa el estado de salud general percibida por cada individuo. El rango de esta escala comprende de 0 (muy mal estado de salud) a 100 (estado de salud óptimo).

Escala Cervantes

La escala Cervantes (187) es específica para mujeres posmenopáusicas y valora el impacto de la menopausia en distintas áreas físicas y psicosociales y, en especial, su repercusión en el bienestar general. Consta de 31 ítems estructurados en 4 dimensiones:

menopausia y salud, sexualidad, dominio psíquico y relación de pareja. Además, la dimensión de menopausia y salud incluye las subdimensiones de sintomatología vasomotora, salud y envejecimiento. En relación con la puntuación global de la escala, el valor más alto corresponde a 155 puntos, lo que se traduce en una baja calidad de vida, mientras que el valor más bajo es de 0 puntos, que indicaría la mejor calidad de vida posible. Todas las dimensiones y subdimensiones tienen un rango de puntuación similar donde el valor más bajo indica una mejor calidad de vida y el valor más alto una peor calidad de vida.

6.6. Otras variables

Variables de laboratorio

En las visitas basal y de seguimiento a los 6 meses, se determinaron los valores plasmáticos de creatinina (mg/L), hemoglobina glicada (%) e insulinemia (mg/dL). La resistencia a la insulina se determinó usando el HOMA-IR (índice de resistencia a la insulina mediante evaluación del modelo homeostático), estimado con la siguiente ecuación: Glucosa (mmol/L) × insulina (mU/mL)/22,5.

También se determinaron la creatinina en orina (mg/dL) y la microalbuminuria (mg/dL).

Evaluación del consumo de chocolate y la dieta habitual

El consumo de chocolate se cuantificó mediante una serie de preguntas sobre la cantidad, el tipo y la frecuencia de consumo del periodo entre cada visita.

La composición nutricional de la dieta habitual que incluye la distribución de macronutrientes y el consumo de energía se evaluó con un recordatorio de 24 horas recogido durante 3 días no consecutivos, previos al día de cada evaluación. Estos datos se registraron y procesaron mediante la EVIDENT App (188).

Consumo de tabaco y alcohol

El consumo de tabaco se valoró con un cuestionario de historia personal de consumo de tabaco y el patrón del mismo.

El consumo de alcohol se recogió mediante un cuestionario de consumo de alcohol en los últimos 7 días que incluirá bebidas específicas y la cantidad consumida en volumen de cada una de ellas.

Actividad física

La actividad física se evaluó mediante el Cuestionario Internacional de Actividad Física (IPAQ, por sus siglas en inglés) en su versión corta y validada en castellano (189). Este

cuestionario evalúa la actividad de los últimos 7 días, clasificando a los sujetos en función de tres niveles de actividad (bajo, moderado y alto) asociados a tres tipos de actividades: caminar, actividades de intensidad moderada y actividades de intensidad vigorosa. La dosis de ejercicio físico se estimó en MET-h/semana.

Adherencia a la intervención

La adherencia a la intervención se calculó como el porcentaje de los días de toma del chocolate respecto al total teórico, según los datos registrados en los calendarios de recogida de cada participante del GI.

7. Procedimiento de recogida de datos y monitorización del estudio

La recolección de datos en cada visita de evaluación la llevó a cabo una enfermera previamente entrenada para ello. Para ello se utilizó un cuestionario de recogida de datos (Anexo IV). Cada participante del estudio se identificó mediante un único código que identificaba los datos recogidos en cada una de las mediciones. Con ello se creó una base de datos a la que únicamente tiene acceso el personal del estudio. El investigador principal llevó a cabo un proceso de limpieza de datos y depuración de la base de datos al finalizar el estudio.

8. Análisis estadístico

8.1. Análisis general

Los resultados se expresaron según la media ± la desviación estándar (DE) o la mediana (rango intercuartílico) en las variables cuantitativas o mediante la distribución de frecuencias en el caso de las cualitativas. La normalidad de las variables se evaluó mediante el test de Kolmogorov-Smirnov. En los casos en los que no se pudo asumir una distribución normal se utilizaron las correspondientes pruebas no paramétricas. Se utilizó el test chi cuadrado o el test exacto de Fisher para analizar la asociación entre variables cualitativas independientes. Mediante la prueba de la *t* de Student o la prueba de la U de Mann-Whitney se compararon las medias entre los dos grupos.

Todos los análisis se realizaron con el programa estadístico SPSS versión 23.0 (IBM Corporation, Armonk, NY, EE.UU.) y se fijó un riesgo alfa de 0,05 como límite de significación estadística.

8.2. Análisis del efecto de la intervención sobre las variables de presión arterial, rigidez arterial y factores de riesgo cardiovascular

El efecto del consumo de chocolate en las variables de presión arterial, rigidez arterial y factores de riesgo cardiovascular se evaluó mediante la prueba de la *t* de Student para la comparación de medias entre dos grupos. Se utilizó la prueba de la *t* de Student para datos apareados para evaluar el cambio dentro del mismo grupo en las variables a los 6 meses desde la evaluación basal. Para comparar los efectos entre los grupos se realizó un análisis de la covarianza (ANCOVA), utilizando los valores basales de los correspondientes valores de seguimiento como covariables.

Se realizaron análisis adicionales teniendo en cuenta la presencia o ausencia de sobrepeso u obesidad como condición basal para evaluar la PAS, PAD y PP utilizando la prueba de la *t* de Student. También se analizaron estas variables teniendo en cuenta la forma en que se consumió el chocolate (solo, con café o té, con otros alimentos a través de un análisis de la varianza (ANOVA), y se realizó un test post hoc cuando se encontraron diferencias estadísticamente significativas.

8.3. Análisis del efecto de la intervención sobre las variables de composición corporal

Para analizar los cambios en las variables a los 6 meses desde la evaluación basal dentro del mismo grupo se utilizó la prueba de la *t* de Student para datos apareados.

Se realizó un ANCOVA utilizando los valores basales de las variables de composición corporal como covariables para comparar los cambios entre los dos grupos. Se estimó el tamaño del efecto en el cambio de la composición corporal mediante el cálculo de la *d* de Cohen.

8.4. Análisis del efecto de la intervención sobre las variables de rendimiento cognitivo

El efecto de la intervención en las variables resultado (variables de rendimiento cognitivo), se evaluó mediante un ANCOVA, utilizando como covariables la edad, el nivel de estudios, el tiempo de menopausia y el consumo de energía diario. Las diferencias entre grupos en todos los casos se presentan como medias e IC 95%. Se estimó el tamaño del efecto en el cambio de las variables de rendimiento cognitivo mediante el cálculo de la *d* de Cohen. Para analizar los cambios a los 6 meses respecto a la evaluación basal en las variables resultado dentro del mismo grupo, se utilizó la prueba de la *t* de Student para datos apareados y se presentan como la media y la desviación estándar.

Se llevaron a cabo análisis según el nivel educativo y la edad como condiciones basales. Para el análisis por edad, la muestra se dividió tomando como referencia la mediana de edad que correspondía a 57,4 años y se evaluó el efecto mediante un ANCOVA ajustando por nivel de estudios, el tiempo de menopausia y el consumo de energía diario. Para evaluar el efecto según el nivel de estudios se conformaron dos grupos (estudios universitarios o de postgrado y estudios primarios o secundarios/bachiller) y se realizó un ANCOVA utilizando como covariables la edad, el tiempo de menopausia y el consumo de energía diario.

8.5. Análisis del efecto de la intervención sobre las variables de calidad de vida

El efecto del consumo de chocolate en las variables principales de calidad de vida se analizó a través de un ANCOVA ajustado por los principales determinantes de la calidad de vida recogidos: edad, nivel de estudios, estado civil, tiempo transcurrido desde el comienzo de la menopausia, consumo energético diario (kcal), consumo basal de chocolate con >70% cacao, actividad física, consumo de alcohol, tabaco, depresión, y dislipemia sin tratamiento farmacológico. Los resultados de estos análisis se presentan mostrando la media marginal estimada y su intervalo de confianza (IC) al 95%. El cambio dentro de cada grupo se analizó con la prueba de la *t* de Student para datos apareados. Para abordar los posibles sesgos debido a la falta de respuesta en algunos ítems de la escala Cervantes, se aplicó la imputación múltiple por ecuaciones encadenadas con 50 conjuntos de datos imputados a los resultados y las covariables (190,191). Las estimaciones de cada conjunto de datos imputados se combinaron siguiendo las reglas descritas por Rubin (192).

9. Aspectos éticos y legales

El estudio fue aprobado por el Comité de Ética de la Investigación con Medicamentos del Área de Salud de Salamanca (CEIm del Área de Salud de Salamanca) en febrero de 2018 (código CEIC: PI11812/2017) (Anexo V). Los participantes recibieron y aceptaron el consentimiento informado (Anexo VI), de acuerdo con la Declaración de Helsinki. Los participantes fueron informados de los objetivos del proyecto y de los riesgos y beneficios de las exploraciones que se les iban a realizar. Ninguna de las exploraciones presentaba riesgos vitales para el tipo de sujetos que se iban a incluir en el estudio. Además, se garantizó en todo momento la confidencialidad de los datos de los sujetos incluidos, conforme lo que dispone la Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales y el Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 abril de 2016 de Protección de Datos (RGPD), y en las condiciones que establece la Ley 14/2007 de Investigación biomédica.

10. Fases de estudio y cronograma

Elaboración del proyecto de Tesis Doctoral: año 2017

Trabajo de campo, recogida de datos: junio 2018 - agosto 2019

Análisis de resultados: a partir de septiembre 2019

Elaboración de publicaciones: a partir de diciembre 2018

Redacción de la memoria de Tesis Doctoral: junio 2020 a marzo 2021

Presentación y defensa de la Tesis Doctoral: mayo 2021

Resultados

Características generales de la población de estudio

Las características clínicas y sociodemográficas de las participantes incluidas en el estudio se muestran en la Tabla 4. La muestra está compuesta por 140 mujeres con una edad media de 57,3 ± 3,6 años. En el momento de la evaluación basal, el tiempo transcurrido desde el inicio de la menopausia era de 6,9 ± 4,2 años. El consumo habitual de chocolate total, así como el consumo específico de chocolate con >70% de cacao, era similar en ambos grupos.

Tabla 4. Características las participantes en el estudio

Variables	Global (*n* = 140)	Grupo de Intervención (*n* = 73)	Grupo Control (*n* = 67)
Edad, años	57,3 ± 3,6	57,1 ± 3,5	57,5 ± 3,8
Estado civil, *n* (%)			
Casada/cohabita	95 (67,9)	48 (65,8)	47 (70,1)
Separada/divorciada	15 (10,7)	8 (11,0)	7 (10,4)
Soltera	24 (17,1)	15 (20,5)	9 (13,4)
Viuda	6 (4,3)	2 (2,7)	4 (6,0)
Nivel de educación, *n* (%)			
Estudios primarios	28 (20,0)	16 (21,9)	12 (17,9)
Estudios secundarios/bachillerato/FP	51 (36,4)	22 (30,1)	29 (43,3)
Estudios universitarios	28 (20,0)	17 (23,3)	11 (16,4)
Estudios de postgrado	33 (23,6)	18 (24,7)	15 (22,4)
Tiempo desde el inicio de la menopausia, años	6,9 ± 4,2	6,9 ± 4,6	6,9 ± 3,6
Hipertensión sin tratamiento farmacológico, *n* (%)	1 (0,7)	1 (1,4)	0 (0,0)
Dislipemia sin tratamiento farmacológico, *n* (%)	18 (12,9)	8 (11,0)	10 (14,9)
Diabetes gestacional, *n* (%)	4 (2,9)	3 (4,1)	1 (1,5)
Terapia hormonal tiroidea, *n* (%)	23 (16,4)	13 (17,8)	10 (14,9)
Fumadora actual, *n* (%)	21 (15,0)	12 (16,4)	9 (13,4)
Consumo de alcohol, g/semana	26,7 ± 39,5	23,1 ± 29,4	30,6 ± 48,1
Consumo de energía, kcal/día	1747,6 ± 379,4	1716,2 ± 357,5	1782,4 ± 402,2
Composición nutricional de la dieta habitual			
Carbohidratos, g/día	170,7 ± 47,5	168,4 ± 45,1	173,4 ± 50,2
Proteínas, g/día	77,4 ± 17,7	76,7 ± 16,7	78,2 ± 18,9
Fibra, g/día	24,9 ± 8,6	24,0 ± 7,5	25,9 ± 9,6
Grasas, g/día	78,5 ± 20,4	77,4 ± 20,7	79,8 ± 20,1
Grasas saturadas, g/día	25,2 ± 7,5	25,1 ± 7,6	25,3 ± 7,5
Actividad física, MET–h/semana	28,6 ± 30,0	31,2 ± 36,8	25,7 ± 20,0
Consumo de chocolate, g/semana[a]	68,8 ± 72,3	68,6 ± 71,1	69,1 ± 74,2
	46 (100–13)	42 (9–109)	50 (21–80)
Consumo de chocolate >70% cacao, g/semana[a]	17,8 ± 34,8	19,9 ± 36,2	15,6 ± 33,2
	0 (24–0)	0 (0–26)	0 (0–24)

Valores expresados como media ± desviación estándar, mediana (rango intercuartílico) o frecuencias. MET, equivalente metabólico.

[a] Para la mejor comprensión de estas variables no paramétricas se han calculado tanto la media ± desviación estándar como la mediana (rango intercuartílico).

La PAS basal en la muestra era de 108,2 ± 15,6 mmHg, mientras que la PP era de 35,7 ± 8,6 mmHg. Los valores basales de presión arterial y de parámetros de laboratorio se pueden consultar en la Tabla 5.

Tabla 5. Características de las variables de laboratorio y presión arterial de las participantes en el estudio

Variables	Global (*n* = 140)	Grupo de Intervención (*n* = 73)	Grupo Control (*n* = 67)
Glucosa, mg/dL	86,4 ± 8,7	86,7 ± 9,0	86,2 ± 8,4
Insulina, mg/dL	7,9 ± 3,2	8,4 ± 3,5	7,5 ± 2,9
CT, mg/dL	206,7 ± 28,0	209,8 ± 28,7	203,3 ± 26,9
Colesterol HDL, mg/dL	66,9 ± 15,3	67,9 ± 17,2	65,8 ± 13,1
Colesterol LDL, mg/dL	124,4 ± 26,8	126,8 ± 26,4	121,7 ± 27,2
Triglicéridos, mg/dL	81,5 ± 32,1	83,0 ± 30,3	79,9 ± 34,1
HOMA-IR	1,7 ± 0,8	1,8 ± 0,9	1,6 ± 0,7
Creatinina, mg/L	0,7 ± 0,1	0,7 ± 0,1	0,7 ± 0,1
PAS, mmHg	108,2 ± 15,6	108,6 ± 16,4	107,8 ± 14,8
PAD, mmHg	72,5 ± 10,5	72,8 ± 10,8	72,1 ± 10,2
FC, l.p.m.	66,7 ± 8,2	66,6 ± 7,9	66,8 ± 8,6
PP, mmHg	35,7 ± 8,6	35,8 ± 9,4	35,6 ± 7,6
PAM, mmHg	84,4 ± 11,8	84,8 ± 12,1	84,0 ± 11,4

CT, colesterol total; FC, frecuencia cardíaca; HDL, lipoproteínas de alta densidad; HOMA-IR, índice de resistencia a la insulina mediante evaluación del modelo homeostático; LDL, lipoproteínas de baja densidad; l.p.m., latidos por minuto; PAS, presión arterial sistólica; PAD, presión arterial diastólica; PAM, presión arterial media; PP, presión de pulso.

Por otro lado, los parámetros de rigidez arterial y función vascular se muestran en la Tabla 6, donde se observa que la VOP en la muestra era de 12,26 ± 1,56 m/s.

Tabla 6. Parámetros de rigidez arterial y función vascular en las mujeres posmenopáusicas participantes

Variables	Global (*n* = 140)	Grupo de Intervención (*n* = 73)	Grupo Control (*n* = 67)
CAVI	7,64 ± 0,87	7,57 ± 0,90	7,71 ± 0,83
VOP, m/s	12,26 ± 1,56	12,19 ± 1,59	12,34 ± 1,53

CAVI, índice vascular cardio-tobillo; VOP, velocidad de la onda del pulso

En cuanto al peso medio de las mujeres participantes, este era de 65,22 ± 9,60 kg, mientras que el IMC correspondía a 25,66 ± 3,46 kg/m^2. Los valores relativos a la composición corporal se encuentran recogidos en la Tabla 7, donde se observa que la masa grasa corporal en la muestra era de 25,82 ± 7,05 kg.

En cuanto a las variables de rendimiento cognitivo, recogidas en la Tabla 8, las mujeres presentaron un desempeño basal en el TMT-B de 93,06 ± 41,10 segundos.

En la Tabla 9 se muestran las puntuaciones para las variables de calidad de vida, en las que se observa una puntuación de 0,892 ± 0,144 en el EQ-5D-3L y una puntuación de 50,24 (1,74) en la escala Cervantes.

Tabla 7. Variables de composición corporal[a]

Variables	Global (*n* = 132)	Grupo de Intervención (*n* = 69)	Grupo Control (*n* = 63)
Peso (kg)	65,22 ± 9,60	65,94 ± 10,34	64,43 ± 8,74
IMC (kg/m^2)	25,66 ± 3,46	26,00 ± 3,75	25,29 ± 3,11
MGC (kg)	25,82 ± 7,05	26,47 ± 7,72	25,10 ± 6,21
PGC (%)	39,01 ± 5,94	39,44 ± 6,32	38,53 ± 5,50
MLG (kg)	39,40 ± 4,20	39,47 ± 4,10	39,32 ± 4,33
MME (kg)	21,17 ± 2,47	21,23 ± 2,43	21,10 ± 2,54
ACT (kg)	28,89 ± 3,08	28,95 ± 3,00	28,83 ± 3,18

ACT, Agua Corporal Total; IMC, Índice de Masa Corporal; MGC, Masa Grasa Corporal; MLG, Masa Libre de Grasa; MME, Masa Muscular Esquelética; PGC, Porcentaje de Grasa Corporal.
[a] La determinación de estas variables con el dispositivo de impedancia Inbody se realizó en 132 mujeres. En las 8 mujeres excluidas no se realizaron estas evaluaciones al concurrir alguna de las circunstancias que describe el fabricante del dispositivo de medición como contraindicaciones para la realización de la prueba.

Tabla 8. Variables de rendimiento cognitivo

Variables	Global (*n* = 140)	Grupo de Intervención (*n* = 73)	Grupo Control (*n* = 67)
TAAVR–RI (palabras)	7,60 ± 1,83	7,70 ± 1,80	7,48 ± 1,88
TAAVR–RD (palabras)	7,02 ± 3,00	6,82 ± 3,16	7,24 ± 2,82
Trail Making Test A (segundos)	40,11 ± 12,79	39,25 ± 13,71	41,06 ± 11,73
Trail Making Test B (segundos)	93,06 ± 41,10	94,00 ± 46,96	92,03 ± 33,91
Dígitos inversos WAIS (puntuación total)	3,24 ± 1,15	3,33 ± 1,26	3,13 ± 1,03
Fluidez fonológica (palabras)	12,28 ± 3,92	12,92 ± 3,94	11,58 ± 3,80
Fluidez categorial (palabras)	20,10 ± 4,80	20,16 ± 5,22	20,03 ± 4,32

Dígitos inversos WAIS, tarea de dígitos inversos de la escala de inteligencia de Wechsler para adultos; TAAVR–RI, Test de Aprendizaje Auditivo Verbal de Rey – recuerdo inmediato; TAAVR–RD, Test de Aprendizaje Auditivo Verbal de Rey – recuerdo diferido

Tabla 9. Variables de calidad de vida

Variables	Global (*n* = 140)	Grupo de Intervención (*n* = 73)	Grupo Control (*n* = 67)
EQ-5D-3L[a,b]	0,892 ± 0,144	0,868 ± 0,159	0,919 ± 0,124
EQ-VAS[a,c]	76,45 ± 13,84	74,7 ± 13,9	78,3 ± 13,6
Escala Cervantes puntuación total[d]	50,24 (1,74)	51,8 (2,5)	48,5 (2,4)

[a] Valores expresados como media ± desviación estándar
[b] Versión de 3 niveles del cuestionario EuroQol-5D. Rango entre 0 (peor calidad de vida) y 1 (mejor calidad de vida)
[c] EQ-VAS, Escala analógica visual del EuroQol-5D. Rango entre 0 (peor calidad de vida) y 100 (mejor calidad de vida)
[d] Rango entre 0 (mejor calidad de vida) y 155 (peor calidad de vida). Expresado como media y error estándar

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Efectos vasculares y cognitivos del chocolate con alto porcentaje de cacao en mujeres posmenopáusicas: protocolo de estudio para un ensayo clínico aleatorizado

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BMJ Open 2018 Dec 14;8(12):e024095

Antecedentes: La ingesta de polifenoles ha demostrado ciertos beneficios para la salud. El objetivo de este estudio es evaluar el efecto de añadir a la dieta habitual una cantidad diaria de chocolate con alto porcentaje de cacao y polifenoles sobre la presión arterial, la función vascular, el rendimiento cognitivo, la calidad de vida y la composición corporal en mujeres posmenopáusicas.

Métodos y análisis: Se trata de un ensayo clínico aleatorizado con dos grupos paralelos en el que se incluirán 140 mujeres entre 50 y 64 años de edad en periodo posmenopáusico definido por amenorrea de al menos 12 meses consecutivos. La variable principal será el cambio en la presión arterial. Las variables secundarias serán los cambios en la función vascular, la calidad de vida, el rendimiento cognitivo y la composición corporal. El grupo de intervención recibirá chocolate con el 99% de cacao con instrucciones de tomar 10 g diarios añadidos a su alimentación habitual durante 6 meses. El aporte nutricional diario de esta cantidad de chocolate es de 59 kcal y 65,4 mg de polifenoles. No se efectuará ninguna intervención sobre el grupo control. Todas las variables serán medidas en la visita basal y a los 3 y 6 meses de la aleatorización, excepto el rendimiento cognitivo y la calidad de vida que solo se evaluarán en la evaluación basal y a los 6 meses. El comienzo del reclutamiento está previsto para el 1 de junio de 2018 y la duración del estudio será hasta el 31 de mayo de 2019.

Ética y difusión: Este estudio fue aprobado por el Comité de Ética de la Investigación con Medicamentos (CEIm) del Área de Salud de Salamanca, España en febrero de 2018. Un listado de verificación de SPIRIT está disponible para este protocolo. El ensayo clínico fue registrado en clinicaltrials.gov perteneciente al US National Library of Medicine - número NCT03492983.

BMJ Open

Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial

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To cite: Garcia-Yu IA, Garcia-Ortiz L, Gómez-Marcos MA, *et al*. Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial. *BMJ Open* 2018;**8**:e024095. doi:10.1136/bmjopen-2018-024095

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-024095).

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Received 9 May 2018
Revised 3 October 2018
Accepted 2 November 2018

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AbstrACt

Introduction The intake of polyphenols has certain health benefits. This study will aim to assess the effect of adding a daily amount of chocolate high in cocoa content and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women.

Methods and analysis Here we plan a randomised clinical trial with two parallel groups involving a total of 140 women between 50 and 64 years in the postmenopausal period, defined by amenorrhoea of at least 12 consecutive months. The main variable will be the change in blood pressure. Secondary variables will be changes in vascular function, quality of life, cognitive performance and body composition. The intervention group will be given chocolate containing 99% cocoa, with instructions to add 10 g daily to their normal diet for 6 months. The daily nutritional contribution of this amount of chocolate is 59 kcal and 65.4 mg of polyphenols. There will be no intervention in the control group. All variables will be measured at the baseline visit and 3 and 6 months after randomisation, except cognitive performance and quality of life, which will only be assessed at baseline and at 6 months. Recruitment is scheduled to begin on 1 June 2018, and the study will continue until 31 May 2019.

Ethics and dissemination This study was approved by the Clinical Research Ethics Committee of the Health Area of Salamanca, Spain ('CREC of Health Area of Salamanca'), in February 2018. A SPIRIT checklist is available for this protocol. The clinical trial has been registered at ClinicalTrials.gov provided by the US National Library of Medicine, number NCT03492983. The results will be disseminated through open access peer-reviewed journals, conference presentations, broadcast media and a presentation to stakeholders.

strengths and limitations of this study

- This study will use commercially available chocolate with a high content of cocoa and polyphenols during the intervention.
- Blood pressure and vascular function will be measured objectively using a sphygmomanometer and a Vasera VS-2000 device (Fukuda Denshi), with body composition measured by impedance analysis, while the quality of life and cognitive performance will be assessed using validated instruments.
- Due to the nature of the intervention, the participants cannot be blinded, although the researchers who perform the measurements and the statistical analysis will be blinded.

IntroduCtIon

Polyphenols are bioactive compounds found in many plants, fruits and vegetables. The beneficial effects on human health associated with the consumption of a diet rich in polyphenols have generated great scientific interest in these substances.[1–3] The action of polyphenols is based on their antioxidant capacity through the uptake of free radicals, the chelation of metals with redox properties and the modulation and inhibition of enzymatic activities.[4]

The most abundant polyphenols in cocoa are flavonoids, which have been linked to a protective effect against cardiovascular disease, decreasing the risk of cardiovascular morbidity and mortality and favouring the prevention of other chronic diseases, such as diabetes mellitus type 2.[1–3 5–7] The ability to reduce cardiovascular risk could be due to an improvement in the elements that define metabolic syndrome, the improvement of vascular endothelial dysfunction, insulin resistance and the inhibition of platelet activation and aggregation.[8 9] However, although current evidence suggests that polyphenols produce an improvement in cardiovascular health, this is insufficient to determine the minimum amount of intake necessary to achieve health benefits.[10]

Cocoa polyphenols and blood pressure

The effect of consuming polyphenols present in chocolate on the blood pressure statistics of healthy individuals is unclear. Cocoa consumption has been associated with an improvement in endothelial function and a decrease in blood pressure in both healthy subjects and those with risk factors and cardiovascular diseases.[11 12] Some studies have observed a dose-dependent relationship between cocoa intake and clinical blood pressure, with higher consumption equated to lower blood pressure and better vascular function.[13 14] Conversely, other research has not obtained significant changes in these parameters related to the supplementation of cocoa or pure polyphenols, such as epicatechin or quercetin.[15 16]

Endothelial dysfunction in postmenopausal women causes changes that favour the development of cardiovascular risk factors and atherosclerosis, which lead to the appearance and maintenance of hypertension.[17 18] A decrease in blood pressure has been observed in this group after daily consumption of cocoa with a flavonol content of 40.12 mg. Below this level, however, no changes have been observed.[19]

Cocoa polyphenols and vascular function

Among healthy individuals, as well as postmenopausal women, the consumption of polyphenols present in cocoa has been associated with a dose-dependent improvement of vascular function, in particular of arterial stiffness measured by pulse wave speed.[13 14 19] One of these studies also suggests that the reduction in arterial stiffness observed in postmenopausal women after consumption of cocoa is independent of the frequency of the intake.[19] However, this relationship is not evident in people with mild hypertension when cardio-ankle vascular index (CAVI) is used as a measure of arterial stiffness.[20]

There is also evidence of the influence of these polyphenols in reducing the augmentation index (AIx). The study by West *et al*,[21] involving subjects with excess weight and moderate obesity, concludes that treatment with dark chocolate decreases AIx in women, although it seems that this association might have a greater effect on the elasticity of the large arteries, especially in subjects with obesity and diabetes mellitus type 2.[22]

Cocoa polyphenols and cognitive performance

There is evidence to suggest that chocolate rich in polyphenols is beneficial for cognitive performance and state since it improves mental processing speed and attenuates the increase of mental fatigue among healthy young adults.[23 24] An improvement in cognitive performance among older age groups after eating chocolate has also been observed,[25] especially in subjects with higher risk of cardiovascular disease.[26] Several studies also show that polyphenol-rich chocolate causes an improvement in executive function, categorical fluency[27] and working memory,[28 29] and a slowing of mental fatigue.[30] Also, a higher frequency of chocolate consumption has been associated with improved cognitive function.[29] Furthermore, a positive influence of cocoa polyphenols on physiological processes has been reported, with a neuroprotective effect[31] and improved cognitive performance.[32] In this regard, it has been suggested that the brain-derived neurotrophic factor plays a role in the cognitive enhancement induced by the flavonoides.[33] Favourable effects on cerebrovascular function have also been observed in postmenopausal women after consumption of chocolate with a high concentration of cocoa.[34]

Cocoa polyphenols and quality of life

The quality of life linked to health is represented by the individual's perception of well-being in various aspects of life, including physical and mental aspects. The effect of chocolate and polyphenols on the quality of life has scarcely been studied, with little available evidence and even less of a conclusive nature. In a study conducted among healthy people, where regular consumption of chocolate was recorded over 1 year, no evidence was found of a clear association between chocolate intake and the physical or mental components of quality of life.[35] Nevertheless, it has been observed that the consumption of dark chocolate might be beneficial for the quality of life of women with fibromyalgia.[36]

Cocoa polyphenols and body composition

The menopause period leads to various changes in the body composition of women.[37] Regarding the connection between cocoa polyphenols and body composition, results diverge. Some clinical trials involving healthy people and overweight or obese patients have not reported significant differences that link chocolate consumption to anthropometric measures.[16 20 21 38] Other studies indicate that chocolate consumption might have positive effects on body composition in adolescents,[39]patients with diabetes[40] or women with obesity.[41] Two recent systematic reviews also indicate that eating chocolate is associated with reduced body mass index (BMI) and waist circumference,[42 43] and one of them also concludes that the amount and the length of time during which it is eaten play a key role in these beneficial effects.[43] Conversely, other studies such as that carried out with the cohort of the Atherosclerosis Risk in Communities study have observed a dose-dependent increase in weight after habitual chocolate consumption.[44]

In sum, the polyphenols present in chocolate seem to have a positive effect on blood pressure, vascular function, cognitive performance and quality of life, especially in populations with increased cardiovascular risk, such as postmenopausal women.[45] However, the conflicting results obtained in different studies suggest that the real contribution of these compounds to health and the underlying mechanisms remain unclear. Moreover, most of these studies have used preparations with high concentrations of polyphenols that are usually not present in the normal diet.

This study aims to evaluate the effect of adding a daily amount of 10 g of chocolate high in cocoa content (99%)

and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women.

MEthods And AnAlysIs

design and setting

This controlled and randomised clinical trial involves two parallel groups. The study will be carried out in the Research Unit of the La Alamedilla Health Centre in Salamanca (Spain), which is part of the Biomedical Research Institute of Salamanca and the Primary Care Prevention and Health Promotion Research Network. The recruitment schedule is set to start on 1 June 2018, and the study will run until 31 May 2019. There will be a baseline assessment and two follow-ups, at 3 and 6 months.

study population

Those subjects who meet the selection criteria and sign the informed consent after receiving information about the objectives and implementation of the study will take part.

Inclusion criteria: women between 50 and 64 years in postmenopause, defined by and checked against amenorrhoea during at least 12 consecutive months.

Exclusion criteria: a personal history of cardiovascular disease; personal history of diabetes mellitus, arterial hypertension or dyslipidaemia under pharmacological treatment; hypocaloric diets; clinically demonstrable neurological and/or neuropsychological disease; treatment with hormone replacement therapy; intolerance and/or allergy to cocoa or any of the components of the supplement.

Participants will be selected using a consecutive sample of women who meet the selection criteria in the general practitioner surgeries of four urban primary care centres in Salamanca, from 1 June, 2018.

Patient and public involvement

Patients and the public were not involved in the design of this study or outcome measures. We hope that the results of the study will be disseminated through press releases and information-sharing meetings with the study participants.

sample size

The size of the sample has been estimated based on the potential modification of the main variable, systolic blood pressure (SBP). Given alpha and beta risks of 0.05 and 0.20 respectively in bilateral contrast and an SD of 5.8 mm Hg, 140 participants (70 per group) will be necessary to detect a minimum difference of 2.9 mm Hg in the SBP between the two groups. A predicted drop-out rate of 10% during follow-up has been taken into account. This estimate has considered the results obtained in a similar study in which a decrease in SBP of 6.5 was observed ±5.8 mm Hg.[14]

randomisation

Participants will be assigned to the intervention group (IG) or control group (CG) at random. The allocation sequence will be generated by an independent researcher using the Epidat V.4.2 program[46] before the inclusion of the first participant, using masked block randomisation. Patients will receive their randomisation number based on the order of their baseline evaluation visit and will remain hidden until the participants are assigned to each group. To ensure that the blinding is maintained, patients will be given clear instructions not to disclose which treatment they have been randomised to while being interviewed by the blind assessors. Information on treatment allocation will be stored in a secure locker in case of emergency unblinding.

Intervention

No type of intervention will be carried out with the CG participants.

IG participants will be given chocolate with 99% cocoa content and asked to eat 10 g daily for 6 months. According to the European Food Safety Authority, 10 g of high-flavanol dark chocolate consumed in the context of a balanced diet could help maintain endothelium-dependent vasodilation.[47] Participants will also be given instructions on eating and keeping the product, with the recommendations, for example, that the chocolate can be consumed in small pieces leaving them unmated in the mouth, without chewing them. Also, a series of recommendations will be given addressing the organoleptic characteristics of the product, as well as the recommendations of trying to consume the product at the same time or refrain from ingesting it dissolved in milk. Also, participants will be given a calendar on which to record the time it was eaten each day. This calendar will be returned to the researchers at each replenishment visit.

This amount of chocolate provides the following daily nutritional contribution: 59 kcal, 0.8 g of carbohydrates, 1.5 g of protein, 5.1 g of fat, of which 3.1 g are saturated fats. The proportion of polyphenols per 10 g is 65.4 mg. The polyphenolic profile of this compound can be seen in table 1. On each visit, IG participants will receive the amount of chocolate they need until the

Table 1 Polyphenols composition of 99% cocoa chocolate

Compounds	Quantity
Protocatechuic acid (mg/g)	0.058±0.008
Procyanidin dimer (B3) (mg/g)	0.176±0.013
Catechin (mg/g)	1.035±0.105
Procyanidin dimer (B2) (mg/g)	1.440±0.055
Epicatechin (mg/g)	2.610±0.075
Procyanidin trimer (C1) (mg/g)	0.853±0.024
Procyanidin A hexoside (mg/g)	0.354±0.007
Quercetin glucoside (mg/g)	0.002±0.000
Quercetin arabinoside (mg/g)	0.003±0.001

next replenishment visit. In addition to the baseline visit, there will be five replenishment visits in months 1, 2, 3 (coinciding with the evaluation visit), 4 and 5. The sole purpose of the replenishment visits will be to supply the amount of chocolate needed until the next visit, without any other intervention being carried out.

Participants in both groups will be instructed to continue with the dietary pattern they usually follow, without changing their eating habits during the study period.

Procedures

For each participant a baseline visit and two follow-up visits at 3 and 6 months after the initial one are scheduled (figure 1). The IG will also make five replenishment visits, in months 1, 2, 3 (coinciding with the first follow-up visit), 4 and 5. In the replenishment visits, participants will be given the amount of chocolate needed until the next visit and will hand in the calendar with the record of the chocolate eaten.

Primary and secondary endpoints

The primary variable will be the decrease in clinical blood pressure, measured with a digital sphygmomanometer. Secondary variables will include vascular function, quality of life, cognitive performance and body composition.

All variables will be measured at 3 and 6 months after randomisation, except for cognitive performance and quality of life, which will be assessed only after 6 months.

blood pressure

Clinical systolic and diastolic blood pressure will be measured with a validated Omron M10-IT sphygmomanometer (Omron Healthcare, Kyoto, Japan). Three measurements will be taken in the dominant arm of the subject in a sitting position after at least 5 min of rest with an appropriately sized cuff, following the recommendations of the European Society of Hypertension.[48] The average of the last two measurements will be recorded.

Vascular function

The Vasera VS-2000 device (Fukuda Denshi) will be used to measure the CAVI and the brachial-ankle pulse wave velocity (ba-PWV) at rest. CAVI is a good indicator of arterial stiffness, providing an accurate estimate of the degree of atherosclerosis without depending on blood pressure.[49] CAVI ≥9 and ba-PWV ≥18.3 will be considered pathological.[50] Pathological CAVI is representative of subclinical atherosclerosis.[51]

Cognitive performance

The instructions are presented visually at the start of the baseline measurement to ensure limiting a learning effect over the subsequent testing periods. Attention and executive functions: Trail Making Test A will be used to measure attention and Trail Making Test B for processing speed and executive functions.[52]

Immediate verbal memory will be assessed with the Rey Auditory Verbal Learning Test. The immediate recall of a list of 15 words is measured in three attempts, followed by delayed verbal memory through the free recall of the words learnt in the first part of the test after 10 min.[53]

Working memory will be assessed with the WAIS Digit Span Backward test.[54]

Phonological fluency will be explored by naming as many words as possible starting with different letters of the FAS Questionnaire in the space of 1 min.[55]

Categorical fluency measures verbal semantic fluency and will be assessed by naming as many animals as possible in 1 min.[56]

Quality of life

The quality of life linked to health will be assessed through the EuroQol 5-D questionnaire. We will use the adapted Spanish version of this questionnaire, which has been validated in the Spanish population.[57] This questionnaire consists of three elements: the assessment by the individuals of their state of health in level of severity by dimension (mobility, personal care, daily activities, pain/discomfort and anxiety/depression), the assessment of their state of health on an analogue visual scale and finally an index of social values obtained for each state of health generated by the instrument.

The quality of life will also be studied using the Cervantes Scale.[58] This questionnaire is specifically designed for menopause and postmenopause and has been validated for Spanish women. Its 31 structured items cover the four dimensions of menopause: menopause and health, sexuality, psychic domain and relationships.

body composition

Body composition will be measured with the Inbody 230 Monitor.[59] This analyser provides data on fat mass and body fat percentage as principal outcomes and also skeletal muscle mass, total body water, fat-free mass, waist-hip ratio, basal metabolism and a segmental analysis.

Body weight will be measured twice with an electronic scale (Scale 7830, Soehnle Professional, Backnang, Germany) after proper calibration (accuracy ±0.1 kg). Height will be measured by recording the average of two readings rounded to the nearest centimetre using a stadiometer (Seca 222, Medical Scale and Measurement System, Birmingham, UK). Both measurements will be made with the subject barefoot and wearing light clothing. BMI will be calculated by dividing weight (kg) by height squared (m^2). Waist circumference will be assessed in accordance with the recommendations of the Spanish Society for the Study of Obesity[60] and will be measured in duplicate before and after inhalation, using a flexible tape parallel to the floor, at the level of the midpoint between the lowest rib and the iliac crest, with the subject standing up and without clothes.

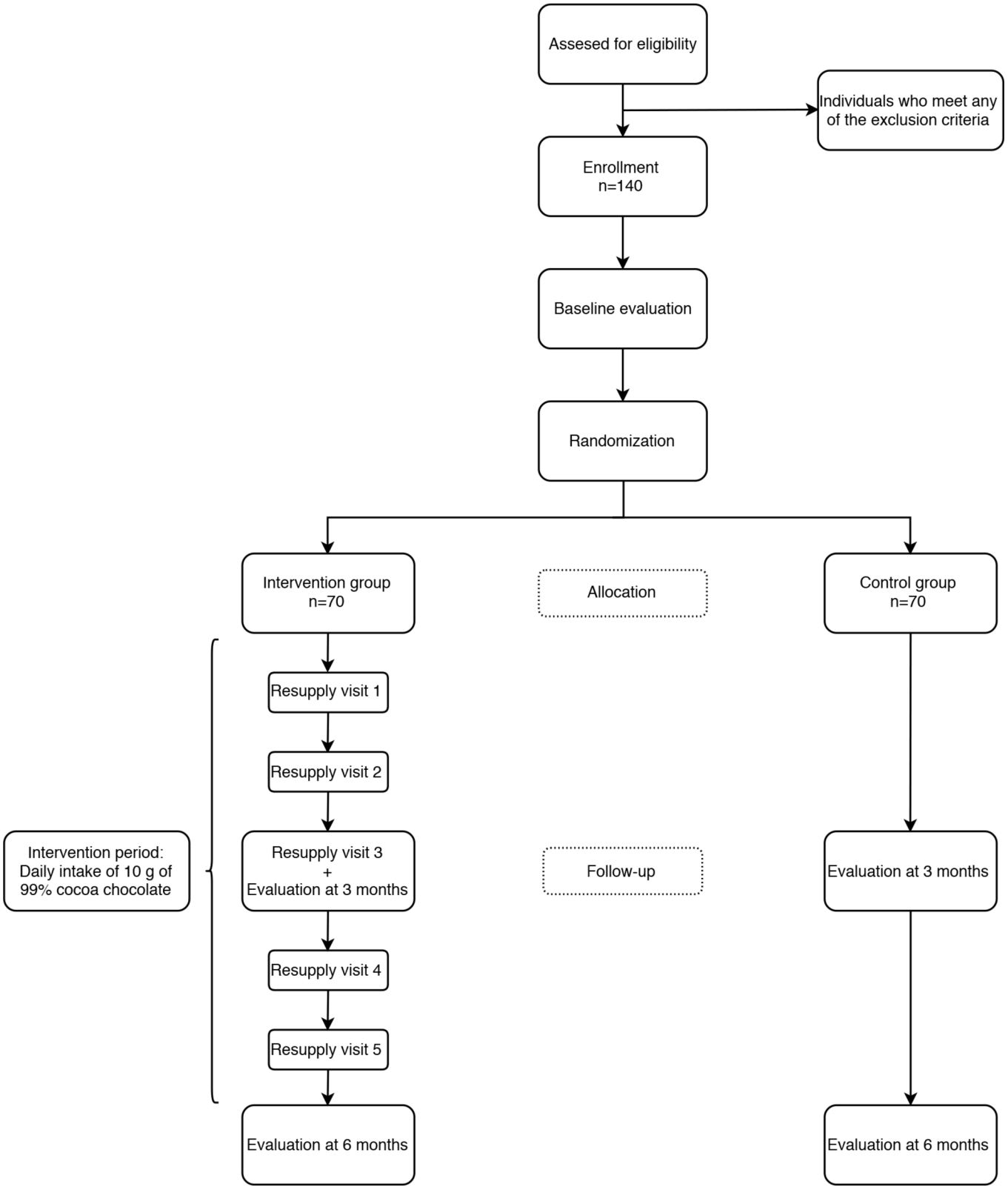

Figure 1 Study flow chart.

other variables

Clinical and sociodemographic variables

At the baseline visit, information on clinical and sociodemographic variables will also be collected via questions about age, marital status, educational level and occupation. The family history of cardiovascular disease and personal history of anxiety and depression, gestational diabetes, hypertension, dyslipidaemia and the prescribed pharmacological treatment (antiaggregants, anticoagulants, thyroid hormone treatment, anxiolytics)

will also be recorded, as well as the taking of non-steroidal anti-inflammatory drugs in the last two weeks.

In subsequent visits, personal histories of cardiovascular disease, diabetes mellitus, arterial hypertension or dyslipidaemia in treatment, as well as the prescribed pharmacological treatment (hypolipidaemic, antihypertensive, antidiabetic) will also be noted.

Evaluation of chocolate consumption and habitual diet

Chocolate consumption will be assessed at each evaluation visit by a series of questions about the amount, type and frequency of consumption in the period between visits.

Nutritional habits will be assessed by a 24 hours log on three non-consecutive days prior to each visit.

Evaluation of other lifestyles

The use of tobacco will be assessed with a questionnaire on the personal history and pattern of smoking.

Alcohol use will be recorded with a questionnaire covering the previous seven days, which will include specific beverages and the amount by volume drunk of each.

Physical activity will be measured using the International Physical Activity Questionnaire in its short version and validated in Spanish.[61] This questionnaire measures activity over the previous seven days, classifying the subjects according to three activity levels (low, moderate and high) with respect to three types of activities: walking, moderate-intensity activities and vigorous-intensity activities. The amount of physical exercise will be estimated in METs-minute/week.

Evaluation of laboratory variables

At baseline and follow-up visits at 6 months, we will measure plasma fasting glucose values (mg/dL), glycated haemoglobin (%), total cholesterol (mg/dL), total triglycerides (mg/dL), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), creatinine (mg/L) and insulinaemia (mg/dL). Creatinine in urine (mg/dL) and microalbuminuria (mg/dL) will also be measured. Insulin resistance will be determined using the Homeostasis Model Assessment Insulin Resistance index estimated using the following equation: Fasting glucose (mmol/L)×insulin (mU/mL)/22.5.

The evaluation visits will be made in the morning between 08:00 and 10:00. Each participant will be informed prior to the visit to fast for at least 12 hours, having avoided during the 24 hours prior to visiting the consumption of polyphenol-rich foods, including cocoa, chocolate, apples and red wine as well as alcoholic drinks or the performance of the programmed physical activity. All evaluation visits, including blood pressure measurements and evaluations of vascular function, will be carried out in a room with standardised lighting and temperature, recommending that patients attend the appointment with a prior rest of at least 8–10 hours.

Data collection procedure, data management and monitoring

Data collection of the baseline and follow-up evaluation visits at 3 and 6 months will be carried out by a nurse specifically trained to do so. The intervention visit after the baseline evaluation will be carried out by another nurse, different from the one who performs the data collection. Each participant will have a unique identification code within the study. All measurements will be compiled in a data collection notebook and kept in a secure place that will remain closed within the health centre. A database will be created in SPSS to which only the members of the research team and the people related to the statistical analyses will have access. The principal investigator or a person designated for this purpose will perform a weekly process of monitoring the study, taking into account the inclusion of patients, cleaning and debugging of databases, and adaptation of the procedures to the protocol.

blinding strategy

Due to the nature of the intervention itself, the participants and the person responsible for delivering the chocolate to IG participants cannot be blinded. However, the person responsible for carrying out the study measurements at each visit and for the statistical analysis will be blind to the intervention.

statistical analysis

General analysis

Results for the quantitative variables will be expressed by mean±SD or by frequency distribution in the case of qualitative variables. The normality of the variables will be assessed using the Kolmogorov-Smirnov test. In cases where a normal distribution cannot be assumed, the corresponding non-parametric tests will be applied. The association between independent qualitative variables will be analysed using the χ^2 test or Fisher's exact test. The means between the two groups will be compared using the Student's t-test or the Mann-Whitney U test, and the Pearson or Spearman correlation coefficients will be calculated to analyse the relationship between quantitative variables.

The analysis of the results for the main variable and the secondary variables will be carried out by intention to treat. Also, a secondary analysis will be made, taking into account chocolate intake adherence (<50% days and >50% days) and other relevant subgroups in relation to their physical activity or previous chocolate consumption.

All analyses will be performed using SPSS V.23.0 (IBM) and an alpha risk of 0.05 will be set as the limit of statistical significance.

Analysis of the intervention's effect on primary and secondary outcomes

To analyse the changes at 3 and 6 months from baseline in the primary outcome (blood pressure) and in the secondary outcomes within the same group, the Student's t-test for paired data or the Wilcoxon test will be used.

The McNemar test will be applied with quantitative or dichotomous variables.

Effects of the intervention will be analysed in a comparison of the changes in blood pressure and the secondary variables between the IG and the CG using analysis of covariance and adjusting for possible confounders, for example, smoking status. Effects of the intervention during follow-up will be studied with an analysis of the variance of repeated measures.

Analysis by subgroups

The effect of the intervention could be influenced by age, sociocultural level and adherence to the study's chocolate intake. The same analyses described above will be performed for each of the subgroups above.

Secondary analyses

A multivariate multiple regression analysis will be performed to identify the variables with the greatest influence on blood pressure changes and the secondary variables analysed.

Methodological limitations

Due to the nature of the intervention, the participating subjects cannot be blinded. However, the researcher who analyses the data and the person who makes the measurements during follow-up visits will be blinded with respect to the group to which the participants belong. The smoking status in the 12 months prior to the time of inclusion could influence the outcome measures related to vascular function and blood pressure; therefore, although these participants will not be excluded, this aspect will be controlled in the statistical analysis. Assessment of the quality of life and lifestyles will be carried out through self-reported data; however, previously validated instruments will be used to obtain these. To make compliance with the intervention in the IG easier, IG participants will be provided with instructions on eating the chocolate and a calendar to record each intake.

EthICs And dIssEMInAtIon

Ethical considerations

The study was approved by the Clinical Research Ethics Committee of the Salamanca Health Area ('CREC of Health Area of Salamanca') in February 2018. A SPIRIT checklist is available for this protocol. The clinical trial has been registered at ClinicalTrials.gov with the identifier NCT03492983.

Participants must provide informed consent in accordance with the Declaration of Helsinki. Subjects will be informed of the objectives of the project and the risks and benefits of the explorations to be carried out, including sample collection. None of the tests will pose risks that could endanger the lives of participants. Confidentiality of participant data will be guaranteed at all times in accordance with the provisions of the Organic Law on the Protection of Personal Data (15/1999 of 13 December LOPD), and under the conditions established by Law 14/2007 of biomedical research.

dissemination plan

The research group plans to achieve rapid and widespread dissemination of results to ensure maximum visibility of this study. To this end, the results of the study will be published in open-access scientific journals with peer review. At least one publication of the main results and others with the secondary results are planned. This will be complemented by the presentation of the results of the study at relevant scientific conferences and seminars of national and international scope. Also, a doctoral thesis based on this project will be prepared. Appropriate dissemination will likewise be carried out through social networks and other media. Moreover, given the involvement of a commercial product, the transfer to clinical practice is expected to be rapid if the results are as expected.

dIsCussIon

In recent years, there has been an increase in attention to polyphenols and their beneficial effects on health, with numerous studies being carried out to assess this.[19 21] Similarly, the therapeutic use of these compounds has been suggested for certain diseases or population groups.[36 62] The menopause increases the risk of developing cardiovascular disease compared with the previous period.[45] However, we have not found any study that assesses the effect of adding commercially available chocolate high in cocoa content to the usual diet in this population. Similarly, no studies have been found that evaluate the effects on cognitive performance, quality of life and body composition of adding commercial chocolate with high cocoa content to the usual diet in postmenopausal women.

This work will provide novel data helpful for the development of strategies in the nutritional education of particularly vulnerable populations, given their high risk of developing cardiovascular disease, including non-pharmacological therapies and strategies that employ lifestyle modification. This intervention might also have implications for the preparation of recommendations in clinical practice guidelines and quality improvement programmes aimed at the care of postmenopausal women.

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Acknowledgements The authors are grateful to all the professionals involved in the ECCAMP study: José I Recio-Rodríguez, José A Maderuelo-Fernández, Luis García-Ortiz, Manuel A Gómez-Marcos, Irene A García-Yu, Rosario Alonso-Domínguez, Sara Mora-Simón, Natalia Sánchez-Aguadero, Jesús González-Sánchez, Cristina Agudo-Conde, Cristina Lugones-Sánchez, Benigna Sánchez-Salgado, Carmen Castaño-Sánchez, Emiliano Rodríguez-Sánchez, Susana González-Manzano, Olaya Tamayo-Morales, and Susana González-Sánchez.

Contributors JI-RR, JAM-F, LG-O and IAG-Y contributed to the conception and design of the study. IAG-Y, JIR-R and JAM-F prepared the manuscript of the study protocol. JIR-R, JAM-F, LG-O, RA-D, SM-S, JG-S, SM-G, ER-S, MAG-M and IAG-Y contributed to the development of the study protocol. JI-RR, JAM-F, LG-O, RA-D, SMS, JGS, SMG, ERS, MGM and IAG-Y provided assistance with statistical methodology and knowledge. JI-RR, JAM-F, LG-O, RA-D, SM-S, JG-S, SM-G, ER-S, MG-M and IAG-Y provided a critical review of the manuscript. All authors have read and accepted the final version of the protocol.

Funding This study was supported in part by grants funded by la Gerencia Regional de Castilla y León (GRS 1583/B/17).

disclaimer Lindt & Sprüngli will provide the necessary chocolate for the implementation of the study. This company will not play any role in the design of the study, data analysis, reporting of results or the decision to present the manuscript for publication.

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval Clinical Research Ethics Committee of the Salamanca Health Area ("CREC of Health Area of Salamanca").

Provenance and peer review Not commissioned; externally peer reviewed.

rEFErEnCEs

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Efectos del chocolate rico en cacao sobre la presión arterial, los factores de riesgo cardiovascular y la rigidez arterial en mujeres posmenopáusicas: ensayo clínico aleatorizado

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Nutrients. 2020 Jun 12;12(6):1758.

El objetivo de este estudio fue evaluar los efectos del consumo de 10 g de chocolate rico en cacao sobre la presión arterial, otros factores de riesgo cardiovascular y la estructura y función vascular en mujeres posmenopáusicas. Un total de 140 mujeres posmenopáusicas participaron en este ensayo clínico paralelo aleatorizado y controlado. Durante 6 meses, el grupo de intervención (GI; *n* = 73) consumió 10 g de chocolate (99% cacao) diarios añadidos a su dieta habitual, mientras que el grupo control (GC; *n* = 67) no recibió ninguna intervención. La presión arterial, la presión de pulso (PP), el índice vascular cardio-tobillo (CAVI), el índice tobillo-brazo (ITB), la velocidad de la onda del pulso brazo-tobillo (VOP), el índice de aumento y las variables de laboratorio se midieron basalmente y a los 6 meses. Los análisis de la covarianza (ANCOVA) ajustados por los valores basales no mostraron diferencias significativas en la presión arterial sistólica (−1,45 mmHg; intervalo de confianza (IC) 95% −4,79 a 1,88; *p* = 0,391) o la VOP (0,18 m/s; IC 95% −0,14 a 0,50; *p* = 0,263) entre grupos. Se observó un descenso en la PP en el GI en comparación con el GC (−2,05 mmHg; IC 95% −4,08 a −0,02; *p* = 0,048). El resto de parámetros de estructura y función vascular, así como las demás variables medidas no mostraron cambios. El consumo de 10 g de chocolate rico en cacao parece que produce una discreta mejora en la salud cardiovascular, aunque tampoco provoca efectos adversos en los parámetros evaluados en mujeres posmenopáusicas a largo plazo.

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Effects of Cocoa-Rich Chocolate on Blood Pressure, Cardiovascular Risk Factors, and Arterial Stiffness in Postmenopausal Women: A Randomized Clinical Trial

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Received: 20 May 2020; Accepted: 10 June 2020; Published: 12 June 2020

Abstract: This study aimed to evaluate the effects of the intake of 10 g of cocoa-rich chocolate on blood pressure, other cardiovascular risk factors, and vascular structure and function in postmenopausal women. A total of 140 postmenopausal women participated in this randomized and controlled parallel clinical trial. For six months, the intervention group (IG; n = 73) consumed daily 10 g of chocolate (99% cocoa) added to their usual food intake, whereas the control group (CG; n = 67) did not receive any intervention. Blood pressure, pulse pressure (PP), cardio-ankle vascular index (CAVI), ankle-brachial index (ABI), brachial-ankle pulse wave velocity (baPWV), augmentation index, and laboratory variables were measured at baseline and six months. ANCOVA analyses adjusted for baseline values revealed no significant differences for systolic blood pressure (−1.45 mm Hg; 95% confidence interval (CI): −4.79, 1.88; p = 0.391) or baPWV (0.18 m/s; 95% CI: −0.14, 0.50; p = 0.263) between groups. A decrease in PP was observed in the IG compared to the CG (−2.05 mm Hg; 95% CI: −4.08, −0.02; p = 0.048). The rest of the vascular structure and function parameters and other measured variables remained unchanged. The daily intake of 10 g of cocoa-rich chocolate seems to provide little improvement to cardiovascular health, but neither does it cause any adverse effects on the parameters evaluated in postmenopausal women in the long term.

Keywords: arterial pressure; vascular stiffness; risk factors; chocolate; postmenopause

1. Introduction

The risk of cardiovascular disease is lower in women than in men [1]. However, with the beginning of menopause, there is an increased risk associated with the estrogen deficiency that takes place in that period [2].

Interventions aimed at the prevention of cardiovascular disease are especially important in populations with increased risk. In the last few years, non-pharmacological treatments and other

prevention strategies are being studied, such as dietary counseling, with the aim of improving the health of people with high risk of suffering from cardiovascular disease [3].

In this regard, flavonoids, which are a group of polyphenols, have been widely studied and their consumption has been related to beneficial effects on health. These benefits include reducing the risk of cerebrovascular disease, lung cancer, type 2 diabetes, or asthma incidence [4] and the risk of colon and rectal cancer [5], as well as preventing ischemic heart disease [6], among others. The beneficial effects of flavonoids have been attributed to their antioxidant properties [7]. Although recent studies provide further explanation of flavonoids' mechanism of action related to reactive oxygen species (ROS) scavenging, immune modulation, cell cycle regulation, epigenetic modification, and genetic regulation of metabolism [8,9].

Different reviews suggest that a polyphenol-rich diet can have protective effects on cardiovascular health [10,11]. Lockyer et al. reported that phenolic-rich olive leaf extract intake decreased blood pressure as well as lipid profile in pre-hypertensive males [12]. In addition, Fuchs et al. concluded that single doses of tea theaflavins and catechins had moderate effects on peripheral microcirculation in healthy subjects [13], while other authors found that a supplementation with green tea catechin extract reduced total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol levels in postmenopausal women [14]. Other studies have evaluated the effects of intaking chocolate and cocoa with a high concentration of polyphenols. The results of a systematic review showed that these products improved flow-mediated dilatation and reduced insulin resistance, having acute and chronic beneficial effects on cardiovascular health [15].

The available evidence suggests that cocoa-rich chocolate can have beneficial effects on arterial stiffness, vascular function, and cardiovascular risk factors in postmenopausal women. In a trial conducted in this population, which evaluated the daily and alternate-day intake of 17 g of flavonoid-rich cocoa, an improvement in arterial stiffness was observed through a decrease in pulse wave velocity (PWV) [16]. Moreover, arterial pressure and pulse pressure (PP) decreased after intervention, as well as some cardiovascular risk factors, such as glucose and triglycerides, with no other changes in the lipid profile. The results of another study showed a decrease in cerebral artery blood flow velocity in postmenopausal women after the intake of dark chocolate (80% cocoa) and milk chocolate (35% cocoa) [17].

However, there are very few clinical trials in the literature focused on evaluating these effects using a commercial compound that reproduces normal clinical conditions. Previous studies have a small sample size and, in addition, a short follow-up time [16,18,19].

The aim of this study was to evaluate the effects of the intake of 10 g of cocoa-rich chocolate (99%) on blood pressure, other cardiovascular risk factors, and vascular structure and function in postmenopausal women.

2. Materials and Methods

2.1. Design and Setting

The design corresponds to a randomized and controlled clinical trial with two separate groups. The study was carried out in the Primary Care Research Service of Salamanca (APISAL) (Salamanca, Spain), which is part of the Biomedical Research Institute of Salamanca (IBSAL) and the Spanish Research Network for Preventive Activities and Health Promotion in Primary Care (redIAPP). The study was conducted between June 2018 and August 2019. This clinical trial was registered at clinicaltrials.gov provided by the US National Library of Medicine as NCT03492983. The results reported in this manuscript are primary outcomes of the study.

2.2. Study Participants and Recruitment

A consecutive sampling was carried out in the doctor's offices of four urban primary care centers in Salamanca (Spain). Women who met the selection criteria and signed the informed consent for

participation were recruited. A total of 140 women aged 50–64 years and in postmenopausal period, defined by amenorrhea for at least 12 consecutive months, were included in the trial. Thirty-two women were not included due to one or more of the exclusion criteria. Exclusion criteria were personal history of cardiovascular disease, personal background of diabetes mellitus, arterial hypertension, or dyslipidemia in pharmacological treatment, hypocaloric diets, clinically demonstrable neurological and/or neuropsychological disease, and treatment with hormone replacement therapy. Women with a usual consumption of more than 210 g of cocoa per week (g/wk) and intolerance and/or allergy to cocoa or any of the components of the supplement were also excluded (Figure 1).

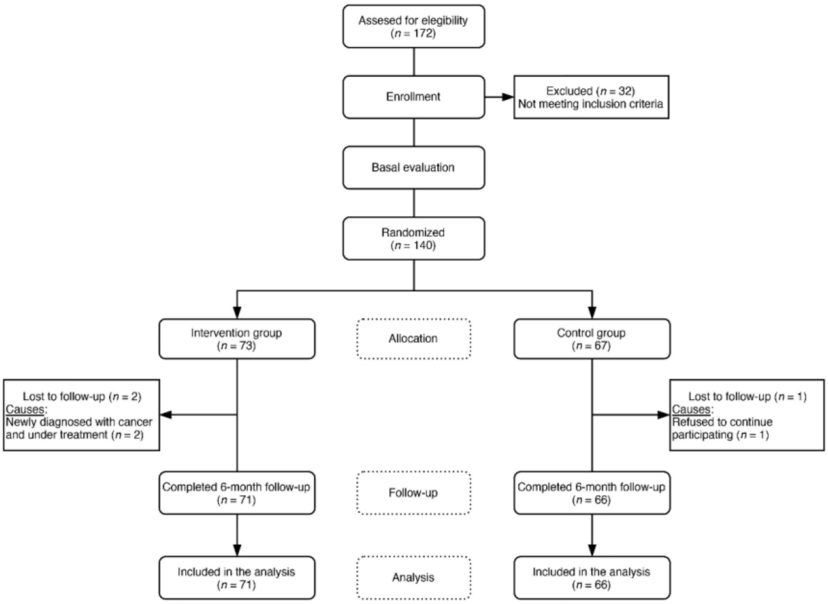

Figure 1. Flow chart of postmenopausal women through the study.

2.3. Sample Size

The size of the sample was estimated based on the potential modification of the main variable, i.e., systolic blood pressure (SBP). Considering given alpha and beta risks of 0.05 and 0.20, respectively, in bilateral contrast and a standard deviation (SD) of 5.8 mm Hg, 140 participants (70 per group) were necessary to detect a minimum difference of 2.9 mm Hg in SBP between the two groups. A predicted drop-out rate of 10% during follow-up was taken into account. This estimate considered the results obtained in a similar study in which a decrease in SBP of 6.5 ± 5.8 mm Hg was observed [19].

2.4. Procedures and Randomization

All participants made a baseline visit and a visit at 6 months after the first visit, in which the study variables were measured (Figure 1). The intervention group (IG) made 5 additional resupply visits at 1, 2, 3, 4, and 5 months from the first visit, during which no other procedure was carried out apart from providing them with the necessary chocolate until the next visit and the collection of a calendar with a record of the chocolate intakes performed.

The participants were randomly distributed into two groups, namely the intervention group (n = 73) and the control group (n = 67). The assignation sequence was generated by an independent researcher using Epidat V.4.2 software [20]. The participants received their randomization number based on the order of their baseline evaluation visit and remained hidden until they were assigned to each group. To ensure that the blinding was maintained, the patients were given clear instructions not to disclose which treatment they had been randomized to while being interviewed by the blind assessors. Information on treatment allocation was stored in a secure locker in case of emergency unblinding.

Due to the characteristics of the intervention, it was not possible to blind all participants. To minimize cross-contamination between groups, the researcher who conducted the evaluations was different from the researcher who carried out the resupply of chocolate for the IG.

2.5. Intervention

The control group (CG) participants did not receive any type of intervention. The IG participants were given chocolate with a cocoa concentration of 99% and the instructions for daily consumption of 10 g of this compound added to their usual food intake. After the basal evaluation, they were given consumption and conservation instructions, recommending the daily intake of chocolate to be at the same time each day. In addition, they were given a calendar to record the time and the intake of each day, which was given back to the researchers in each resupply visit.

The daily nutritional contribution of 10 g of this chocolate, as stated by the manufacturer, is 59 kcal, 0.8 g of carbohydrates, 1.5 g of proteins, and 5.1 g of fat, of which 3.1 g are saturated fat. The determination and quantification of individual phenolic compounds (mg/g) in the cocoa were carried out by high-performance liquid chromatography coupled to a photodiode array detector and mass spectrometer (HPLC–DAD–ESI/MS). The composition in polyphenols was quantified from the areas of their chromatographic peaks by comparison with calibration curves prepared with the external standards of each compound. The compounds procyanidin trimer and procyanidin A were quantified by the procyanidin dimer B2 calibration curve, due to the lack of standards neither commercial nor isolated in the laboratory. The contribution of polyphenols in 10 g of this product is 65.4 mg. The polyphenolic profile of this compound is shown in Table 1. The participants of both groups were requested to continue with the dietary pattern they usually followed without modifying their eating habits during the study period.

Table 1. Polyphenol composition of 10 g of 99% cocoa chocolate used in the intervention.

Compounds	Quantity, mg/10 g
Protocatechuic acid	0.58
Procyanidin dimer (B3)	1.76
Catechin	10.4
Procyanidin dimer (B2)	14.4
Epicatechin	26.1
Procyanidin trimer (C1)	8.53
Procyanidin A hexoside	3.54
Quercetin glucoside	0.02
Quercetin arabinoside	0.03

2.6. Main Outcomes

2.6.1. Blood Pressure Measurements

SBP, diastolic blood pressure (DBP), and heart rate (HR) were measured using a validated Omron M10-IT sphygmomanometer (Omron Healthcare, Kyoto, Japan). Three measurements were made following the recommendations of the European Society of Hypertension [21]. Then, PP was calculated as the difference between SBP and DBP, whereas the mean blood pressure was estimated using the following formula: DBP + 1/3 (SBP-DBP).

2.6.2. Evaluation of Vascular Structure and Function

The cardio-ankle vascular index (CAVI), ankle-brachial index (ABI), and brachial-ankle pulse wave velocity (baPWV) were evaluated using a Vasera VS-2000 (Fukuda Denshi, Tokyo, Japan) device, following the manufacturer's instructions.

The CAVI and the baPWV values were calculated using the equation as published by Shirai et al. [22]. The ABI was calculated by dividing the higher of the two ankle systolic pressures by the highest measurement of the two systolic pressures in the arm [23].

Vascular function was evaluated through central augmentation index (cAIx), central augmentation index corrected for a heart rate of 75 bpm (cAIx75), and peripheral augmentation index (pAIx). These measurements were carried out using a wrist-worn device, developed by Microsoft Research (Redmond, WA, USA), which includes an applanation tonometer placed over the radial artery [24]. The CAIx was normalized to a standard HR of 75 bpm using the equation published by the manufacturers of the Sphygmocor device (AtCor Medical Pty Ltd, West Ryde, Australia) [25]. The PAIx was calculated following the equation proposed by Munir et al. [26].

2.6.3. Laboratory Variable Assessment

The following laboratory variables were measured: plasma fasting glucose values (mg/dL), plasma lipid profile (TC (mg/dL), total triglycerides (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), LDL cholesterol (mg/dL)), as well as creatinine (mg/L) and serum insulin (mg/dL) concentrations. Insulin resistance was determined using the Homeostasis Model Assessment Insulin Resistance index (HOMA-IR), which was estimated using the following equation: Fasting glucose (mmol/L) × insulin (mU/mL)/22.5. These laboratory variables were evaluated after blood extraction in at least 10–12 h of fasting, between 08:00 a.m. and 10:00 a.m. The participants were requested to avoid the consumption of chocolate and other polyphenol-rich products 24 h prior to the blood extraction.

2.6.4. Other Measurements

1. Body Weight and Body Mass Index

Body weight was measured twice with an electronic scale (Scale 7830, Soehnle Professional, Backnang, Germany) after proper calibration (accuracy ± 0.1 kg). Height was measured by recording the average of two readings rounded to the nearest centimeter using a stadiometer (Seca 222, Medical Scale and Measurement System, Birmingham, UK). Both measurements were made with the subject barefoot and wearing light clothing. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Based on baseline BMI cutoff value, subjects were classified into subgroups of overweight (BMI 25–29.9 kg/m^2) and obese condition (BMI ≥ 30 kg/m^2) [27].

2. Clinical and Sociodemographic Variables

At the baseline visit, information on clinical and sociodemographic variables was collected via questions about age, marital status, and educational level. The personal history of gestational diabetes, untreated hypertension and dyslipidemia, and the prescribed pharmacological treatment were recorded, as well as the time before diagnosis of menopause.

3. Adherence to the Intervention

Adherence was calculated as the percentage of days of chocolate intake with respect to the theoretical total days, according to the data recorded in the calendars of each IG participant.

4. Evaluation of Chocolate Consumption and Habitual Diet

Chocolate consumption was assessed at each evaluation visit by a series of questions about the amount, type, and frequency of consumption in the period between visits.

The nutritional composition of the habitual diet, which includes the distribution of macronutrients and energy consumption, was evaluated with a reminder of 24 h recorded for 3 non-consecutive days, prior to each evaluation day. These data were recorded and processed using the EVIDENT app [28].

The methods used for measuring other variables, such as physical activity, alcohol consumption, and smoking habits, are described in the previously published study protocol [29].

2.7. Data Collection Procedure, Data Management, and Monitoring

The collection of data in each evaluation visit was conducted by a nurse who had been previously trained for the task. Each participant was identified through a unique code that identified the data gathered in each of the measurements. With this, a database was created, which could only be accessed by the researchers of the study. The principal investigator carried out a data cleaning and clearing process in the database at the end of the study.

2.8. Ethical Considerations

The study was approved by the Clinical Research Ethics Committee of the Salamanca Health Area (CREC of the Health Area of Salamanca) in February 2018 (ethic approval code: PI11812/2017). The participants provided a signed informed consent, in accordance with the Declaration of Helsinki. The subjects were informed of the objectives of the project as well as the risks and benefits of the explorations to be carried out. The confidentiality of the participants' data was guaranteed at all times, in accordance with the provisions of the Organic Law 3/2018, December 5th, of Personal Data Protection and guarantee of digital rights, and Regulation (EU) 2016/679 of the European Parliament and of the 27 April 2016 Council of Data Protection (RGDP), and under the conditions established by the national law 14/2007 of biomedical research.

2.9. Statistical Analyses

The statistical analysis was carried out following the study protocol [29]. The data were verified for normal distribution and most data were considered normally distributed. The characteristics of the study population are presented as mean and standard deviation for the continuous variables and as distribution of frequencies for the qualitative variables. To evaluate the comparability in the baseline evaluation between the two study groups, the chi-squares test was used for qualitative variables and the Student's *t*-test was used to compare the means between the two groups.

The effects of chocolate consumption on the outcome measures (blood pressure, arterial stiffness, and cardiovascular risk factors), were evaluated using the Student's *t*-test to compare the means between the two groups. To analyze the changes at 6 months from baseline in the outcome measures within the same group, the Student's *t*-test for paired data was used. Analysis of covariance (ANCOVA) was performed to compare the effects, using the pre-values as covariates of the corresponding post-values. Intergroup differences are presented as means and 95% confidence interval (CI).

Subgroup analyses were conducted considering the presence or absence of overweight or obesity as baseline condition to evaluate SBP, DBP, and PP, using the Student's *t*-test. These variables were also analyzed based on the way in which the chocolate was consumed (plain, with coffee/tea, with other foods, etc.) through ANOVA, and a post hoc test was conducted when a significant difference was found.

All analyses were performed using SPSS V.23.0 (IBM Corp., Armonk, NY, USA), establishing an alpha risk of 0.05 as the limit of statistical significance.

3. Results

3.1. Baseline Characteristics of the Study Groups

Of the 140 women included in the study (73 in the IG and 67 in the CG), there were two losses in the follow-up of the IG due to newly diagnosed cancer that required treatment and one loss in the CG who had refused to continue participating. Therefore, 137 women completed the study and were included in the analysis, with 71 in the IG and 66 in the CG (Figure 1).

Table 2 shows the baseline characteristics of the participants. As can be seen, no differences were observed between the two groups. The mean age in the IG and CG was 57.1 ± 3.5 and 57.5 ± 3.8 y, respectively. At the time of the baseline evaluation, the time lapsed from the beginning of menopause was similar in IG and CG. The consumption of chocolate as well as the consumption of chocolate with over 70% cocoa were also similar in both groups.

Table 2. Baseline characteristics of the postmenopausal women included in the study [1].

Variables	Intervention Group (*n* = 73)	Control Group (*n* = 67)
Age, y	57.1 ± 3.5	57.5 3.8
Civil status, *n* (%)		
Married/cohabitant	48 (65.8)	47 (70.1)
Separated/divorced	8 (11.0)	7 (10.4)
Single	15 (20.5)	9 (13.4)
Widow	2 (2.7)	4 (6.0)
Education level, *n* (%)		
Elementary education	16 (21.9)	12 (17.9)
Middle-High school	22 (30.1)	29 (43.3)
Bachelor	17 (23.3)	11 (16.4)
Postgraduate	18 (24.7)	15 (22.4)
Time from menopause onset, y	6.9 ± 4.6	6.9 3.6
Untreated hypertension, *n* (%)	1 (1.4)	0 (0.0)
Untreated dyslipidemia, *n* (%)	8 (11.0)	10 (14.9)
Gestational diabetes, *n* (%)	3 (4.1)	1 (1.5)
Thyroid hormone treatment, *n* (%)	13 (17.8)	10 (14.9)
Current smoker, *n* (%)	12 (16.4)	9 (13.4)
Alcohol consumption, g/week	23.1 ± 29.4	30.6 ± 48.1
Energy, kcal/day	1720 ± 357	1780 ± 402
Carbohydrates, g/day	168 ± 45.1	173 ± 50.2
Proteins, g/day	76.7 ± 16.7	78.2 ± 18.9
Fiber, g/day	24.0 ± 7.5	25.9 ± 9.6
Fats, g/day	77.4 ± 20.7	79.8 ± 20.1
Saturated fats, g/day	25.1 ± 7.6	25.3 ± 7.5
Physical activity, MET-h/week	31.2 ± 36.8	25.7 ± 20.0
Chocolate intake, g/week	68.6 ± 71.1	69.1 ± 74.2
>70% cocoa chocolate intake, g/week	19.9 ± 36.2	15.6 ± 33.2

[1] Values are means ± SDs or frequencies (percent). MET, metabolic equivalent of task.

The mean adherence to the intervention was 97.6% ± 3.34%, with a minimum of 80.6% and a maximum of 100%.

3.2. Cardiovascular Risk Factors and Blood Pressure

The results of the effects on cardiovascular risk factors and blood pressure adjusted for baseline values are shown in Table 3. No significant differences were observed between groups for SBP ($p = 0.391$) or DBP ($p = 0.622$). There was a decrease in PP in the IG in contrast to the increase observed for this parameter in the CG, after adjustment for pre-values ($p = 0.048$). The levels of TC ($p = 0.758$), LDL cholesterol ($p = 0.556$), or HDL cholesterol ($p = 0.795$) did not significantly differ between groups. Likewise, there were no differences in body weight between the study groups or in the levels of glucose, insulin, or HOMA-IR.

Table 3. Cardiovascular risk factors and blood pressure in postmenopausal women participants [1].

	Intervention Group (n = 71)				Control Group (n = 66)							
Characteristic	**Baseline**	**6 Months**	**Change**	p [2]	**Baseline**	**6 Months**	**Change**	p [2]	**Intergroup Difference (IG-CG)** [3]	p [3]	**Adjusted Intergroup Difference (IG-CG)** [4]	p [4]
Body weight, kg	65.1 ± 10.3	64.9 ± 10.3	−0.2 ± 2.2	0.438	64.9 ± 8.6	64.5 ± 8.9	−0.4 ± 2.7	0.272	0.16 (−0.67, 0.98)	0.708	0.16 (−0.66, 0.99)	0.696
BMI, kg/m^2	25.7 ± 3.8	25.6 ± 3.7	−0.1 ± 0.9	0.502	25.6 ± 3.1	25.4 ± 3.2	−0.1 ± 1.0	0.250	0.07 (−0.25, 0.40)	0.652	0.08 (−0.24, 0.40)	0.627
Glucose, mg/dL	86.4 ± 8.8	86.6 ± 10.0	0.2 ± 7.9	0.820	86.2 ± 8.5	87.2 ± 8.7	1.0 ± 7.4	0.270	−0.80 (−3.39, 1.79)	0.542	−0.74 (−3.19, 1.70)	0.549
Insulin, mg/dL	8.2 ± 3.4	8.3 ± 5.1	0.1 ± 5.1	0.869	7.5 ± 2.9	7.8 ± 3.6	0.3 ± 3.0	0.434	−0.19 (−1.63, 1.24)	0.791	0.11 (−1.27, 1.48)	0.879
TC, mg/dL	211 ± 28.5	212 ± 34.6	1.3 ± 23.4	0.635	204 ± 26.6	205 ± 30.2	0.9 ± 18.5	0.700	0.44 (−6.71, 7.60)	0.902	1.12 (−6.06, 8.30)	0.758
HDL cholesterol, mg/dL	68.2 ± 17.3	67.0 ± 15.9	−1.2 ± 14.0	0.479	65.8 ± 13.2	65.0 ± 12.9	−0.9 ± 7.5	0.356	−0.32 (−4.16, 3.52)	0.870	0.45 (−3.00, 3.91)	0.795
LDL cholesterol, mg/dL	128 ± 26.4	130 ± 29.1	2.7 ± 16.9	0.175	122 ± 26.9	124 ± 29.3	1.6 ± 17.7	0.468	1.15 (−4.69, 7.00)	0.696	1.73 (−4.07, 7.54)	0.556
Triglycerides, mg/dL	83.4 ± 30.6	83.1 ± 34.7	−0.3 ± 27.4	0.938	80.0 ± 34.3	80.3 ± 28.5	0.2 ± 24.1	0.935	−0.49 (−9.24, 8.22)	0.911	0.63 (−7.35, 8.60)	0.876
HOMA-IR	1.8 ± 0.8	1.8 ± 1.4	0.1 ± 1.4	0.676	1.6 ± 0.7	1.7 ± 0.9	0.1 ± 0.7	0.292	−0.02 (−0.42, 0.36)	0.896	0.03 (−0.35, 0.41)	0.874
Creatinine, mg/L	0.7 ± 0.1	0.7 ± 0.1	0.0 ± 0.1	0.918	0.7 ± 0.1	0.7 ± 0.1	0.0 ± 0.1	0.242	0.02 (−0.02, 0.05)	0.336	0.03 (0.00, 0.05)	0.084
SBP, mm Hg	108 ± 16.4	106 ± 14.1	−1.8 ± 10.2	0.152	108 ± 15.0	108 ± 14.4	−0.2 ± 12.0	0.919	−1.62 (−5.39, 2.16)	0.398	−1.45 (−4.79, 1.88)	0.391
DBP, mm Hg	72.6 ± 10.7	72.4 ± 10.0	−0.3 ± 7.3	0.757	72.2 ± 10.3	71.4 ± 10.1	−0.7 ± 7.9	0.463	0.45 (−2.13, 3.03)	0.732	0.59 (−1.76, 2.94)	0.622
HR, bpm	66.5 ± 7.6	66.3 ± 8.0	−0.2 ± 6.0	0.804	66.7 ± 8.6	65.7 ± 7.6	−1.0 ± 7.6	0.298	0.80 (−1.51, 3.11)	0.495	0.74 (−1.31, 2.79)	0.479
PP, mm Hg	35.6 ± 9.5	34.1 ± 7.4	−1.5 ± 7.2	0.088	35.6 ± 7.6	36.1 ± 7.7	0.6 ± 7.1	0.518	−2.07 (−4.50, 0.37)	0.095	−2.05 (−4.08, −0.02)	0.048
MAP, mm Hg	84.5 ± 12.1	83.7 ± 11.0	−0.8 ± 7.7	0.403	84.0 ± 11.5	83.5 ± 11.2	−0.5 ± 8.9	0.629	−0.24 (−3.05, 2.56)	0.865	−0.10 (−2.63, 2.44)	0.939

[1] Values are means ±SDs and differences are means (95% CI). BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; HDL, high-density lipoproteins; HOMA-IR, homeostasis assessment model for insulin resistance; HR, heart rate; LDL, low-density lipoproteins; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; TC, total cholesterol. [2] Intragroup comparison by the paired Student's *t*-test. [3] These values are unadjusted. Intergroup comparison by the Student's *t*-test. [4] These values are adjusted for baseline values. Results are based on ANCOVA.

Considering the way in which the chocolate was consumed (plain, with coffee/tea, with other foods, etc.), no significant differences were found for PP in the CG (0.55 mm Hg; 95% CI: −1.19, 2.30) compared to the participants who consumed the chocolate with coffee or tea (−1.75 mm Hg; 95% CI: −5.27, 1.77) and to those who consumed it without mixing it with other foods or liquids (−1.92 mm Hg; 95% CI: −4.35, 0.52) (p = 0.329).

Moreover, in this subgroup categorization, no changes were observed on SBP in the subgroup of participants who had chocolate on its own (−2.51 mm Hg; 95% CI: −6.45, 1.42) and in those who had it with other foods (−1.25 mm Hg; 95% CI: −6.71, 4.21) (p = 0.800). No statistically significant differences were found between groups in any case (Figure 2).

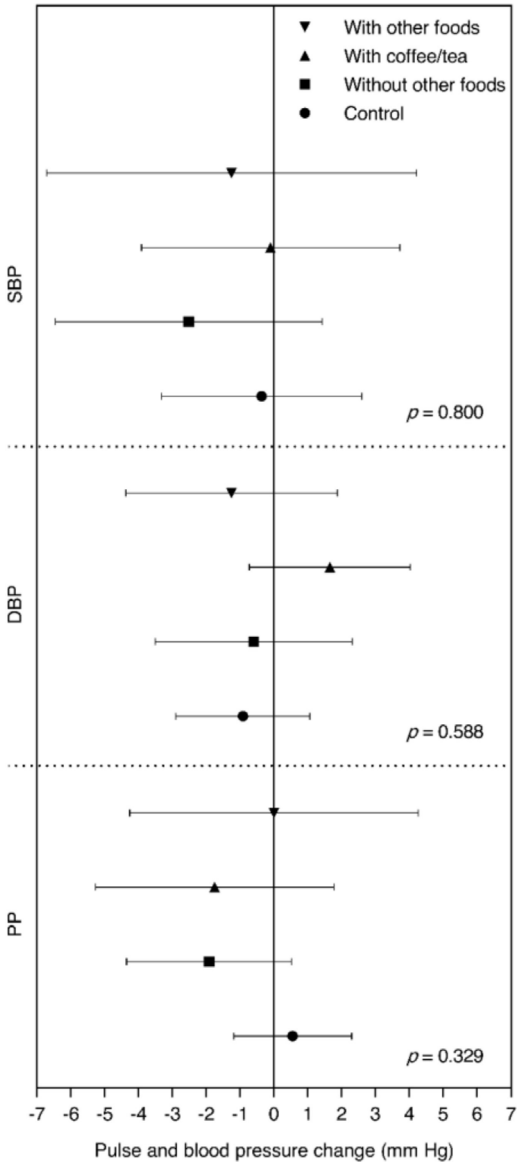

Figure 2. Changes in diastolic blood pressure (DBP), systolic blood pressure (SBP), and pulse pressure (PP) by the mode of chocolate intake in postmenopausal women participants. Values are differences in means (95% CI). n = 14 in subgroup that consumed chocolate with other foods, n = 20 in subgroup that consumed chocolate with coffee/tea, n = 36 in subgroup that consumed chocolate without other foods, n = 66 in control group. p-values from ANOVA for differences in DBP, SBP, and PP between all subgroups are shown.

In the subgroup analysis based on the presence or absence or overweight or obesity as baseline condition, a sharp decrease of SBP was observed in the subjects of the IG with overweight or obesity (−4.64 mm Hg; 95% CI: −8.32, −0.96), in contrast to the increase observed in the CG (1.13 mm Hg; 95% CI: −2.15, 4.41) (p = 0.020).

Similarly, PP decreased in the subjects of the IG with overweight or obesity (−3.88 mm Hg; 95% CI: −6.09, −1.68), in contrast to the increase in the subjects of the CG with overweight or obesity (1.28 mm Hg; 95% CI: −1.34, 3.89) (p = 0.003) (Figure 3).

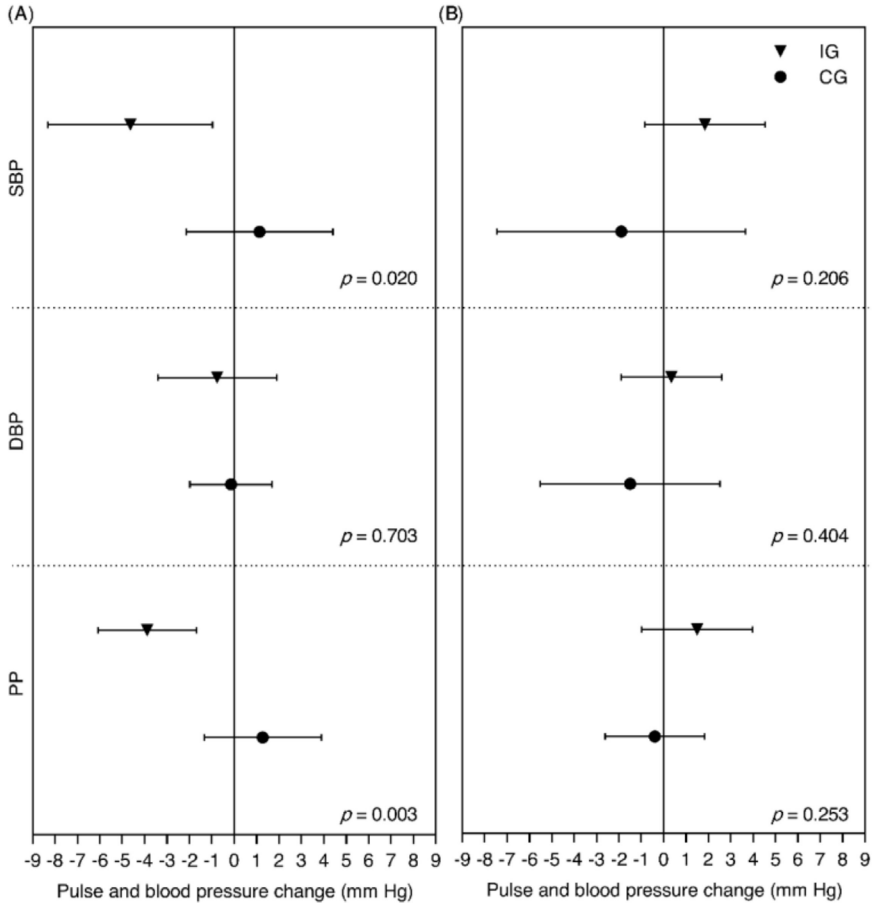

Figure 3. Subanalysis of changes in diastolic blood pressure (DBP), systolic blood pressure (SBP), and pulse pressure (PP) by overweight or obesity as a baseline condition in postmenopausal women participants. (**A**) Subgroup with overweight/obesity (intervention group (IG): n = 39; control group (CG): n = 38). (**B**) Subgroup without overweight/obesity (IG: n = 31; CG: n = 28). Values are differences in means (95% CI). p-values from Student's t-test for differences in DBP, SBP, and PP between IG and CG are shown.

3.3. *Arterial Stiffness Parameters and Vascular Function*

After adjusting for baseline values, no intergroup differences were found for any of the arterial stiffness parameters or vascular function (Table 4).

Table 4. Arterial stiffness parameters and vascular function in postmenopausal women participants [1].

	Intervention Group (n = 71)				Control Group (n = 66)							
Characteristic	**Baseline**	**6 Months**	**Change**	p [2]	**Baseline**	**6 Months**	**Change**	p [2]	**Intergroup Difference (IG-CG)** [3]	p [3]	**Adjusted Intergroup Difference (IG-CG)** [4]	p [4]
CAVI	7.55 ± 0.91	7.78 ± 0.86	0.23 ± 0.67	0.005	7.70 ± 0.83	7.74 ± 0.87	0.04 ± 0.63	0.580	0.18 (−0.03, 0.40)	0.100	0.14 (−0.06, 0.34)	0.175
ABI	1.09 ± 0.07	1.11 ± 0.07	0.02 ± 0.07	0.064	1.10 ± 0.07	1.10 ± 0.07	0.01 ± 0.08	0.523	0.01 (−0.02, 0.03)	0.477	0.01 (−0.01, 0.03)	0.480
baPWV, m/s	12.1 ± 1.53	12.3 ± 1.55	0.14 ± 0.92	0.220	12.3 ± 1.53	12.3 ± 1.68	-0.08 ± 1.01	0.538	0.21 (−0.11, 0.54)	0.200	0.18 (−0.14, 0.50)	0.263
CAIx	41.6 ± 20.5	47.3 ± 29.1	5.77 ± 35.06	0.196	43.2 ± 19.4	47.9 ± 18.9	4.79 ± 22.3	0.092	0.98 (−9.38, 11.3)	0.852	−0.34 (−8.96, 8.27)	0.937
CAIx75	31.2 ± 15.4	35.5 ± 21.8	4.33 ± 26.3	0.196	32.4 ± 14.6	36.0 ± 14.2	3.59 ± 16.7	0.092	0.73 (−7.03, 8.50)	0.852	−0.26 (−6.72, 6.20)	0.937
PAIx	100.8 ± 16.1	103.5 ± 19.1	2.70 ± 26.0	0.407	101.6 ± 16.2	102.6 ± 18.0	0.97 ± 22.9	0.740	1.73 (−6.91, 10.4)	0.693	0.87 (−5.69, 7.42)	0.794

[1] Values are means ±SDs and differences are means (95% CI). ABI: ankle-brachial index; ba-PWV: brachial-ankle pulse wave velocity; cAIx: central augmentation index; cAIx75: central augmentation index corrected for a heart rate of 75 bpm; CAVI: cardio-ankle vascular index; pAIx: peripheral augmentation index. [2] Intragroup comparison by the paired Student's *t*-test. [3] These values are unadjusted. Intergroup comparison by the Student's *t*-test. [4] These values are adjusted for baseline values. Results are based on ANCOVA.

4. Discussion

4.1. Main Findings

The results did not show differences in SBP or DBP between groups, although a decrease in PP was observed in the IG compared to the CG. The levels of TC, LDL cholesterol, and HDL cholesterol, as well as the body weight, were similar in the two groups. Likewise, no differences were found in the levels of glucose, insulin, or HOMA-IR. No relevant changes were observed regarding the evaluation variables of vascular structure and function.

4.2. Discussion of Blood Pressure Results

Okamoto et al. [16], who administered a cocoa compound daily or in alternate days to postmenopausal women randomized in two groups, observed that values of SBP, DBP, mean arterial pressure (MAP), and PP decreased significantly with respect to the control group. A PP decrease associated with a greater consumption of chocolate has also been observed in studies with healthy adults [30]. Moreover, in a meta-analysis of randomized controlled trials, it was concluded that chocolate and cocoa appear to reduce both SBP and DBP after chronic intake [31]. In this regard, the results of this study are in line with previous evidence.

The decrease in SBP and PP observed in the participants with overweight or obesity of the IG with respect to those of the CG is in contrast to the results of another study, in which it was observed that the acute consumption of cocoa increased 4 mm Hg the BP at rest in healthy overweight subjects [18]. However, we must take into account that, in our study, the acute effects were not evaluated, but those in the medium term, and that the study population only included postmenopausal women.

Despite the fact that no significant differences were found, the results suggest that the intake of chocolate on its own or mixed with other foods or drinks could influence the possible effects of this compound, in the sense that the intake of other foods along with chocolate could interfere in the possible effects of chocolate on health. Nevertheless, it would be necessary to carry out other studies that could provide evidence on this.

The sample size of this trial was estimated based on the potential modification of the SBP, but it was not addressed to detect differences in the subgroup analysis. Therefore, it is possible that the sizes of the subgroups based on the presence or absence of overweight or obesity as baseline condition and by the mode of chocolate intake were inadequate for such analysis.

4.3. Discussion of the Results of Other Cardiovascular Risk Factors

With respect to metabolic risk factors, no changes were observed in the lipid profile in a similar way as in other studies with postmenopausal women [16], although a study with hypertensive patients with impaired glucose tolerance showed a significant decrease in the levels of TC and LDL cholesterol after the consumption of dark chocolate [32]. Furthermore, Okamoto et al. observed a significant decrease in triglycerides and glucose after the intake of cocoa [16], whereas our results showed no changes in these parameters. Previous studies indicate an improvement in the levels of insulin and HOMA-IR [15,32]; however, the results of the present study showed no differences in these parameters in any of the two groups or between them. Body weight remained unaltered after the consumption of dark chocolate, as shown by other studies performed in populations with augmented risk [16,18,33].

4.4. Discussion of the Results of Arterial Stiffness Parameters and Vascular Function

There is evidence that supports the improvement of arterial stiffness through the decrease of PWV after the consumption of cocoa, both in healthy subjects [19,30] and in postmenopausal women [16]. However, the results of the present study did not show significant changes.

Previous studies suggest that the consumption of chocolate with a high concentration of cocoa may improve vascular function in postmenopausal women [17], although Marsh et al. evaluated

the acute effects, whereas the present study analyzed the effects in the medium term. Furthermore, the beneficial effects of a greater consumption of chocolate on the increase rate have been observed in healthy subjects [30] and in overweight women [18]. On the other hand, the parameters of vascular function measured in our study did not show relevant changes.

Our study has a larger sample of postmenopausal women compared to other similar studies with a relatively small sample size [16,17]. Moreover, the follow-up time of six months, which is in contrast to that of other studies in which the intervention lasted a few weeks, allowed us to evaluate the effects in the medium term.

The adherence to chocolate consumption reached in our study was practically total or very high, which is in line with that reported by other studies of similar characteristics [19,34–36].

With respect to the product used in the intervention, it is a commercially available compound with certain unmodifiable characteristics. Moreover, the contribution of 10 g of chocolate suits the recommendations of the European Food Safety Authority, which states that the consumption of this amount of high-flavanol dark chocolate included in a balanced diet could help maintain endothelium-dependent vasodilation [37]. However, other studies used a greater amount of chocolate, e.g., 50–100 g/day [31], or a product specifically manufactured for the purpose of the research, creating compounds with a very high content of polyphenols [17,19]. In this way, the possible effects can be potentiated with the great contribution of polyphenols, although this type of intervention does not fit a real clinical and easily reproducible context.

Likewise, there were no modifications or restrictions in the habitual diet of the participants in the present study, unlike in other studies [16]; the chocolate was simply added to the diet of the IG's participants. This, along with the fact that the chocolate used is commercially available, allowed evaluating an intervention that could be applied in the usual clinical practice with a product that is accessible to the general population.

4.5. Limitations

The present study has several limitations. First, the amount of chocolate administered to the IG contained a low concentration of polyphenols compared to other studies. The polyphenol contribution of this amount of chocolate could be insufficient to show relevant changes in the effect size.

Moreover, it would have been interesting to evaluate the bioavailability of polyphenols by measuring, for example, epicatechin in plasma, with the aim of establishing a correlation with the results; however, this was not feasible.

The blinding of the participants was not possible due to the nature of the intervention, although the researchers who made the measurements and those who conducted the statistical analyses were blinded.

5. Conclusions

The daily intake of 10 g of cocoa-rich chocolate seems to provide little improvement to cardiovascular health, but neither does it cause any adverse effects on the parameters evaluated in postmenopausal women in the long term. However, it is necessary to carry out further clinical trials that include a greater number of subjects, easily reproducible in the usual clinical practice, and which evaluate the effects of the intake of cocoa-rich chocolate in the long term in populations with augmented cardiovascular risk, such as postmenopausal women.

Author Contributions: Conceptualization, J.I.R.-R., J.A.M.-F., L.G.-O. and I.A.G.-Y.; methodology, L.G.-O., J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; validation, M.A.G.-M. and E.R.-S.; formal analysis, J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; investigation, M.A.G.-M., E.R.-S., C.A.-C. and J.G.-S.; resources, C.A.-C. and J.G.-S.; data curation, E.R.-S., C.A.-C. and J.G.-S.; writing—original draft preparation, I.A.G.-Y., J.I.R.-R. and J.A.M.-F.; writing—review and editing, L.G.-O., M.A.G.-M., E.R.-S., C.A.-C. and J.G.-S.; supervision, J.I.R.-R., J.A.M.-F., L.G.-O.; project administration, J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; funding acquisition, J.A.M.-F. and J.I.R.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported in part by grants funded by Gerencia Regional de Salud de Castilla y León (GRS 1583/B/17). It was also supported by the Institute of Health Carlos III of the Ministry of Science and Technology (Spain) through the Network for Prevention and Health Promotion in Primary Care (redIAPP, RD16/0007), co-financed with the European Union's ERDF.

Acknowledgments: The authors are grateful to all the volunteers for their participation, and the professionals involved in the study: José I Recio-Rodríguez, José A Maderuelo-Fernández, Luis García-Ortiz, Manuel A Gómez-Marcos, Irene A García-Yu, Rosario Alonso-Domínguez, Sara Mora-Simón, Natalia Sánchez-Aguadero, Jesús González-Sánchez, Cristina Agudo-Conde, Cristina Lugones-Sánchez, Benigna Sánchez-Salgado, Carmen Castaño-Sánchez, Emiliano Rodríguez-Sánchez, Susana González-Manzano, Olaya Tamayo-Morales, and Susana González-Sánchez.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. Lindt & Sprüngli provided the necessary chocolate for the implementation of the study. This company did not play any role in the design of the study, the data analysis, the reporting of results, or the decision to present the manuscript for publication.

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British Journal of Nutrition

Chocolate rico en cacao y composición corporal en mujeres posmenopáusicas. Ensayo clínico aleatorizado

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British Journal of Nutrition. 2021 Mar 14;125(5):548-556.

Durante la menopausia, las mujeres experimentan una serie de cambios fisiológicos que incluyen la redistribución del tejido graso. Este estudio se diseñó para evaluar el efecto sobre la composición corporal de añadir 10 g de chocolate rico en cacao diarios a la dieta habitual de mujeres posmenopáusicas. Se realizó un ensayo clínico aleatorizado y controlado de dos brazos de 6 meses de duración. Las mujeres posmenopáusicas (57,2 ± 3,6 años, *n* = 132) se reclutaron en centros de salud de atención primaria. Las participantes del grupo control (GC) no recibieron ninguna intervención. Las del grupo de intervención (GI) recibieron 10 g diarios de chocolate con 99% de cacao añadidos a su dieta habitual durante 6 meses. Esta cantidad aporta 59 kcal y 65,4 mg de polifenoles. Los resultados principales fueron las diferencias entre grupos de las variables de composición corporal, medidos mediante impedanciometría al finalizar la intervención. El efecto principal de la intervención mostró una reducción favorable al GI respecto al GC en la masa grasa corporal (−0,63 kg [IC 95% −1,15 a −0,11], *p* = 0,019), (*d* de Cohen = −0,450) y en el porcentaje de grasa corporal (−0,79% [IC 95% −1,31 a −0,26], *p* = 0,004), (*d* de Cohen = −0,539). Se observó un descenso no significativo en el índice de masa corporal (−0,20 kg/m^2 [IC 95% −0,44 a 0,03], *p* = 0,092), (*d* de Cohen = −0,345). Tanto la masa grasa corporal como el porcentaje de grasa corporal mostraron un descenso en el GI para los 3 segmentos corporales analizados (tronco, extremidades superiores e inferiores). La adición de 10 g de chocolate rico en cacao a la dieta habitual de mujeres posmenopáusicas reduce su masa grasa corporal y su porcentaje de grasa corporal sin modificar su peso.

Registro del ensayo: ClinicalTrials.gov, número NCT03492983. Fecha de registro: 10 Abril, 2018.

British Journal of Nutrition (2021), 125, 548–556 doi:10.1017/S0007114520003086

Cocoa-rich chocolate and body composition in postmenopausal women: a randomised clinical trial

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(Submitted 19 June 2020 – Accepted 27 July 2020 – First published online 4 August 2020)

Abstract

During menopause, women undergo a series of physiological changes that include a redistribution of fat tissue. This study was designed to investigate the effect of adding 10 g of cocoa-rich chocolate to the habitual diet of postmenopausal women daily on body composition. We conducted a 6-month, two-arm randomised, controlled trial. Postmenopausal women (57·2 (SD 3·6) years, n 132) were recruited in primary care clinics. Participants in the control group (CG) did not receive any intervention. Those of the intervention group (IG) received 10 g daily of 99 % cocoa chocolate in addition to their habitual diet for 6 months. This quantity comprises 247 kJ (59 kcal) and 65·4 mg of polyphenols. The primary outcomes were the between-group differences in body composition variables, measured by impendancemetry at the end of the study. The main effect of the intervention showed a favourable reduction in the IG with respect to the CG in body fat mass (−0·63 kg (95 % CI −1·15, −0·11), $P = 0·019$; Cohen's $d = -0·450$) and body fat percentage (−0·79 % (95 % CI −1·31, −0·26), $P = 0·004$; Cohen's $d = -0·539$). A non-significant decrease was also observed in BMI (−0·20 kg/m² (95 % CI −0·44, 0·03), $P = 0·092$; Cohen's $d = -0·345$). Both the body fat mass and the body fat percentage showed a decrease in the IG for the three body segments analysed (trunk, arms and legs). Daily addition of 10 g of cocoa-rich chocolate to the habitual diet of postmenopausal women reduces their body fat mass and body fat percentage without modifying their weight.

Key words: Body composition: Postmenopause: Chocolate: Body fat distribution: Body weight changes

The parameters that have been traditionally measured in the study of body composition are body weight, BMI and other indirect measurements of body fat distribution and abdominal obesity, such as waist perimeter and the waist height and waist hips indices[1]. More recently, the proliferation and validation of different impedancemetry devices[2] have allowed evaluating the composition and distribution of body fat and relating body fat mass and lean mass to mortality. In this sense, the review conducted by Lee *et al.*[3] in 2018 suggests that an increase in the content of fat mass and/or a decrease in lean mass could be associated with an increase in mortality.

During menopause, women undergo a series of physiological changes that include an increase in the levels of total cholesterol and LDL-cholesterol, with the consequent increase in cardiovascular risk[4]. The increase of age is also associated with a redistribution of fat tissue, with an increment in central localisation, which favours greater abdominal obesity and, ultimately, greater frequency of metabolic complications[5].

Among the interventions that assess the changes in body composition, some have shown beneficial results in body distribution and composition in postmenopausal women using isolated physical activity programmes[6,7]. Other interventions, such as the one designed by Seimon *et al.*[8], combine physical activity with energy restriction and with a slight increase of protein intake, obtaining improvements in body weight and fat mass. This moderate increase in the intake of proteins has also been reported by other authors as a possible cause of body fat percentage decrease[9]. The modification of the amounts of

Abbreviations: CG, control group; IG, intervention group; SBP, systolic blood pressure.

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macronutrients in the habitual diet was pointed out in the Women's Health Initiative Dietary Modification Trial[10], where the group of postmenopausal women assigned to an intervention based on lower fat intake (<20 % energy) obtained a decrease in body fat percentage and body fat mass after 1 year of follow-up.

However, the effect of polyphenols on the modification of body composition in postmenopausal women is still unclear. In the trial carried out by Choquette *et al.*[11], the addition of 70 mg/d of isoflavones to the habitual diet of the participants caused a reduction in leg fat percentage, although there were no changes for this parameter in other body parts or in the body as a whole. A daily supplement in the form of a chocolate snack[12] has been reported to decrease both body fat mass and percentage in overweight/obese premenopausal women. However, this study combined the supplement with energy restriction, thereby the isolated effect of the supplement could not be determined.

The aim of the present study was to analyse the effect of the daily addition of 10 g of chocolate with a high concentration of cocoa (99 %) to the habitual diet, for 6 months, on the body composition of postmenopausal women.

Methods

Design and setting

Controlled randomised trial with two parallel groups. The sample recruitment and the evaluation visits were conducted between June 2018 and August 2019. This clinical trial was registered in ClinicalTrials.gov (NCT03492983), and its protocol has been published[13]. This manuscript includes results on body composition as a secondary outcome from the intervention study. Results on blood pressure, as the main outcome of the trial, have been previously published[14].

Study participants and recruitment

The sample recruitment was carried out in the doctor's offices of four urban primary healthcare centres of Salamanca, Spain, through a consecutive sampling of women who met the inclusion criteria. The evaluations were performed in the Primary Care Research Service of Salamanca, which is part of the Spanish Research Network for Preventive Activities and Health Promotion in Primary Care and of the Biomedical Research Institute of Salamanca. The study included 140 women aged between 50 and 64 years and in the period of postmenopause, defined as amenorrhoea for at least twelve consecutive months. Potential participants were excluded based on the following criteria: personal history of CVD; personal history of diabetes mellitus, high blood pressure or dyslipidaemia under pharmacological treatment; hypoenergetic diets; clinically proven neurological and/or neuropsychological disease; treatment with hormone replacement therapy; habitual weekly consumption of over 210 g of cocoa; cocoa intolerance and/or allergy; intolerance and/or allergy to any of the compounds of the study supplement. The final sample consisted of 132 women. The evaluation of body composition through impedancemetry was not performed in the eight women who were excluded from the analysis, since they met some of the circumstances described by the manufacturer of the measuring device, which advised against the realisation of such procedure in them. These situations include people with pacemakers or any other electronic medical device in their bodies; people who could have difficulties in the analysis, such as being under 110 cm in height; people with limb amputations or people carrying metal prostheses.

Sample size

The sample size was estimated based on the estimated changes in the main outcome of this trial, that is, systolic blood pressure (SBP). To detect a minimum difference of 2·9 mmHg in SBP between the two groups, 140 participants (seventy per group) were needed, considering given α- and β-risks of 0·05 and 0·20, respectively, in bilateral contrast and a standard deviation (SD) of 5·8 mmHg and assuming a predicted drop-out rate of 10 % during follow-up. This estimate considered the results obtained in a similar study in which a decrease in SBP of 6·5 (SD 5·8) mmHg was observed[15]. This estimate powers to detect a difference of 0·8 % or higher in body fat percentage as statistically signifi- cant ($P < 0·05$).

Procedures and randomisation

All participants had a baseline assessment and a follow-up evaluation 6 months after the former, in which the study variables were measured (Fig. 1). The intervention group (IG) also conducted five chocolate re-supply visits at 1, 2, 3, 4 and 5 months after the baseline evaluation. No procedures were performed in these visits other than the provision of the necessary chocolate until the next visit and the collection of a calendar with the record of the chocolate intakes of the participants.

The postmenopausal women were randomly assigned to two groups: an IG and a control group (CG). The assignation sequence was generated by an independent researcher using the Epidat 4.2 software[16]. The participants received their randomisation number based on the order of their baseline evaluation visit; such number remained hidden until they were assigned to each group. To ensure that the blinding was maintained, the participants were given clear instructions not to disclose which treatment they had been randomised to while being interviewed by the blind assessors. The information about treatment allocation was stored in a secure locker in case of emergency unblinding.

Due to the characteristics of the intervention, it was not possible to blind the participants. To minimise contamination between groups, the researcher who conducted the evaluations was different from the one who re-supplied the chocolate to the IG.

Intervention

The participants of the CG did not receive any type of intervention. Those of the IG were provided with chocolate (99 % cocoa) and the instructions for the daily intake of 10 g of this supplement in addition to their habitual diet for 6 months. In the first re-supply visit, they were instructed in how to consume and store the chocolate, recommending that the daily intake should be at

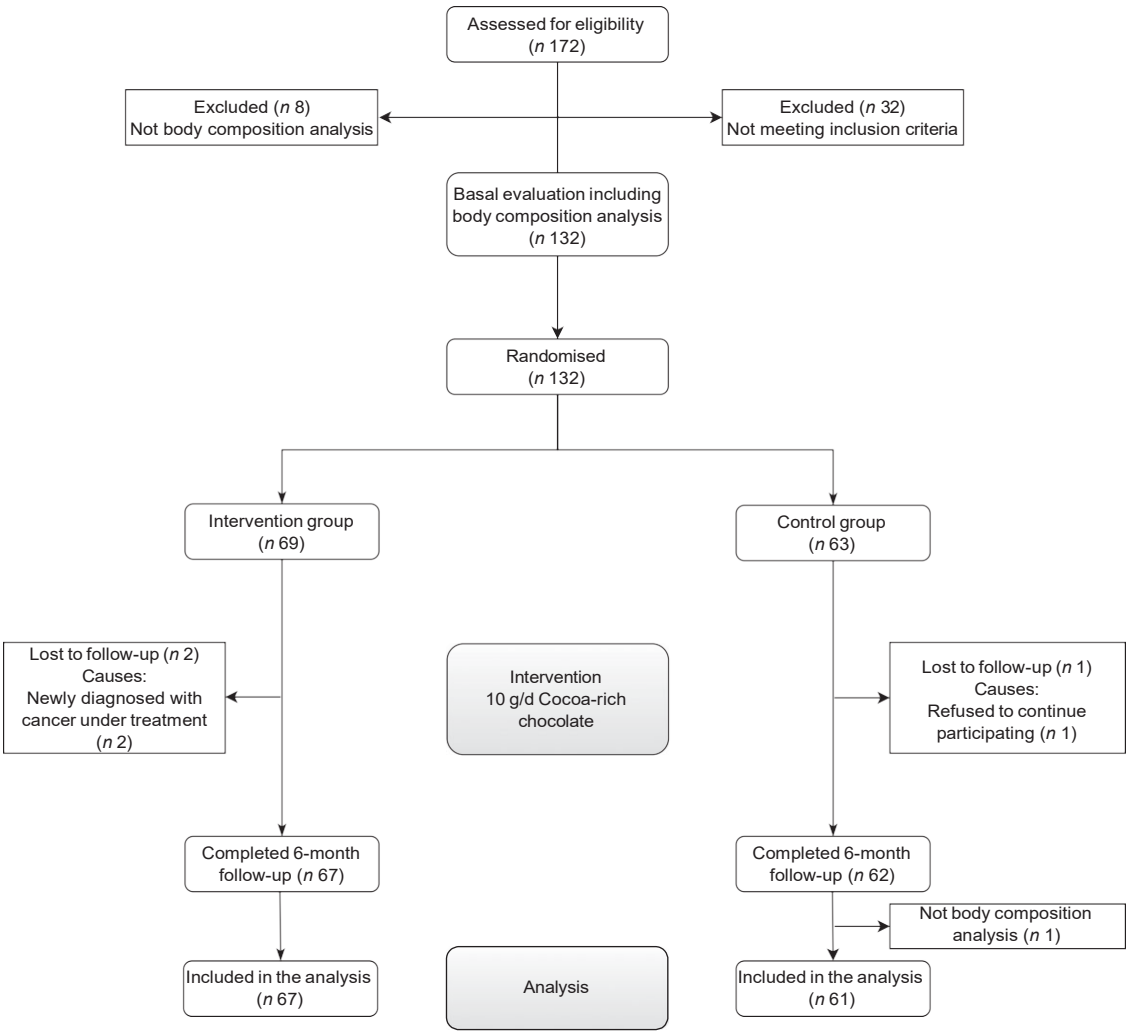

Fig. 1. Flow chart of the analysis of body composition of the participants.

the same time of the day. Moreover, a calendar was given to each participant, in which they were asked to record the time and date of each intake; these calendars were given back to the researchers in each re-supply visit.

The daily nutritional intake of 10 g of this chocolate is 247 kJ (59 kcal), 0·8 g of carbohydrates, 1·5 g of protein and 5·1 g of fat, of which 3·1 g are saturated fat. The intake of polyphenols per 10 g of this product is 65·4 mg. The polyphenolic profile per 10 g of this compound consists in 26·1 mg epicatechin, 10·4 mg catechin, 0·58 mg protocatechuic acid, 1·76 mg procya- nidin dimer (B3), 14·4 mg procyanidin dimer (B2), 8·53 mg pro- cyanidin trimer (C1), 3·54 mg procyanidin A hexoside, 0·02 mg quercetin glucoside and 0·03 mg quercetin arabinoside. The par- ticipants of both groups were instructed to follow their usual dietary pattern without modifying their eating habits during the study period.

Main outcomes

Analysis of body composition. The measurements of body composition by impedancemetry were performed using an Inbody 230 multifrequency analyser (Inbody)[2]. This is a segmental impedance device with which ten measurements were performed using two different frequencies (20 and 100 kHz) in each segment (right arm, left arm, trunk, right leg and left leg). The data were calculated by the algorithm of the manufacturer and include fat mass, fat-free mass, skeletal muscle mass, total body water, proteins and minerals. Furthermore, the device calculated the total body weight (kg). To determine the body composition measurements, the recommendations of the manufacturer were followed: the test was conducted before or two hours after a meal; the participants were asked to go to the bathroom before the beginning of the analysis, to avoid biological errors derived from including the weight of urine and/or faeces; they were asked to do no physical exercise before the analysis; they had to stand for 5 min before the analysis, avoiding to lie down and sitting for long periods, since this could induce changes in the results due to the tendency of water to move towards the lower limbs when the person stands up; the analysis was not performed after a shower or sauna session, or during menstruation; lastly, the room was kept at a stable temperature of 20–25°C, maintaining the same conditions in both evaluation sessions.

Body height and BMI. Body height was measured by recording the average of two readings rounded to the nearest centimetre using a stadiometer (Seca 222, Medical Scale and Measurement System). BMI was calculated by dividing weight (kg) by height squared (m^2).

Nutritional composition of the habitual diet and consumption of chocolate. The nutritional composition of the habitual diet of the participants was estimated using a 24-h record of three non-consecutive days before each visit, including business and non-business days. The data were processed using the EVIDENT II application[17], which has been validated for the estimation of energy intake (kcal) and macronutrient intake (carbohydrates, lipids, proteins, cholesterol and fibre). The nutritional intake calculations include the energy intake from the intervention. The consumption of chocolate was evaluated in each visit (baseline and at 6 months) through a series of questions about the amount, type and frequency of consumption in the period between visits.

Blood pressure. SBP and diastolic blood pressure were measured using a validated Omron M10-IT sphygmomanometer (Omron Healthcare), following the recommendations of the European Society of Hypertension[18].

Other measurements

Serum insulin concentrations (mg/dl) were measured, and insulin resistance was determined using the homoeostasis model assessment of insulin resistance index (HOMA-IR). At the baseline evaluation, the researchers gathered information of clinical and sociodemographic variables, such as gestational diabetes, hypertension and dyslipidaemia under treatment, pharmacological treatment and time elapsed since menopause diagnosis. A more detailed description of the methodology used and of the measuring of other physical variables, such as alcohol consumption and/or smoking, can be found in the previously published study protocol[13].

Data collection, data management and monitoring

The collection of data in each evaluation visit was carried out by a nurse who was previously trained for this purpose. Each participant was identified with a unique code, which referred to the data gathered in each of the measurements. These codes and data were used to create a database that could only be accessed by the researchers of the study. The principal investigator conducted a data cleansing process at the end of the study.

Ethical considerations

The study was approved by the Clinical Research Ethics Committee of the Health Area of Salamanca ('CREC of the Health Area of Salamanca') in February 2018. The participants signed an informed consent in accordance with the Declaration of Helsinki. They were informed about the objectives of the project and the risks and benefits of the explorations to be carried out. The confidentiality of the participants' data was guaranteed at all times in accordance with the provisions of the Organic Law 3/2018, of 5th December, of Personal Data Protection and guarantee of digital rights, and Regulation 2016/679 of the European Parliament and of the Council of 27th April 2016 for Data Protection, and under the conditions established by Spanish Law 14/2007 of biomedical research.

Statistical analyses

The statistical analyses were carried out following the study protocol[13]. The data were checked for normal distribution, and most data were considered normally distributed. The characteristics of the population are presented as mean and standard deviation for the continuous variables and as frequency distribution for the qualitative variables. For the baseline data, the χ^2 test and the Student's t test were used to compare the qualitative variables and the means, respectively, between the two groups. Paired t tests were performed to compare changes from baseline (Post *v.* Pre) within the same group. ANCOVA with baseline body composition variables as covariates was used to compare the changes between the two groups. The effect size was calculated by the standardised mean difference (Cohen's d). All analyses were performed using SPSS version 23.0 (IBM Corp), and an α-risk of 0·05 was established as the limit of statistical significance.

Results

Baseline characteristics of the participants

This study included 132 women (sixty-nine in IG and sixty-three in CG). The results of the effect of the intervention on the variables of body composition include 128 cases (sixty-seven in IG and sixty one in CG). There were three losses in the second evaluation (two in IG and one in CG). Moreover, another woman from the CG was not included in the analyses, since the impedancemetry analysis was not conducted in her at 6-month follow-up (Fig. 1). In the baseline evaluation, no differences were observed in any of the clinical or sociodemographic variables analysed (age, time elapsed since the beginning of menopause, history of hypertension and dyslipidaemia under treatment, gestational diabetes, pharmacological treatment, physical activity, smoking, SBP, diastolic blood pressure, serum insulin and HOMA-IR values). The baseline consumption of chocolate was similar in both groups (IG 69·6 (SD 71·8) *v.* CG 71·8 (SD 69·6) g/week), with no differences in the weekly consumption of chocolate with over 70 % cocoa (Table 1).

Intragroup and intergroup differences in variables of body composition

The IG did not show changes in body weight after the intervention (baseline evaluation 65·66 (SD 10·36) kg; evaluation at 6 months 65·34 (SD 10·33) kg), although the most relevant results show a significant decrease in body fat mass (baseline 26·27 (SD 7·72) kg; 6 months 25·80 (SD 7·65) kg; $P = 0·030$) and body fat percentage (baseline 39·30 (SD 6·33) %; 6 months 38·75 (SD 6·60) %; $P = 0·005$). No significant differences were observed in the rest of the analysed variables (total body water, proteins, minerals and skeletal muscle mass) (Table 2).

Table 1. Baseline characteristics of the study population
(Mean values and standard deviations; numbers and percentages)

Variables	Intervention group (*n* 69)		Control group (*n* 63)	
	Mean	SD	Mean	SD
Age (years)	57·2	3·6	57·3	3·8
Time from menopause onset (years)	6·8	4·5	6·8	3·7
Untreated hypertension				
n	1		0	
%	1·6		0·0	
Untreated dyslipidaemia				
n	8		10	
%	12·7		14·5	
Gestational diabetes				
n	3		1	
%	4·8		1·4	
Thyroid hormone treatment				
n	13		9	
%	20·6		13·0	
Current smoker				
n	10		8	
%	15·9		11·6	
Alcohol consumption (g/week)	23·4	30·0	31·8	49·3
Physical activity (METS/min per week)	1934·5	2257·0	1565·1	1220·1
Systolic blood pressure (mmHg)	108·4	16·7	107·9	15·2
Diastolic blood pressure (mmHg)	72·8	11·0	72·4	10·4
Insulin (mg/dl)	8·6	3·4	7·3	2·7
Homoeostasis assessment model for insulin resistance	1·9	0·9	1·6	0·6
Chocolate intake (g/week)	69·6	71·8	71·8	69·6
>70 % cocoa chocolate intake (g/week)	20·4	36·9	15·1	33·7

On the other hand, within the CG, there were no relevant differences in any of the analysed variables of body composition between the baseline measurements and the measurements at 6 months. Body weight did not change (baseline evaluation 64·34 (SD 8·79) kg; evaluation at 6 months 64·52 (SD 8·91) kg; the same result was obtained for body fat percentage (baseline 38·57 (SD 5·50) %; 6 months 38·81 (SD 5·38) %).

The main effect of the intervention showed a favourable decrease in the IG for both body fat mass (−0·63; 95 % CI −1·15, −0·111 kg; $P = 0·019$) (Cohen's $d = -0·450$) and body fat percentage (−0·79; 95 % CI −1·31, −0·26 %; $P = 0·004$) (Cohen's $d = -0·539$). A favourable decrease was also observed in the IG for BMI, although it was not significant (−0·20; 95 % CI −0·44, 0·03 g/m²; $P = 0·092$) (Cohen's $d = -0·345$). No significant differences were found between groups in minerals, proteins, total body water or skeletal muscle mass.

Changes in body composition by body segment

Both body fat mass and fat percentage showed a decrease in the IG for the three body segments analysed (trunk, arms and legs). On the other hand, the CG did not show such reduction. The differences in mean values showed a favourable change in the IG ($P < 0·05$) for all measurements, except for trunk fat mass ($P = 0·065$). The decrease was more pronounced in body fat percentage (trunk −0·73 (95 % CI −1·20, −0·26); arms −0·9 (95 % CI −1·59, −0·43); legs −0·76 (95 % CI −1·21, −0·31)). The intervention also showed favourable results in the IG for body fat mass in arms and legs, with no significant change in the trunk (Table 3).

Changes in nutritional composition

The intervention did not alter the nutritional composition of the groups. There were no differences in the intake of energy (kJ), carbohydrates, lipids or fibre. However, there was an increase in the consumption of proteins in the IG (baseline 76·5 (SD 16·8) g 6 months 82·4 (SD 17·4) g). There were no differences between the two groups in any of the variables of nutritional composition (Table 4).

Other variables measured remained unchanged. No differences in SBP or diastolic blood pressure were observed between groups. There were no significant changes in serum insulin (−0·37 mg/dl; 95 % CI −1·88, 1·14; $P = 0·631$) and in HOMA-IR (−0·07; 95 % CI −0·48, 0·34; $P = 0·743$) between the two groups.

Discussion

The main finding of this study is the beneficial effect of the daily consumption of 10 g of cocoa-rich chocolate on the body composition of postmenopausal women. After 6 months of intervention, a decrease of both body fat mass and fat percentage was achieved with respect to the CG. No relevant differences were observed in terms of body weight, BMI or other aspects of body composition. Moreover, there were no changes in the habitual dietary intake.

This is one of the first studies conducted in humans to show the beneficial effects that the compounds of cocoa can have on markers of body composition and fat distribution. Among the main compounds present in cocoa, polyphenols have shown

Table 2. Change in body composition variables
(Mean values and standard deviations; 95 % confidence intervals)

	IG (*n* 67)							CG (*n* 61)												
	Baseline		6 months		Change			Baseline		6 months		Change			Intergroup difference (IG – CG), unadjusted			Intergroup difference (IG – CG), adjusted			
	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	95 % CI	*P*†	Mean	95 % CI	*P*‡	Cohen's *d*
Weight (kg)	65·66	10·36	65·34	10·33	−0·32	1·97	0·187	64·34	8·79	64·52	8·91	0·17	1·33	0·321	−0·49	−1·08, 0·10	0·104	−0·48	−1·08, 0·12	0·117	−0·291
BMI (kg/m^2)	25·93	3·78	25·79	3·69	−0·15	0·79	0·144	25·30	3·12	25·37	3·14	0·08	0·51	0·246	−0·22	−0·45, 0·02	0·067	−0·20	−0·44, 0·03	0·092	−0·345
Body fat mass (kg)	26·27	7·72	25·80	7·65	−0·47	1·74	0·030	25·09	6·21	25·29	6·13	0·20	1·18	0·200	−0·67	−1·20, −0·14	0·013	−0·63	−1·15, −0·11	0·019	−0·450
Percent body fat (%)	39·30	6·33	38·75	6·60	−0·56	1·58	0·005	38·57	5·50	38·81	5·38	0·24	1·38	0·181	−0·80	−1·31, −0·28	0·003	−0·79	−1·31, −0·26	0·004	−0·539
Fat-free mass (kg)	39·39	4·12	39·54	4·20	0·15	0·90	0·174	39·25	4·38	39·23	4·60	−0·03	0·92	0·824	0·18	−0·14, 0·49	0·273	0·18	−0·14, 0·49	0·278	0·197
Skeletal muscle mass (kg)	21·18	2·44	21·29	2·48	0·11	0·54	0·115	21·07	2·57	21·05	2·69	−0·02	0·55	0·798	0·12	−0·07, 0·31	0·201	0·12	−0·07, 0·32	0·206	0·238
Total body water (kg)	28·90	3·02	29·00	3·08	0·10	0·66	0·200	28·78	3·22	28·74	3·38	−0·03	0·67	0·719	0·14	−0·10, 0·37	0·253	0·13	−0·10, 0·37	0·260	0·195
Estimated proteins (kg)	7·68	0·81	7·71	0·81	0·03	0·19	0·273	7·64	0·86	7·65	0·89	0·01	0·19	0·646	0·01	−0·05, 0·08	0·681	0·01	−0·05, 0·08	0·677	0·105
Estimated minerals (kg)	2·82	0·31	2·83	0·32	0·01	0·08	0·184	2·83	0·33	2·83	0·35	0·00	0·07	0·958	0·01	−0·01, 0·04	0·346	0·01	−0·01, 0·04	0·342	0·133

IG, intervention group; CG, control group.
* Intragroup comparison by the paired Student's *t* test.
† Intergroup comparison unadjusted.
‡ Intergroup comparison adjusted by baseline body composition variables.

Table 3. Change in body composition variables by segments (trunk, arms and legs)
(Mean values and standard deviations; 95 % confidence intervals)

	IG (*n* 67)							CG (*n* 61)									
	Baseline		6 months		Change			Baseline		6 months		Change			Intergroup difference (IG – CG)		
	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	95 % CI	*P*†
Body fat mass (kg)																	
Trunk	13·53	3·90	13·30	3·94	−0·23	1·02	0·071	13·04	3·24	13·10	3·15	0·06	0·64	0·501	−0·28	−0·59, 0·02	0·065
Arms	2·09	0·86	2·02	0·81	−0·07	0·22	0·016	1·93	0·65	1·95	0·64	0·02	0·13	0·225	−0·09	−0·15, −0·02	0·008
Legs	3·70	1·05	3·64	1·01	−0·06	0·24	0·031	3·51	0·83	3·57	0·82	0·06	0·22	0·042	−0·12	−0·20, −0·04	0·003
Percent body fat (%)																	
Trunk	40·20	5·99	39·63	6·35	−0·57	1·39	0·001	39·72	5·20	39·89	5·11	0·17	1·26	0·310	−0·73	−1·20, −0·26	0·002
Arms	46·54	6·97	45·85	7·39	−0·69	1·64	0·001	46·03	6·16	46·35	6·06	0·32	1·69	0·142	−1·01	−1·59, −0·43	0·001
Legs	37·13	5·85	36·63	5·97	−0·50	1·30	0·003	36·46	5·07	36·73	5·09	0·27	1·26	0·106	−0·76	−1·21, −0·31	0·001

IG, intervention group; CG, control group.
* Intragroup comparison by the paired Student's *t* test.
† Intergroup comparison by the Student's *t* test.

Table 4. Change in nutritional composition
(Mean values and standard deviations; 95 % confidence intervals)

	IG (*n* 67)							CG (*n* 61)							Intergroup difference (IG – CG)		
	Baseline		6 months		Change			Baseline		6 months		Change					
	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	Mean	SD	*P**	Mean	95 % CI	*P*†
Energy (kcal/d)‡	1708	367	1733	345	25	387	0·605	1797	409	1803	348	7	353	0·883	18	−112, 149	0·782
Carbohydrates (g/d)	168·1	46·8	163·3	44·1	−4·8	56·2	0·497	174·5	50·6	179·3	39·2	4·8	48·4	0·443	−9·6	−28·1, 9·0	0·310
Proteins (g/d)	76·5	16·8	82·4	17·4	6·0	19·3	0·016	78·4	19·5	81·8	15·6	3·4	19·0	0·170	2·6	−4·2, 9·3	0·454
Fats (g/d)	76·6	20·4	78·3	19·1	1·7	23·0	0·555	80·9	20·3	78·4	18·4	−2·5	18·1	0·280	4·2	−3·1, 11·5	0·257
Cholesterol (mg/d)	296·2	87·9	321·3	96·7	25·0	102·7	0·054	314·1	119·8	324·7	118·0	10·5	111·3	0·463	14·5	−23·2, 52·2	0·448
Fibre (g/d)	23·8	7·5	22·6	6·5	−1·2	7·7	0·194	25·4	9·5	25·8	9·2	0·4	10·4	0·768	−1·6	−4·9, 1·6	0·313

IG, intervention group; CG, control group.
* Intragroup comparison by the paired Student's *t* test.
† Intergroup comparison by the Student's *t* test.
‡ To convert kcal to kJ, multiply by 4·184.

high antioxidant capacity and potential coadjuvant capacity in certain metabolic mechanisms. In a trial conducted in rats, small doses of cocoa extract supplements were enough to counteract obesity and type 2 diabetes, providing new ideas about the possible application of cocoa supplements in the management of metabolic syndrome[19]. Likewise, another laboratory trial concluded that cocoa and its flavonoids could improve endothelial dysfunction and contribute with their reducing effect on arterial pressure[20]. Although polyphenols and their impact on obesity are still poorly known, some studies have suggested that they can have a positive effect on glucose regulation, adipogenesis, lipolysis, lipid metabolism and appetite control[21–24].

Improvements in insulin sensitivity, with decreased insulin levels and HOMA-IR, have been observed after dark chocolate ingestion in previous studies conducted in healthy persons[25]. Whilst others have not shown differences in these parameters after chocolate intake[26], which is consistent with our findings. Desideri *et al.*[27] observed an improvement in HOMA-IR, but no differences in plasma insulin levels after cocoa flavanols ingestion in elderly subjects with mild cognitive impairment. This discrepancy may be explained by differences in the methods used in every research. This significant finding could be explained by other effects on gastrointestinal peptide hormones or neurohormonal regulation of gastrointestinal function, satiety and satiation; however, these were not measured in this trial. It would be interesting for future studies to explore these as possible mechanistic effects.

There is evidence of an increase in percent body fat, as well as a central and visceral redistribution of fat mass, with ageing[28]. Additionally, menopause is an important change in women in terms of body composition. Toth *et al.*[29,30] associated menopause with an increase of abdominal fat and quantified these changes in 49 % more abdominal fat with respect to premenopausal women. The meta-analysis carried out by Ambikairajah *et al.*[31] showed an increase in body fat percentage (2·88 %; 95 % CI 2·13, 3·63 %) and trunk fat percentage (5·49 %; 95 % CI 3·91, 7·06 %) between premenopausal and postmenopausal women, though the change in fat mass quantity was attributable predominantly to increasing age. Furthermore, their results showed a decrease in total leg fat percentage and an increase in measures of central fat suggesting changes in fat mass distribution during menopause. In addition, it has to be mentioned that Mahabir *et al.*[32] reported that a one-unit change for body fat percentage was associated with a substantial change in serum leptin. There is evidence suggesting that this hormone could be a potential biomarker for breast cancer risk in women, especially overweight/obese and postmenopausal women[33] and that higher levels of circulating leptin have been associated with increased severity of non-alcoholic fatty liver disease[34]. In turn, such changes in the regional distribution of fat have been correlated to an increase in cardiometabolic risk in this population[35]. Therefore, it is necessary to know the variation of fat distribution among postmenopausal women as a consequence of interventions such as the one presented in this study. Aerobic physical activity combined with resistance exercises in 3 weekly sessions have shown to reduce body fat mass and fat percentage in all body segments, that is, trunk, arms and legs[11]. The intervention conducted by Choquette *et al.*[11] also included a group with a

polyphenol supplement (isoflavones), which only showed such reduction in the legs. Therefore, it is worth highlighting the decrease achieved in our study for body fat mass in general and in the trunk, arms and legs, which shows the magnitude of the results presented in this manuscript. The findings of our trial may be clinically relevant and even more considering that these changes occurred after a nutritional intervention with a commercially available chocolate. Moreover, this intervention showed no adverse effects on any of the parameters evaluated.

In the present study, there were no significant changes in body weight or in BMI after the 6 months of intervention. The results are in line with those of a recent meta-analysis of thirty-five randomised clinical trials about the impact of cocoa on body weight and BMI[36]. That meta-analysis suggested that cocoa supplements do not have a significant effect on body weight (−0·108 kg, 95 % CI −0·262, 0·046, $P = 0·168$) or on BMI (−0·014 kg/m², 95 % CI −0·105, 0·077, $P = 0·759$). However, a subgroup analysis revealed that body weight and BMI decreased with cocoa supplements of ≥30 g of chocolate per d in trials of 4–8 weeks. In our work, we observed a change in body composition, with no effect on body weight, although a greater decrease (−0·49 kg (95 % CI −1·08, 0·10)) was observed with respect to the meta-analysis conducted by Kord-Varkaneh *et al.*[36]. Therefore, the daily contribution of energy content from the chocolate supplement (10 g; 247 kJ (59 kcal)) did not seem to have a negative influence on body weight. Likewise, it did not appear to alter the mean energy intake of the participants (75 kJ (18 kcal) (95 % CI −112, 149)). Moreover, no relevant increase was observed in total body water, fat-free mass or skeletal muscle mass. All these parameters showed a slight increase in the IG.

The use of a placebo control in dietary studies must be attempted if possible. Similar studies evaluating the effects of chocolate intake have used white chocolate as a suitable placebo. Providing a placebo compound to the CG could have controlled for some confounding factors, such as preload effect on energy content taken during subsequent meal and would probably have allowed to assess the effects considering the polyphenol composition of the chocolate. However, this would also have potentially affected other metabolic variables and would not have allowed to evaluate the effects of the intake of the chocolate as a whole. Supporting this, Almoosawi *et al.*[37] pointed out the possibility of adverse effects occurring with polyphenol-poor chocolate placebo, reporting that in the absence of polyphenols, high-fat products such as chocolate may adversely affect metabolism, causing deleterious effects. Nevertheless, the clinical trial that this study is part of aimed to evaluate the effect of adding a daily amount of 10 g of chocolate high in cocoa content (99 %) and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women. The chocolate used in the intervention is commercially available; this makes the results of this study more accessible than those of other studies in which laboratory supplements are used. Moreover, this provided a real clinical context and allowed us to assess the potential benefits, as well as potential harms, of the intake of this compound as a whole, as pretended.

The limitations of this study include, on the one hand, the limited amount of polyphenols in the amount of chocolate administered to the IG with respect to other studies. However, this intervention attained the study objective by providing a commercial supplement to the habitual diet. This makes the results of this study more accessible than those of other studies in which laboratory supplements are used, although the intake of polyphenols from this amount of chocolate may not be enough to show relevant changes in the magnitude of the effect. On the other hand, it was not possible to blind the participants due to the nature of the intervention.

In conclusion, the daily addition of 10 g of cocoa-rich chocolate to the habitual diet of postmenopausal women reduces body fat mass and body fat percentage without altering body weight. Future studies should focus on the mechanisms through which the main compounds of cocoa produce these effects, in order to determine the most suitable amount and duration of the intake of these compounds.

Acknowledgements

The authors are grateful to all the volunteers for their participation, and the professionals involved in the study: J. I. R.-R., J. A. M.-F., L. G.-O., M. A. G.-M., I. A. G.-Y., Rosario Alonso-Domínguez, Sara Mora-Simón, Natalia Sánchez-Aguadero, Jesús González-Sánchez, Cristina Agudo-Conde, C. L.-S., Benigna Sánchez-Salgado, Carmen Castan˜o-Sánchez, E. R.-S., Susana González-Manzano, Olaya Tamayo-Morales and Susana González-Sánchez.

This study was supported in part by grants funded by Gerencia Regional de Salud de Castilla y Leo´n (GRS 1583/B/17). Lindt & Sprüngli provided the necessary chocolate for the implementation of the study. This company did not play any role in the design of the study, data analysis, reporting of results or the decision to present the manuscript for publication.

I. A. G.-Y., J. A. M.-F. and J. I. R.-R. designed the study; I. A. G.-Y., J. I. R.-R., L. G.-O., E. R.-S., M. A. G.-M. and C. L.-S. conducted research; I. A. G.-Y., J. A. M.-F. and J. I. R.-R. analysed data; I. A. G.-Y., J. A. M.-F. and J. I. R.-R. had primary responsibility for final content. All authors read and approved the final manuscript.

The authors declare that they have no conflicts of interest.

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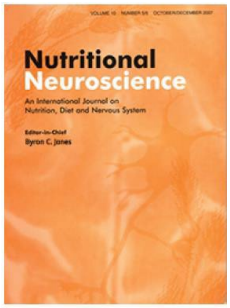


Nutritional Neuroscience
An International Journal on Nutrition, Diet and Nervous System

Efectos del chocolate rico en cacao sobre el rendimiento cognitivo en mujeres posmenopáusicas. Ensayo clínico aleatorizado

Irene A. Garcia-Yu, Luis Garcia-Ortiz, Manuel A. Gomez-Marcos, Emiliano Rodriguez-Sanchez, Sara Mora-Simon, Jose A. Maderuelo-Fernandez, Jose I. Recio-Rodriguez

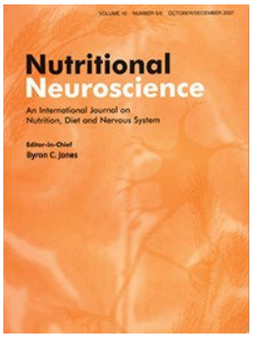
Nutritional Neuroscience. 2020 Nov 15;1-12.

Objetivos: El objetivo de este estudio fue evaluar los efectos sobre el rendimiento cognitivo de añadir 10 g de chocolate rico en cacao (99%) a la dieta habitual en mujeres posmenopáusicas.

Métodos: Se trata de un ensayo clínico aleatorizado controlado paralelo, en el que se reclutaron un total de 140 mujeres posmenopáusicas de 50-64 años. El grupo intervención (n = 73) consumió 10 g diarios de chocolate (99% cacao) añadidos a su dieta habitual durante 6 meses, mientras que el grupo control (n = 67) no recibió ninguna intervención. La atención y las funciones ejecutivas, la memoria verbal, la memoria de trabajo, la fluidez fonológica, la fluidez categorial y las variables clínicas se evaluaron basalmente y a los 6 meses.

Resultados: El tiempo de ejecución del Trail Making Test B mostró un descenso de −12,08 s (IC 95% −23,99 a −0,18; p = 0,047) en el grupo de intervención en comparación con el grupo control, tras ajustar por la edad, el nivel de educación, el tiempo desde el inicio de la menopausia y el consumo energético diario (d de Cohen = −0,343). No se observaron cambios en la atención, la memoria verbal inmediata y demorada, la fluidez fonológica y categorial ni la memoria de trabajo.

Conclusiones: El consumo de chocolate rico en cacao (99%) añadido a la dieta habitual podría asociarse con una ligera mejora en el rendimiento cognitivo con respecto a la flexibilidad cognitiva y la velocidad de procesamiento en mujeres posmenopáusicas, sin observar cambios en las demás variables de rendimiento cognitivo evaluadas.



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ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial

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To cite this article: Irene A. Garcia-Yu , Luis Garcia-Ortiz , Manuel A. Gomez-Marcos , Emiliano Rodriguez-Sanchez , Sara Mora-Simon , Jose A. Maderuelo-Fernandez & Jose I. Recio-Rodriguez (2020): Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial, Nutritional Neuroscience, DOI: 10.1080/1028415X.2020.1840119

To link to this article: https://doi.org/10.1080/1028415X.2020.1840119

NUTRITIONAL NEUROSCIENCE
https://doi.org/10.1080/1028415X.2020.1840119

Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial

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ABSTRACT

Objectives: The aim of this research was to evaluate the effects of adding 10 g of cocoa-rich chocolate (99%) to the habitual diet on cognitive performance in postmenopausal women.

Methods: Following a randomised controlled parallel clinical trial, a total of 140 postmenopausal women aged 50–64 were recruited. The intervention group (n = 73) consumed daily 10 g of chocolate (99% cocoa) in addition to their usual food intake for 6 months, whereas the control group (n = 67) did not receive any intervention. Attention and executive functions, verbal memory, working memory, phonological fluency, category fluency and clinical variables were assessed at baseline and 6 months.

Results: Trail Making Test B execution time showed a decreased of −12.08 s (95% CI: −23.99, −0.18; p = 0.047) in the intervention group compared to the control group, after adjusting for age, educational level, time elapsed from the beginning of menopause and daily energy consumption (Cohen's d = −0.343). Attention, immediate or delayed verbal memory, phonological or category fluency, and working memory remained unchanged.

Conclusions: The consumption of cocoa-rich (99%) chocolate in addition to the habitual diet could be related to a slight improvement in cognitive performance regarding cognitive flexibility and processing speed in postmenopausal women, with no changes in the rest of the cognitive performance variables evaluated.

Trial registration: This clinical trial has been registered at clinicaltrials.gov as NCT03492983.

KEYWORDS
Chocolate; polyphenols; postmenopause; cognition; executive function; attention; memory

Introduction

The consumption of chocolate or cocoa-rich products has been shown to have multiple beneficial effects on health [1–4]. Cognitive function is one of the studied aspects that could improve with the intake of this type of compounds. Polyphenols are believed to act both as neuroprotectors, potentially improving cognitive performance through a signalling cascade activation mechanism in the brain, and on the vascular system, leading to beneficial changes in the cerebrovascular blood flow [5–7].

The available evidence regarding the effects of cocoa on cognitive performance is discrepant. Some studies have reported changes in cognitive performance, reflected by improvements in executive functions and verbal fluency [8], as well as working memory [9,10], and a decrease of mental fatigue [11]. Furthermore, greater chocolate consumption has been associated with better cognitive function [12]. Similarly, the intake of chocolate has been related to lower risk of dementia [13] and of cognitive decline [14]. On the other hand, the findings of other studies have shown no improvements in cognitive performance after the consumption of cocoa [15,16].

Positive effects on cognitive function associated with the intake of cocoa have been observed in different population subgroups, such as young adults [10,17] and elderly subjects, both without cognitive alterations [8,18] and with mild cognitive impairment [19]. In postmenopausal women, it appears that the change in the levels of estrogens may affect their cognitive state [20]. In this population group, improvements have been

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Supplemental data for this article can be accessed at https://doi.org/10.1080/1028415X.2020.1840119

reported in cerebral blood flow velocity and conductance responses after the consumption of cocoa-rich chocolate [21]. However, the evidence about the effects of chocolate consumption on cognitive function in postmenopausal women is scarce.

The aim of this study was to evaluate the effects of adding 10 g of cocoa-rich chocolate (99%) to the habitual diet on cognitive performance in postmenopausal women.

Materials and methods

Design and setting

This was a randomised, controlled clinical trial with two parallel groups carried out between June 2018 and August 2019 in the Research Unit for Primary Care of Salamanca (APISAL) (Spain), which is part of the Biomedical Research Institute of Salamanca (IBSAL) and the Spanish Research Network for Preventive Activities and Health Promotion in Primary Care (REDIAPP). This clinical trial has been registered at clinicaltrials.gov as NCT03492983. This manuscript presents results on cognitive performance as a secondary outcome of the trial. Results on blood pressure, as the main outcome of the intervention study [22], as well as results on body composition, as a secondary outcome [23], have been previously published.

Study participants and recruitment

A consecutive sampling was carried out in the doctor's offices of four primary healthcare centres in the city of Salamanca (Spain) to recruit women who met the selection criteria and signed the informed consent for participation. A total of 140 women in the range of 50–64 years of age and in the postmenopausal period, defined by amenorrhea for at least 12 consecutive months, were included in the study. Thirty-two women were excluded from the trial, due to one of the following criteria: personal history of cardiovascular disease; personal history of diabetes mellitus, arterial hypertension or dyslipidaemia under pharmacological treatment; hypocaloric diets; clinically demonstrable neurological and/or neuropsychological disease; treatment with hormone replacement therapy; habitual consumption of over 210 grams per week (g/week) of cocoa; cocoa intolerance and/or allergy or similar reactions to any of the components of the supplement (Figure 1).

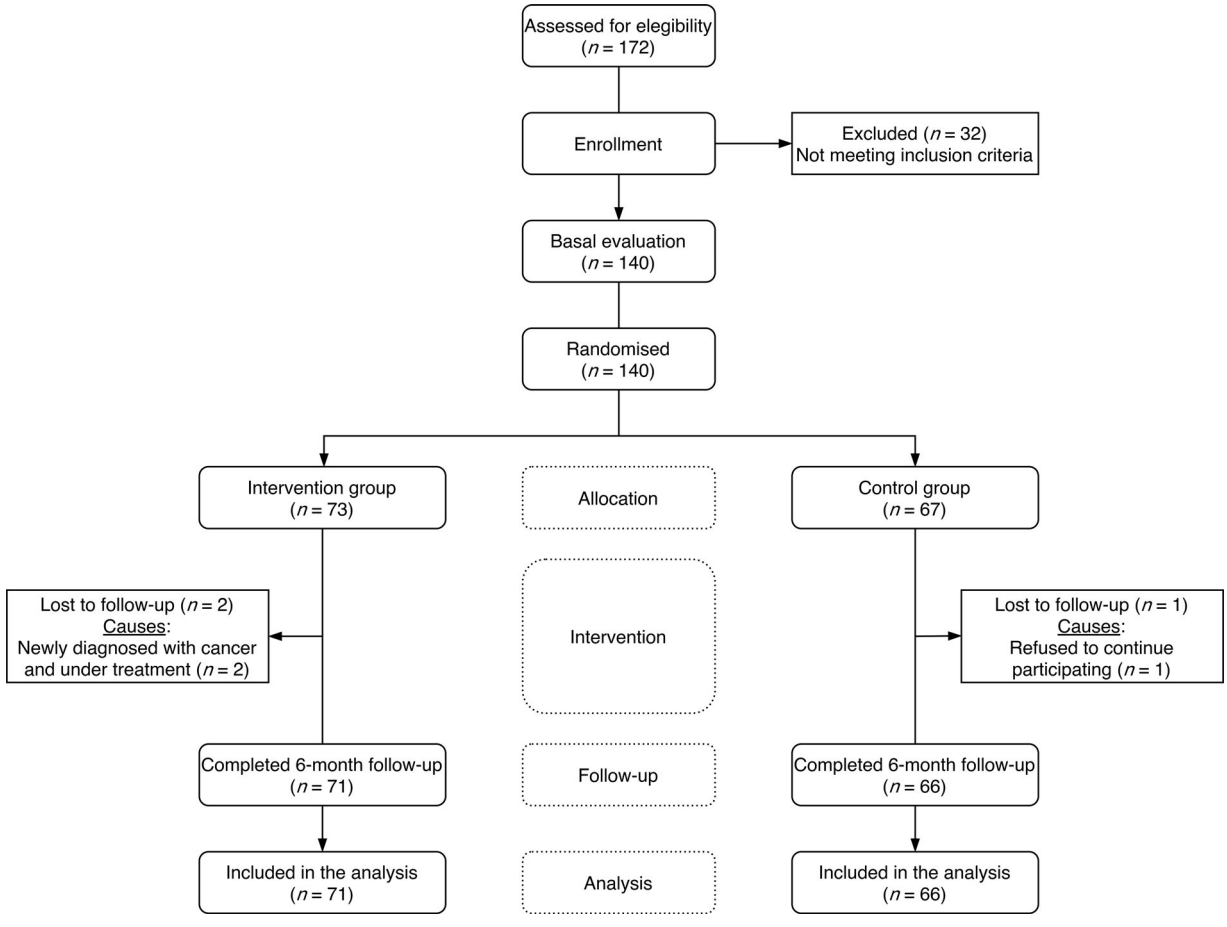

Figure 1. Flow chart of participants through the study.

Sample size

The sample size was estimated considering the change in arterial systolic pressure as the main variable of this clinical trial. To detect a minimum difference of 2.9 mm Hg in systolic arterial pressure between the two groups, 140 participants were required (70 per group), assuming an alpha risk of 0.05, a beta risk of 0.20 in a two-sided contrast, and a standard deviation of 5.8 mm Hg. In addition, a follow-up loss rate of 10% was assumed. These calculations were based on a similar study results that showed a decrease of 6.5 ± 5.8 mm Hg in systolic arterial pressure [24]. With the 140 participants included in this study, a hypothesis testing power of 80% was achieved, which allowed detecting a statistically significant difference in the mean score of the Trail Making Test B of 15 s between the intervention group and the control group, assuming an alpha risk of 0.05 in a bilateral contrast.

Procedures and randomisation

All participants paid a baseline visit and a follow-up visit 6 months after the baseline evaluation, in which the study variables were recorded (Figure 1). The intervention group paid 5 additional resupply visits 1, 2, 3, 4 and 5 months after the baseline visit, in which they were given the necessary chocolate until the next visit. Moreover, in this resupply visits, the participants of the intervention group handed a calendar with the record of the chocolate intakes done, with no other procedures involved.

The participants were randomly assigned to two parallel groups: an intervention group (IG) of 73 participants and a control group (CG) of 67 participants. The randomisation was conducted by an independent researcher using the Epidat 4.2 software [25]. The participants received the randomisation number according to the baseline visit, which was hidden until they were all assigned to a group. The information related to the treatment allocation was kept in a safe box in case of emergency unmasking.

To ensure the blinding of the study, the participants were clearly instructed not to reveal which treatment they had been assigned to in the interviews conducted by the blinded researchers. The characteristics of the intervention did not allow for the participants to be blinded. The evaluation and chocolate resupply visits in IG were conducted by different researchers in order to minimise contamination between groups.

Intervention

The CG participants did not receive any intervention. The IG participants were given chocolate with a cocoa concentration of 99% and the instructions for the consumption of 10 g of this compound as an addition to their habitual food intake every day for 6 months. After the baseline intervention, the IG participants received instructions about the consumption and storage of the chocolate supplement, recommending them to take the daily dose at the same time of the day. They were also given a calendar to record the time and intake of each day, which was handed back to the researchers in each resupply visit. The daily nutritional value of 10 g of this cocoa-rich supplement is 59 Kcal, 0.8 g carbohydrates, 1.5 g of protein and 5.1 g of fat, of which 3.1 g are saturated. The polyphenol contribution per 10 g of this product is 65.4 mg. The polyphenol profile of this supplement is shown in the previously published study protocol [26]. All participants were requested to maintain their eating and diet habits during the study period.

Main outcomes

Cognitive performance was evaluated through a brief neuropsychological test.

Attention and executive functions

Attention was measured with Trail Making Test A (TMT-A), and processing speed and cognitive flexibility (as a component of executive functions) were evaluated with Trail Making Test B (TMT-B) [27]. Furthermore, this test allows assessing visuomotor speed, visual tracking, motor function and working memory [28,29]. TMT-A consists in linking a series of numbers in ascending order, whereas TMT-B consists in linking a series of numbers and letters alternatively, with the numbers following the ascending order and the letters following the order of the alphabet. In both tests, the obtained score is based on the time (quantified in seconds) that the subject took to complete the task.

Verbal memory

Verbal memory was evaluated using the abbreviated version of the Rey Auditory Verbal Learning Test (RAVLT) [30]. Immediate verbal memory was measured based on the capacity of the participant to immediately remember a list of 15 words in 3 attempts (RAVLT-IR). The outcome variable was the number of words remembered in the third attempt. After 10 min, delayed verbal memory was measured based on the capacity of the participant to freely remember the words learned in the first part of the evaluation (RAVLT-DR).

Working memory

Working memory was assessed with the WAIS Digit Span Backward test [31]. This test consists of six categories, each with two series of numbers. The series of each category have one more number than the previous category, beginning with two numbers. The participant must immediately and inversely repeat each series. The test ends when the subject makes mistakes in the two series of one category. The score is equal to the last category of which at least one of the series of numbers was correctly followed.

Phonological fluency

Phonological fluency was evaluated with the FAS Questionnaire, which consists in naming as many words beginning with F, A and S as possible in one minute [32]. The obtained score is equal to the number of words pronounced correctly, counting out repetitions, derived words and proper nouns.

Category fluency

Category fluency measures controlled association semantic verbal fluency. This test consists in naming as many animals as possible in one minute [33]. The obtained score is equal to the sum of words pronounced correctly, counting out repetitions and derived words.

Other measurements

Sociodemographic variables

In the baseline visit, questionnaires were used to gather information about sociodemographic variables, which included questions about age, marital status and educational level. Marital status was recorded in the following categories: married/coexists, separated/divorced, single and widowed. The educational level was recorded in the following categories: primary studies, middle-high school, university studies, postgraduate studies.

Clinical variables

In the baseline visit, questionnaires were used to gather the participants' personal history of gestational diabetes, untreated hypertension and dyslipidaemia and the prescribed pharmacological treatment, as well as the time elapsed from the beginning of menopause.

Adherence to the intervention

Adherence was calculated as the percentage of days of chocolate intake with respect to the theoretical total percentage, based on the data recorded in the calendars of each IG participant.

Evaluation of chocolate consumption and habitual diet

The habitual consumption of chocolate was assessed in each of the evaluation visits through a series of questions about the amount, type and frequency of consumption in the periods between visits. To evaluate the nutritional composition of the habitual diet, which includes the distribution of macronutrients and energy consumption, a 24-hour recall was used; this reminder was recorded in 3 non-consecutive days, prior to the day of each evaluation. These data were recorded and processed using the EVIDENT app [34].

The study protocol includes the description of the method used to measure other variables that were also recorded, such as physical activity, alcohol consumption and smoking [26].

Data collection procedure, data management and monitoring

In each evaluation visit, the data were collected by a nurse, who had been previously trained for this task. Each participant of the study was identified with a unique code, which in turn identified the data collected in each of the measurements. A database was created with all the collected data, which could only be accessed by the researchers of the study.

Ethical considerations

The study was approved by the Clinical Research Ethics Committee of the Salamanca Health Area ('CREC of Health Area of Salamanca') in February 2018. The participants signed an informed consent document, in accordance with the Declaration of Helsinki. They were informed of the objectives of the project and the risks and benefits of the explorations to be carried out. The confidentiality of the participant data was guaranteed at all times in accordance with the provisions of the Organic Law 3/2018, of December 5th, on Personal Data Protection and guarantee of digital rights, and EU Regulation 2016/679 of the European Parliament and of the Council of April 27th 2016 on Data Protection (RGDP), and under the conditions established by national law 14/2007 of biomedical research.

Statistical analyses

The statistical analyses were carried out following the study protocol [26]. The data were checked for normal distribution and most data were considered normally distributed. The characteristics of the sample are presented as mean and standard deviation or median

(interquartile range) for the continuous variables, whereas the qualitative variables are expressed using frequency distribution. To evaluate the comparability between the two study groups in the baseline evaluation, the chi-square test and Student's *t*-test were used for the qualitative and quantitative variables, respectively. Fisher's exact test and Mann–Whitney *U* test were used for qualitative and quantitative variables with non-normal distribution, respectively.

The effect of the intervention on the outcome variables (cognitive performance variables) was evaluated through a covariance analysis (ANCOVA), using age, educational level, time elapsed from the beginning of menopause and daily energy consumption as covariates. The differences between groups in every case are presented as mean and 95% CI. The effect size was estimated in the change of the cognitive performance variables through the calculation of Cohen's *d*. To analyse the intragroup change in the outcome variables at 6 months with respect to the baseline evaluation, the Student's *t*-test was used for paired data, which are presented as mean and standard deviation.

Subgroup analyses were carried out based on educational level and age as baseline conditions. For the subanalysis based on age, the sample was divided taking the median of age as reference, which was 57.4 years, and the effect was evaluated through an ANCOVA adjusting for educational level, time elapsed from the beginning of menopause and daily energy consumption. To evaluate the effect in the subanalysis based on educational level, two subgroups were made (university or postgraduate studies and primary or middle-high school), and an ANCOVA was conducted using age, time elapsed from the beginning of menopause and daily energy consumption as covariates.

All analyses were performed using SPSS V.23.0 (IBM Corp, Armonk, NY) and establishing an alpha risk of 0.05 as the limit of statistical significance.

Results

Baseline characteristics of the study population

The study included 140 women: 73 in IG and 67 in CG. Three participants were lost in the follow-up: two from IG due to a newly diagnosed cancer and under treatment, and one in CG who refused to continue participating. Therefore, a total of 137 women completed the study and were included in the analysis: 71 in IG and 66 in CG (Figure 1).

The baseline characteristics of the participants showed no differences between the two groups (Table 1). Most of the women, both in IG (65.8%) and in CG (70.1%) were married or coexisted. In both groups, the educational level of most of the participants was middle-high school (30.1% in IG, 43.3% in CG). In IG, 23.3% of the women had university studies and 24.7% had postgraduate studies, whereas these percentages were 16.4% and 22.4%, respectively, in CG. The habitual consumption of chocolate, as well as the specific consumption of chocolate with over 70% of cocoa, was similar in both groups. Average adherence to the intervention (%) was 97.61 ± 3.34, with a minimum and maximum adherence of 80.56% and 100%, respectively.

Table 1. Baseline characteristics of the study population.

Variables	Intervention group (*n* = 73)	Control group (*n* = 67)	*p*[a]
Age (years)	57.1 ± 3.5	57.5 ± 3.8	0.555
Marital status (*n*, %)			0.574
Married/coexists	48 (65.8%)	47 (70.1%)	
Separated/divorced	8 (11.0%)	7 (10.4%)	
Single	15 (20.5%)	9 (13.4%)	
Widowed	2 (2.7%)	4 (6.0%)	
Educational level (*n*, %)			0.417
Primary studies	16 (21.9%)	12 (17.9%)	
Middle-high school	22 (30.1%)	29 (43.3%)	
University studies	17 (23.3%)	11 (16.4%)	
Postgraduate studies	18 (24.7%)	15 (22.4%)	
Time from menopause onset (years)	6.9 ± 4.6	6.9 ± 3.6	0.990
Untreated hypertension (*n*, %)	1 (1.4%)	0 (0.0%)	0.340
Untreated dyslipidemia (*n*, %)	8 (11.0%)	10 (14.9%)	0.484
Gestational diabetes (*n*, %)	3 (4.1%)	1 (1.5%)	0.621
Thyroid hormone treatment (*n*, %)	13 (17.8%)	10 (14.9%)	0.646
Current smoker (*n*, %)	12 (16.4%)	9 (13.4%)	0.619
Alcohol consumption (g/week)	23.1 ± 29.4	30.6 ± 48.1	0.262
Energy intake (kcal/day)	1720 ± 357	1780 ± 402	0.306
Physical activity (MET–h/week)	31.2 ± 36.8	25.7 ± 20.0	0.275
Chocolate intake (g/week)	42 (9–109)	50 (21–80)	0.925
>70% cocoa chocolate intake (g/week)	0 (0–26)	0 (0–24)	0.395

Notes: Values expressed as mean ± standard deviation, median (interquartile range) or frequencies (percent).
Abbreviations: MET, metabolic equivalent of task.
[a]Intergroup comparison by the Student's *t*-test or the Mann–Whitney *U* test for quantitative variables and by the chi-square test or the Fisher's exact test for the qualitative variables.

Results for baseline and 6-month evaluation of energy and nutrients intake of habitual diet are shown in Table 2.

Cognitive performance variables

TMT-A execution time decreased in both groups, with no differences between them. On the other hand, IG showed a TMT-B execution time decrease of −12.52 ± 35.40 s, whereas the change in CG was −0.97 ± 31.47 s, which implies a difference of −12.08 (−23.99, −0.18) seconds ($p = 0.047$) between the two groups after adjusting for age, educational level, time elapsed from the beginning of menopause and daily energy consumption. The Cohen's *d* value for this difference was −0.343 (Table 3).

Table 2. Energy intake and nutrients of habitual diet in postmenopausal women participants.

	IG (n = 71)				CG (n = 66)					
	Baseline	6 months	Change	p^a	Baseline	6 months	Change	p^a	Intergroup difference (IG-CG)[b]	p^b
Energy intake (kcal/day)	1712 ± 365	1722 ± 353	9.5 ± 389.1	0.840	1782 ± 402	1794 ± 339	12.2 ± 351.2	0.780	−2.7 (−129.6, 124.2)	0.967
Carbohydrates (g/day)	167.8 ± 45.6	162.3 ± 43.4	−5.5 ± 55.2	0.414	172.5 ± 50.0	177.9 ± 39.0	5.4 ± 48.1	0.367	−10.9 (−28.6, 6.9)	0.227
Proteins (g/day)	76.2 ± 16.8	81.7 ± 17.7	5.5 ± 19.2	0.020	78.3 ± 19.0	81.5 ± 15.4	3.2 ± 18.9	0.176	2.3 (−4.2, 8.8)	0.491
Fiber (g/day)	24.0 ± 7.7	22.7 ± 6.4	−1.4 ± 7.6	0.146	25.4 ± 9.5	25.4 ± 9.1	−0.2 ± 10.6	0.888	−1.2 (−4.3, 2.0)	0.463
Fats (g/day)	77.1 ± 20.7	77.7 ± 20.1	0.6 ± 23.0	0.823	80.1 ± 20.1	77.9 ± 18.1	−2.2 ± 17.8	0.315	2.9 (−4.2, 9.9)	0.425
Saturated fats (g/day)	25.1 ± 7.7	25.0 ± 7.8	−0.1 ± 8.5	0.920	25.5 ± 7.4	24.4 ± 6.4	−1.2 ± 7.5	0.219	1.0 (−1.7, 3.8)	0.451

Notes: Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group.
[a]Intragroup comparison by the Paired Student's t-test.
[b]Intergroup comparison by the Student's t-test.

Table 3. Cognitive performance variables in postmenopausal women participants.

	IG (n = 71)				CG (n = 66)								
	Baseline	6 months	Change	p^a	Baseline	6 months	Change	p^a	Intergroup difference (IG-CG)[b]	p^b	Adjusted Intergroup difference (IG-CG)[c]	p^c	Cohen's d
RAVLT-IR (words)	7.70 ± 1.82	8.57 ± 1.96	0.87 ± 1.82	0.000	7.47 ± 1.89	8.44 ± 2.12	0.97 ± 1.70	0.000	−0.10 (−0.70, 0.49)	0.737	−0.05 (−0.65, 0.56)	0.872	−0.057
RAVLT-DR (words)	6.79 ± 3.18	8.25 ± 2.96	1.46 ± 2.87	0.000	7.17 ± 2.78	8.35 ± 2.84	1.18 ± 2.16	0.000	0.28 (−0.58, 1.15)	0.518	0.19 (−0.69, 1.07)	0.665	0.117
Trail Making Test A (seconds)	39.32 ± 13.82	35.61 ± 11.95	−3.72 ± 12.96	0.018	40.77 ± 11.58	37.12 ± 12.31	−3.65 ± 10.74	0.007	−0.07 (−4.11, 3.97)	0.974	−0.10 (−4.34, 4.13)	0.961	0.000
Trail Making Test B (seconds)	94.00 ± 47.36	81.48 ± 31.99	−12.52 ± 35.40	0.004	91.61 ± 33.99	90.64 ± 32.64	−0.97 ± 31.47	0.803	−11.55 (−22.90, −0.20)	0.046	−12.08 (−23.99, −0.18)	0.047	−0.343
Digit Span Backwards (total score)	3.33 ± 1.26	3.20 ± 1.06	−0.13 ± 1.15	0.354	3.12 ± 1.03	3.12 ± 0.95	0.00 ± 1.16	1.000	−0.13 (−0.52, 0.26)	0.519	−0.10 (−0.51, 0.31)	0.635	−0.083
Phonological fluency (words)	13.11 ± 3.82	13.56 ± 4.22	0.45 ± 3.43	0.273	11.59 ± 3.83	12.15 ± 4.21	0.56 ± 3.54	0.203	−0.11 (−1.29, 1.07)	0.854	0.06 (−1.14, 1.25)	0.926	−0.028
Category fluency (words)	20.27 ± 5.14	22.52 ± 5.40	2.25 ± 5.43	0.001	20.12 ± 4.29	22.44 ± 4.32	2.32 ± 3.96	0.000	−0.06 (−1.68, 1.55)	0.937	0.26 (−1.40, 1.92)	0.758	0.000

Notes: Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group, RAVLT-IR: Rey Auditory Verbal Learning Test−Immediate Recall, RAVLT−DR: Rey Auditory Verbal Learning Test−Delayed Recall.
[a]Intragroup comparison by the Paired Student's t-test.
[b]Intergroup comparison by the Student t test.
[c]Intergroup comparison by ANCOVA adjusted for age, educational level, time from menopause onset and energy intake.

The results obtained in RAVLT-IR, RAVLT-DR and category fluency show a score increase in both groups, with no differences between them. With respect to the scores obtained in the WAIS Digit Span Backwards and FAS tests, no changes were found.

Subgroup analysis by age

The results of the subanalysis by age, adjusted for educational level, time elapsed from the beginning of menopause and daily energy consumption, are shown in Table 4.

The TMT-B execution time decreased in the IG participants with respect to CG both in the group of women aged ≤57.40 years and in that of women aged >57.40 years, showing no statistically significant differences.

No relevant changes were found in these subgroups in any of the cognitive performance variables measured.

Subgroup analysis by educational level

The women with university studies showed a difference of −10.39 (−24.15, 3.36) seconds in the TMT-B execution time between the two groups after adjusting for age, time elapsed from the beginning of menopause and daily energy consumption, although this difference was not statistically significant (p = 0.136), as was observed in the group of women without university studies (−12.11 s; −30.62, 6.41; p = 0.196) (Table 5).

Similarly, no relevant changes were observed in the scores of RAVLT-IR or RAVLT-DR, and no differences were found between groups based on educational level in neither TMT-A nor any of the cognitive performance variables evaluated.

Discussion

The findings of this study show a decrease of TMT-B execution time in IG after the daily consumption of 10 g of cocoa-rich (99%) chocolate, which suggests a slight improvement in the cognitive performance related to cognitive flexibility and processing speed, as components of executive functions. However, no relevant differences were found in attention, immediate or delayed verbal memory, phonological or category fluency, or working memory.

The effect of cocoa on cognitive performance has been studied by different authors, although the obtained results are heterogeneous. The findings of the present clinical trial, in which there was an improvement in the execution time of a test that explores cognitive flexibility with a moderate effect size, are in line with those reported in other studies. Nurk et al. [18] observed

Table 4. Subanalysis of cognitive performance variables by age in postmenopausal women participants.

	Age ≤57.4 years						Age >57.4 years					
	IG (n = 38)		CG (n = 33)				IG (n = 35)		CG (n = 34)			
	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p[a]	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p[a]
RAVLT-IR (words)	7.50 ± 1.57	8.85 ± 1.90	7.52 ± 1.75	8.66 ± 2.13	0.10 (−0.78, 0.98)	0.817	7.92 ± 2.02	8.27 ± 2.00	7.45 ± 2.02	8.23 ± 2.12	−0.33 (−1.17, 0.50)	0.425
RAVLT-DR (words)	6.42 ± 2.76	8.54 ± 2.73	7.12 ± 2.61	8.58 ± 2.88	0.26 (−1.01, 1.54)	0.681	7.26 ± 3.53	7.94 ± 3.20	7.35 ± 3.05	8.12 ± 2.83	−0.10 (−1.27, 1.07)	0.865
Trail Making Test A (seconds)	36.82 ± 9.41	34.16 ± 11.97	38.79 ± 12.64	34.70 ± 10.75	1.26 (−3.79, 6.30)	0.62	41.89 ± 16.97	37.18 ± 11.90	43.26 ± 10.49	39.55 ± 13.43	−1.66 (−8.83, 5.50)	0.64
Trail Making Test B (seconds)	90.39 ± 47.65	77.51 ± 30.89	85.24 ± 34.52	82.79 ± 27.32	−11.36 (−27.45, 4.74)	0.16	97.91 ± 46.57	85.79 ± 33.07	98.62 ± 32.44	98.48 ± 35.93	−14.30 (−32.50, 3.91)	0.12
Digit Span Backwards (total score)	3.32 ± 1.32	3.22 ± 1.18	3.12 ± 1.14	3.24 ± 0.94	−0.21 (−0.79, 0.36)	0.46	3.34 ± 1.21	3.18 ± 0.92	3.15 ± 0.93	3.00 ± 0.97	0.05 (−0.57, 0.67)	0.86
Phonological fluency (words)	13.42 ± 4.27	13.81 ± 4.34	11.67 ± 3.96	12.24 ± 4.35	−0.25 (−2.00, 1.50)	0.77	12.37 ± 3.53	13.29 ± 4.14	11.50 ± 3.70	12.06 ± 4.14	0.54 (−1.09, 2.16)	0.51
Category fluency (words)	21.11 ± 5.10	24.30 ± 5.40	21.36 ± 3.87	23.70 ± 3.96	1.38 (−1.23, 3.98)	0.29	19.14 ± 5.23	20.59 ± 4.76	18.74 ± 4.40	21.18 ± 4.35	−0.78 (−2.86, 1.30)	0.45

Notes: Subgroups were created based on median age (57.40 years). Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group.
[a]Intergroup comparison by ANCOVA adjusted for educational level, time from menopause onset and energy intake.

Table 5. Subgroup analysis of cognitive performance variables by educational level in postmenopausal women participants.

	University studies (Bachelor, Postgraduate)						Non-university studies (Elementary education, Middle-High school)					
	IG (n = 35)		CG (n = 25)				IG (n = 36)		CG (n = 41)			
	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a
RAVLT-IR (words)	8.41 ± 1.83	9.26 ± 1.86	8.04 ± 2.21	9.24 ± 2.35	−0.43 (−1.46, 0.61)	0.41	7.05 ± 1.52	7.91 ± 1.84	7.13 ± 1.55	7.96 ± 1.83	0.12 (−0.61, 0.84)	0.74
RAVLT-DR (words)	7.54 ± 3.75	9.71 ± 2.90	8.42 ± 2.98	9.64 ± 2.66	0.82 (−0.71, 2.34)	3	6.16 ± 2.35	6.83 ± 2.27	6.48 ± 2.47	7.56 ± 2.68	−0.19 (−1.21, 0.82)	9
Trail Making Test A (seconds)	35.69 ± 11.57	33.11 ± 10.02	36.50 ± 9.94	35.68 ± 16.06	−2.68 (−9.18, 3.81)	0.28	42.53 ± 14.82	38.03 ± 13.25	43.95 ± 11.97	38.00 ± 9.46	1.17 (−4.60, 6.94)	0.70
Trail Making Test B (seconds)	75.51 ± 28.65	65.09 ± 20.32	77.57 ± 22.38	78.72 ± 26.99	−10.39 (−24.15, 3.36)	0.13	111.03 ± 54.00	97.42 ± 33.41	101.20 ± 36.90	97.90 ± 33.94	−12.11 (−30.62, 6.41)	0.19
Digit Span Backwards (total score)	3.97 ± 1.18	3.56 ± 0.96	3.54 ± 0.99	3.64 ± 0.86	−0.39 (−1.10, 0.32)	0.27	2.74 ± 1.03	2.86 ± 1.05	2.88 ± 0.98	2.80 ± 0.87	0.23 (−0.27, 0.73)	0.36
Phonological fluency (words)	14.66 ± 3.31	15.03 ± 3.94	13.38 ± 2.95	13.16 ± 4.11	0.86 (−1.15, 2.86)	0.39	11.32 ± 3.84	12.14 ± 4.04	10.44 ± 3.87	11.54 ± 4.21	−0.15 (−1.64, 1.35)	0.84
Category fluency (words)	21.97 ± 4.89	24.20 ± 5.98	22.23 ± 4.22	23.44 ± 4.44		0.20	18.50 ± 5.01	20.89 ± 4.24	18.63 ± 3.82	21.83 ± 4.18		0.45

Notes: Values are means ± SDs and differences are means (95% CI).
Abbreviations: IG: Intervention Group, CG: Control Group, RAVLT-IR: Rey Auditory Verbal Learning Test–Immediate Recall, RAVLT-DR: Rey Auditory Verbal Learning Test–Delayed Recall.
[a]Intergroup comparison by ANCOVA adjusted for age, time from menopause onset and energy intake.

that regular consumers of chocolate performed better in the cognitive tests, with executive functions being among the most favoured functions. Similarly, other authors have reported an improvement in executive functions, shown by the reduction in the time required to complete TMT-A and TMT-B, after the 8-week consumption of two polyphenol-rich cocoa compounds against another compound with low polyphenol concentration in elderly subjects, both without cognitive deterioration [8] and with mild cognitive impairment [19]. On the other hand, the authors of another clinical trial did not observe a short-term benefit in executive functions after the consumption of chocolate [16].

The exact causes that trigger these effects on cognitive performance are still unknown, although different mechanisms have been proposed. Some studies suggest that an increase in cerebral blood flow caused by the polyphenols of cocoa can improve the results of cognitive performance tasks [10,35]. Likewise, Scholey et al. [4] state that the improvement in endothelial function and blood flow caused by polyphenols could be related to these effects. It has also been suggested that brain-derived neurotrophic factor (BDNF) could act as a mediator in the cognitive improvement after the intake of cocoa [7]. Other authors suggest that a decrease in insulin resistance may be involved in the appearance of these effects on cognitive performance [8,19]. Additionally, since the modification in the levels of estrogens may affect postmenopausal women cognitive state [20], estrogen is thought to have a key role on cognitive changes during menopause with a neuroprotective effect [36]. Evaluation of hormone levels was not carried out in the present study, however it should be considered for further research.

Regarding education, a large percentage of the women of both groups showed a high level, which was higher in IG. However, our findings do not show statistically significant differences between the two groups based on their educational level, unlike previous studies which report that people with a higher educational level show better cognitive performance [28,37].

It seems that the intervention did not affect other cognitive functions, which is in line with the results of other clinical trials, such as the one conducted in postmenopausal women by Marsh et al. [21] and the one conducted by Pase et al. [15]. On the other hand, other studies have reported beneficial effects in other cognitive aspects, such as working memory and attention [4,12]. Nurk et al. [18] observed an improvement in verbal memory related to the consumption of chocolate in an observational study. Similarly, other authors have reported an improvement in phonological fluency after the intake of polyphenol-rich cocoa

compounds in elderly people [8,19]. Furthermore, Karabay et al. [17] suggested that cocoa flavanols could improve certain aspects of attention. Likewise, Grassi et al. [9] found an increase in working memory after the consumption of flavonols with sleep deprivation.

It is important to take into account that the repetition of the tests 6 months after the baseline visit may have introduced a learning component, inducing a better performance in the tests conducted in the follow-up visit for both groups, despite the fact that this is the time period recommended to avoid the learning effect.

The clinical trial that this study is part of aimed to evaluate the effect of adding a daily amount of 10 g of chocolate high in cocoa content (99%) and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women. With a randomised clinical trial design, this intervention aimed to assess the effects of the chronic intake of cocoa-rich chocolate on a population with specific characteristics, as it is postmenopausal women. This trial had a larger number of participants compared to other studies [21,38,39] assessing the effect of cocoa rich chocolate on cognitive function, although the sample size could be insufficient for the contrast of this variable since it was not estimated based on this outcome. Moreover, the study intervention consisted on the addition of an amount of commercially available chocolate, which was not specifically designed for this purpose and has specific and unmodifiable characteristics, to the habitual diet. This provided a real clinical context and enabled the assessment of the potential benefits, as well as potential harms, of the intake of this product as a whole, as intended. Hence, this makes the results of this trial more accessible than those of other studies [8,21] using non-commercialized compounds elaborated specifically for research purposes that are not available in a real context. The amount of 10 g of cocoa-rich (99%) chocolate used in the intervention fits with the recommendations of the European Food Safety Authority [40] and coincides with the data provided by Nurk et al. [18], who reported a maximum beneficial effect on cognitive performance with the daily consumption of approximately 10 g of chocolate. Although the mean baseline chocolate intake was about 70 g/week (10 g/day) in both groups (median (interquartile range): 42 (9−109) g/week in the IG and 50 (21−80) g/week in the CG), it is important to note that this includes dark chocolate, milk chocolate and white chocolate. Nonetheless, the mean >70% cocoa chocolate (dark chocolate) intake was less than 20 g/week in both groups (median (interquartile range): 0 (0−26) g/week in the IG and 0 (0−24) g/week in the CG). The daily consumption of 10 g of cocoa-rich (99%) chocolate added to the habitual diet in the intervention group fits with the EFSA recommendations and ensures that every participant in this group complies with these recommendations. However, the chocolate used in this trial contains a lower concentration of polyphenols than the compounds used in other studies which are specifically designed for this purpose [4,8]. Moreover, dietary polyphenol intake could be very high, as observed in previous studies with an estimated mean total intake of 820 ± 323 mg polyphenols/day in the PREDIMED cohort [41], and 1193 ± 510 mg/day in the SU.VI.MAX cohort [42]. The cocoa polyphenol contribution of the amount of chocolate used in the intervention seems to be low compared with the amount of polyphenols consumed in the diet; therefore, it may be insufficient to show important changes in the effect size and should be considered for further research.

The population sample in which this study was carried out presents special characteristics. Postmenopausal women may have cognitive difficulties [20] related to hormonal changes, which are typical of this period. Therefore, it is important to develop interventions such as the one conducted in this trial, that is, with the aim of improving cognitive function in this population group without causing adverse effects.

Regarding the limitations of this study, it is worth highlighting that it was not possible to blind the participants due to the nature of the intervention, although the blinding of the researchers who recorded the measurements and conducted the statistical analyses was ensured. Lack of consideration of the amount of polyphenols consumed by individual participants as a criterion for inclusion and the lack of control of polyphenol consumption during the study could be another limitation. However, this was not possible since the tool used to assess the nutritional composition of the habitual diet does not provide data on the polyphenol content of the diet or the specific foods consumed. Dietary supplements intake was not recorded as well. Although we could assume that randomisation had balanced the groups with respect to dietary intake as well as polyphenol intake, this should be considered in future trials.

To conclude, the results of this study suggest that the consumption of cocoa-rich (99%) chocolate in addition to the habitual diet could be related to a slight improvement in cognitive performance regarding cognitive flexibility and processing speed in postmenopausal women, with no changes in the rest of the cognitive performance variables evaluated. However, it is necessary to carry out further studies that allow clarifying the possible benefits of consuming

cocoa-rich chocolate in cognitive performance for this population group.

Acknowledgements

The authors are grateful to all the volunteers for their participation, and the professionals involved in the study: José I Recio-Rodríguez, José A Maderuelo-Fernández, Luis García-Ortiz, Manuel A Gómez-Marcos, Irene A García-Yu, Rosario Alonso-Domínguez, Sara Mora-Simón, Natalia Sánchez-Aguadero, Jesús González-Sánchez, Cristina Agudo-Conde, Cristina Lugones-Sánchez, Benigna Sánchez-Salgado, Carmen Castaño-Sánchez, Emiliano Rodríguez-Sánchez, Susana González-Manzano, Olaya Tamayo-Morales, and Susana González-Sánchez.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported in part by grants funded by the Gerencia Regional de Salud de Castilla y León (GRS 1583/B/17).

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Chocolate rico en cacao y calidad de vida en mujeres posmenopáusicas. Ensayo clínico aleatorizado

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Nutrients 2020 Sep 10;12(9):2754.

La menopausia tiene un impacto negativo sobre la calidad de vida (CdV). El objetivo de este estudio fue analizar el efecto sobre la CdV de añadir 10 g al día de chocolate con una alta concentración de cacao (99%) a la dieta habitual, durante 6 meses, en una muestra de mujeres posmenopáusicas. Las mujeres posmenopáusicas (n = 140) de 50 a 64 años de edad fueron aleatorizadas a recibir 10 g al día de chocolate rico en cacao añadidos a su dieta habitual o no recibir ningún suplemento. Todas las variables se midieron al inicio del estudio y tras 6 meses de intervención. La CdV se evaluó utilizando la versión de 3 niveles del EuroQol-5D (EuroQoL-5D-3L), la escala visual analógica del EuroQol (EQ-VAS) y la escala Cervantes. Los análisis de la covarianza (ANCOVA) ajustados por los principales determinantes de la CdV considerados en este estudio no mostraron cambios en la puntuación global de la CdV evaluada con el EuroQoL-5D-3L. El grupo de intervención mostró un incremento de 6,0 puntos (intervalo de confianza (IC) 95% 0,4 a 11,7) en el EQ-VAS en comparación con el grupo control (p = 0,036). No se observaron cambios significativos entre grupos en la puntuación global de la CdV ni en las dimensiones o subdimensiones medidas con la escala Cervantes. El aporte diario adicional de 10 g de chocolate rico en cacao en mujeres posmenopáusicas podría tener un leve impacto en su percepción de su estado de salud, aunque sin modificar la calidad de vida relacionada con la salud o las dimensiones que la componen.

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Cocoa-Rich Chocolate and Quality of Life in Postmenopausal Women: A Randomized Clinical Trial

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Received: 17 August 2020; Accepted: 7 September 2020; Published: 10 September 2020

Abstract: Menopause has a negative impact on quality of life (QoL). The aim of the present study was to analyse the effect on QoL of adding 10 g per day of chocolate with a high concentration of cocoa (99%) to the habitual diet, for 6 months, in a sample of postmenopausal women. Postmenopausal women (n = 140) aged 50–64 years were randomised to either an addition of 10 g per day of cocoa-rich chocolate to their usual diet or no supplement addition. All variables were measured at baseline and after six months of intervention. QoL was evaluated using the 3-level version of EuroQol-5D (EuroQoL-5D-3L), the EuroQol Visual Analogue Scale (EQ-VAS) and the Cervantes scale. Analysis of covariance (ANCOVA) analyses adjusted for the main determinants of QoL considered in this study showed no changes in the global score of QoL evaluated with the EuroQoL-5D-3L. The intervention group showed an increase of 6.0 points (95% confidence interval (CI): 0.4, 11.7) in the EQ-VAS compared to the control group (p = 0.036). No significant changes were observed between groups in the global score of QoL nor in the dimensions and subdimensions measured with the Cervantes scale. The additional daily contribution of 10 g of cocoa-rich chocolate in postmenopausal women could have a slight impact on their perception toward their health state, although without modifying the health-related QoL or the dimensions that compose it.

Keywords: chocolate; postmenopause; quality of life; randomised controlled trial

1. Introduction

Menopause has a negative impact on quality of life (QoL), with a gradual decrease from the premenopausal period to the postmenopausal period [1], in terms of both physical and mental health [2,3]. Such decrease in the QoL of postmenopausal women is associated with the appearance of genitourinary [4–6] and, especially, vasomotor [1] symptoms, such as hot flushes [7]. Moreover, in many cases, other psychological factors appear together during the postmenopausal period, such as depression, which worsen the perception toward QoL [8].

The consumption of chocolate, especially dark chocolate, has been associated with small, yet beneficial changes in the cardiovascular health of postmenopausal women [9]. However,

very few studies have analysed its relationship with mental health and/or components of QoL. Balboa-Castillo et al. [10] analysed a cohort of 4599 individuals (average age: 54.1 years, 50.8% women), with no evidence of correlation between QoL and a greater or lower consumption of 10 g/day of chocolate, although these authors did not include an analysis based on age and sex. In women, the consumption of chocolate could have a significant positive impact on sexual function, especially on sexual desire [11]. A greater consumption of chocolate has been associated with a higher score in the Centre for Epidemiologic Studies Depression Scale (CES-D) [12], in which 16 or more points often represent a positive screening result, although no cause–effect relationship has been established between showing more signs of depression and a greater consumption of chocolate [13].

The benefits of dark chocolate have been attributed to a type of polyphenols known as flavonoids, which include flavonols and other polyphenols, such as epicatechin and catechin. In this line, some studies [14] have related the consumption of certain flavonol- and polyphenol-rich products, such as propolis [15], fruits and vegetables [16], to QoL, with rather inconclusive results. In women, the habitual consumption of coffee has been associated with slight improvement in the mental dimension of QoL [17]. Similarly, in postmenopausal women, the consumption of fermented soy has been related to an improvement in QoL [18], whereas the Mediterranean diet, which includes a large variety of polyphenol-rich products, has not been clearly associated with an improvement in QoL in older adults [19].

To sum up, despite the worsening of QoL that takes place during menopause and the indications about the possible positive effect that the consumption of chocolate can have on such deterioration, there are very few studies that approached this topic, obtaining divergent and poorly clarifying results [10,11,13]. Beyond the evaluation of pharmacological and/or nutritional therapies during menopause, it is fundamental to determine the real impact of these on QoL [20].

This study aimed to assess the effect of adding 10 g per day of chocolate with a high concentration of cocoa (99%) to the habitual diet, for 6 months, on QoL, in a sample of postmenopausal women.

2. Materials and Methods

2.1. Design and Setting

This is a controlled, randomised clinical trial with two parallel groups. The participants were recruited in the doctor offices of four urban primary care centres of Salamanca (Spain) through consecutive sampling. The evaluation period was from June 2018 to August 2019. It was conducted in the Primary Care Research Unit of Salamanca (APISAL), which is part of the Spanish Research Network for Preventive Activities and Health Promotion in Primary Care (redIAPP) and the Institute of Biomedical Research of Salamanca (IBSAL). The clinical trial has been registered in clinicaltrials.gov as NCT03492983. The protocol of the trial has been published [21].

This manuscript presents results on quality of life as a secondary outcome from the clinical trial. Results on blood pressure, as the main outcome of the trial [22], as well as results on body composition, as a secondary outcome [23], have been previously published.

2.2. Study Participants

The study included 140 women aged 50–64 years in the postmenopausal period, defined as amenorrhea for at least 12 consecutive months. The exclusion criteria were: personal history of cardiovascular disease, diabetes mellitus, arterial hypertension or dyslipidaemia under pharmacological treatment, hypocaloric diets, clinically demonstrable neurological and/or neuropsychological disease, hormone replacement therapy, habitual consumption of over 210 g per week (g/week) of cocoa and intolerance and/or allergic reaction to cocoa or any of components of the supplement.

2.3. Sample Size

Sample size estimation was carried out considering the change in arterial systolic pressure as the main variable of this clinical trial. To detect a minimum difference of 2.9 mm Hg in systolic arterial pressure between the two groups, 140 participants were required (70 per group), assuming an alpha risk of 0.05, a beta risk of 0.20 in a two-sided contrast and a standard deviation (SD) of 5.8 mm Hg. A follow-up loss rate of 10% was also assumed. These calculations were based on the results obtained in a similar study that reported a decrease of 6.5 ± 5.8 mm Hg in systolic arterial pressure [24]. With the 140 participants included in this study, we obtained a power of 72% for the hypothesis test to detect a statistically significant difference of 6 points in the mean score of the EuroQol Visual Analogue Scale (EQ-VAS) between study groups, assuming an alpha risk of 0.05 in a two-sided contrast.

2.4. Procedures and Randomisation

The study variables were measured in all participants at baseline and at 6-month follow-up (Figure 1). The women in the intervention group paid another 5 visits for chocolate resupply at 1, 2, 3, 4 and 5 months after the baseline visit. In these resupply visits, held in the health centre, no other procedure was carried out, except for the supply of the necessary chocolate until the next visit and the gathering of a calendar with the record of the chocolate intakes performed.

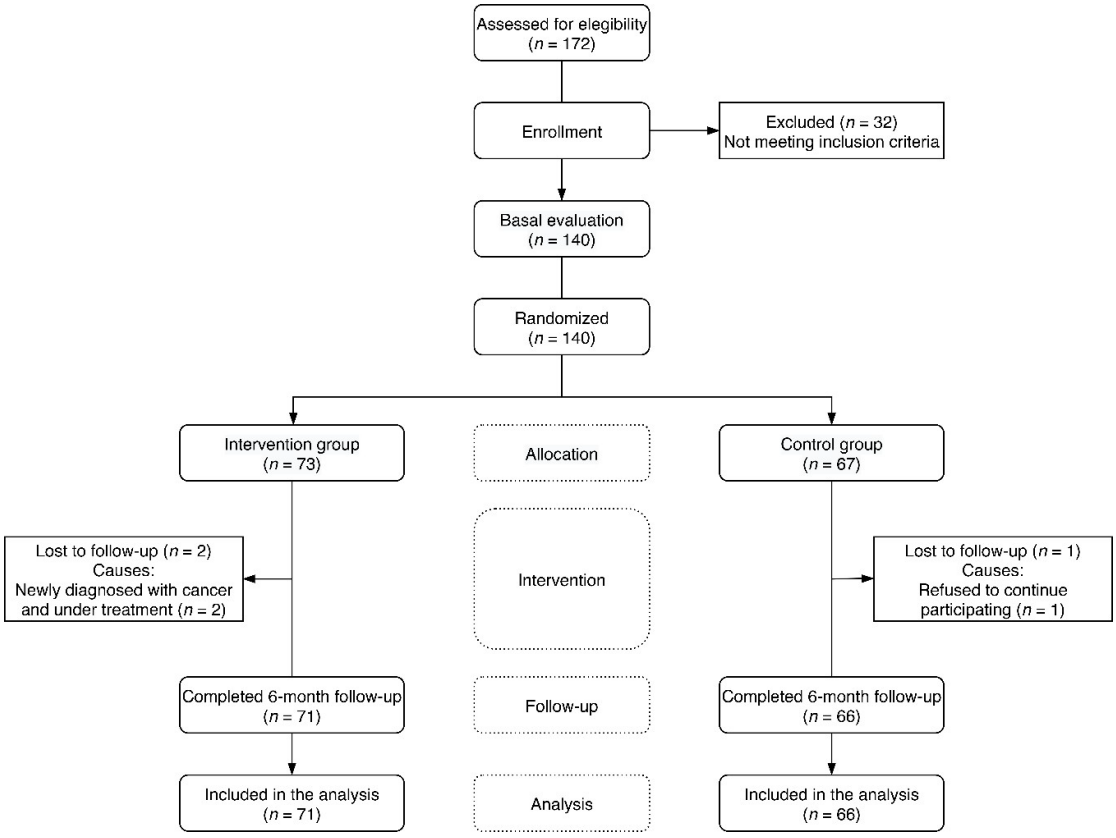

Figure 1. Flow diagram of postmenopausal women through the study.

Participants were randomised into two groups: intervention group (IG) and control group (CG). The randomisation sequence was carried out by an independent researcher using the Epidat 4.2 software [25]. Depending on the order of the baseline visit, participants received their randomisation number, which was hidden until all the women were assigned to a group. To ensure the blinding, the participants were clearly requested not to reveal their group to the blinded researchers during the interviews.

The characteristics of the intervention impeded the blinding of the participants. With the aim of minimising contamination between groups, the evaluation and chocolate resupply visits in the IG were conducted by different researchers. The information related to the randomisation of the treatment was kept in a safe in case of emergency unblinding.

2.5. Intervention

Participants in the CG did not receive any intervention, while participants in the IG were supplied with cocoa-rich chocolate (99%) and were instructed to consume 10 g daily of this supplement added to their habitual food intake. The intervention period was 6 months. During the first supply visit, the IG participants were provided with instructions about the consumption and storage of the chocolate supplement, recommending them to take the daily dose at the same time of the day. Additionally, women in the IG were asked to record the time of each daily intake in a calendar provided by the researchers, which was returned to them at each resupply visit.

The daily nutritional contribution of 10 g of this chocolate is 59 kcal, 0.8 g of carbohydrates, 1.5 g of protein and 5.1 g of fat, of which 3.1 g correspond to saturated fat. The contribution of polyphenols per 10 g of this product is 65.4 mg. All participants were asked to continue with their usual dietary pattern without modifying their eating habits during the study period.

2.6. Main Outcomes

Quality of life (QoL) was evaluated using two tools: the 3-level version of EuroQol-5D (EuroQoL-5D-3L) and the Cervantes scale.

EuroQol [26,27] is a health state measurement that includes two tools: the EuroQol-5D (EQ-5D) and the EuroQol Visual Analogue Scale (EQ-VAS). In the present study, the 3-level version of EQ-5D (EQ-5D-3L) was used, which descriptively analyses five dimensions (mobility, self-care, usual activities, pain and discomfort and anxiety and depression) on a scale of three possible answers categorised from 1 to 3 (1 "I have no problems", 2 "I have some problems", 3 "I have serious problems"). The results are presented through a descriptive analysis of each of the five dimensions and through the estimation of a general health state index between 0 (worst health state) and 1 (best health state) [28]. This EQ-5D summary index is derived by applying a formula that essentially attaches values (weights) to each of the levels in each dimension. The index can be calculated by deducting the appropriate weight from 1, the value for full health.

The EQ-VAS is a visual analogue scale that evaluates the general health state perceived by each individual. The range of this scale is between 0 (very bad health state) and 100 (optimal health state).

The Cervantes scale [29] was specifically designed for postmenopausal women. It assesses the impact of menopause on different physical and psychosocial characteristics and, especially, its effect on general wellbeing. It consists of 31 items structured in 4 dimensions: menopause and health, sexuality, mental domain and relationship. Furthermore, the dimension "menopause and health" includes three subdimensions: vasomotor symptoms, health and ageing. Regarding the global score of the scale, the highest score is 155 points, which represents low QoL, whereas the lowest score is 0 points, which represents the best QoL possible. All dimensions and subdimensions have a similar score range, where the lowest value indicates the best QoL and the highest value indicates the worst QoL.

2.7. Other Measurements

During the baseline evaluation, information about the clinical and sociodemographic variables was gathered. This information included the marital status, the education level and the time elapsed from the onset of menopause. Moreover, the history of the following morbidities was also recorded: gestational diabetes, hypertension and dyslipidaemia without pharmacological treatment and diagnosed depression. A more detailed description of the methodology and of the assessment of other variables, such as physical activity, alcohol consumption and smoking, is included in the trial protocol [21].

2.8. Data Collection Procedure, Data Management and Monitoring

The data collection in the evaluation visits was conducted by a previously trained nurse: this person was not the researcher in charge of making the randomisation and later analysis of the data. A unique identification code was used for every participant of the study in order to identify the data recorded in each measurement. Thus, a database was created, which could only be accessed by the professionals who worked in the study.

2.9. Ethical Considerations

The study was approved by the Clinical Research Ethics Committee of the Salamanca Health Area ('CREC of the Health Area of Salamanca') in February 2018 (ethic approval code: PI11812/2017). Informed consent was provided by each participant in accordance with the Declaration of Helsinki. All participants received information on the objectives of the project and the risks and benefits of the explorations to be conducted. The confidentiality of the participants' data was guaranteed at all times in accordance with the provisions of Organic Law 3/2018, of 5 December, on Personal Data Protection and digital rights, and European Regulation 2016/679 of the European Parliament and of the Council of 27 April 2016 on General Data Protection (GDPR), and under the conditions established by Spanish Law 14/2007 on biomedical research.

2.10. Statistical Analyses

The statistical analyses were performed according to the study protocol [21]. The data were checked for normal distribution and most of the data were considered normally distributed. The characteristics of the study population are presented as mean and standard deviation (SD) or median (interquartile range) for the continuous variables and as frequency distribution for the qualitative variables. To evaluate the comparability in the baseline evaluation between the two groups, the chi-square test and Fisher's exact test were used for the qualitative variables. In addition, a Student's *t*-test and Mann–Whitney U test were applied to compare the mean values between groups. The effect of chocolate consumption on the main variables of QoL was analysed through an analysis of covariance (ANCOVA), adjusted for the main determinants of QoL gathered in the interviews: age, education level, marital status, time elapsed since the beginning of menopause, daily calorie intake (kcal), baseline consumption of chocolate with 70% cocoa, physical activity, alcohol consumption, smoking, depression and untreated dyslipidaemia. The results of these analyses are presented as estimated marginal mean and its 95% confidence interval (CI). The change in each group was analysed using a Student's *t*-test for paired data. To address the possible bias due to a lack of response in some items of the Cervantes scale, we applied the multiple imputation by chained equations with 50 sets of data imputed to the results and covariables [30,31]. The estimations of each set of imputed data were combined following the guidelines described by Rubin [32]. An alpha risk of 0.05 was established as the limit of statistical significance. All analyses were performed using SPSS V.23.0 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics of the Study Participants

A total of 140 women (IG: n = 73, CG: n = 67) were included in this study. Three participants were lost in the evaluation at 6 months from the beginning of the study (IG: n = 2, CG: n = 1). The two losses in IG corresponded to two women who were diagnosed with cancer under pharmacological treatment during the study period, whereas the one from CG was a woman who decided to abandon the study in the follow-up visit (Figure 1). At baseline, no differences were found in any of the clinical or sociodemographic variables analysed (age, marital status or education level), nor in the time elapsed from the beginning of menopause. The baseline consumption of chocolate (g/week) was similar in both groups, as well as the rest of the analysed lifestyles: smoking, alcohol consumption, daily calorie intake and physical activity. Similarly, no differences were found in the baseline evaluation in terms of

the number and percentage of women with a history of gestational diabetes, depression, untreated dyslipidaemia or hypertension (Table 1). Daily energy intake and nutrients of habitual diet, as well as chocolate intake, remained unchanged during the intervention in both groups.

Table 1. Baseline characteristics of postmenopausal women participants in the study.

	Intervention Group (*n* = 73)	**Control Group (*n* = 67)**
Age, years	57.1 ± 3.5	57.5 ± 3.8
Marital status, *n* (%)		
Married/cohabitant	48 (65.8)	47 (70.1)
Separated/divorced	8 (11.0)	7 (10.4)
Single	15 (20.5)	9 (13.4)
Widowed	2 (2.7)	4 (6.0)
Education level, *n* (%)		
Elementary education	16 (21.9)	12 (17.9)
Middle–High school	22 (30.1)	29 (43.3)
Bachelor	17 (23.3)	11 (16.4)
Postgraduate	18 (24.7)	15 (22.4)
Time from menopause onset, years	6.9 ± 4.6	6.9 ± 3.6
Chocolate intake, g/week	42 (9–109)	50 (21–80)
>70% cocoa chocolate intake, g/week	0 (0–26)	0 (0–24)
Lifestyles		
Smokers, *n* (%)	12 (16.4)	9 (13.4)
Adequate alcohol consumption, *n* (%)	71 (97.3)	65 (97.0)
Physical activity light intensity, MET h/week	18.5 ± 9.4	18.8 ± 11.3
Energy, kcal/day	1720 ± 357	1780 ± 402
Morbidities, *n* (%)		
Untreated dyslipidaemia	8 (11.0)	10 (14.9)
Untreated hypertension	1 (1.4)	0 (0)
Gestational diabetes	3 (4.1)	1 (1.5)
Depression	15 (20.5)	15 (22.4)

Values expressed as mean ±standard deviation, median (interquartile range) or frequencies. Abbreviations: MET, metabolic equivalent of task.

3.2. Changes in QoL Measured with EuroQol

A descriptive analysis of the five dimensions evaluated in the EQ-5D-3L (mobility, self-care, usual activities, anxiety/depression and pain/discomfort) both in the baseline evaluation and at the 6-month evaluation visit is shown in Table 2. No differences were observed in the baseline evaluation between the groups in any of the dimensions.

In a model adjusted for the main determinants of QoL, the group that consumed 10 g of cocoa-rich chocolate daily showed a non-significant increase of 0.044 in the EQ-5D-3L score (95% CI: −0.012, 0.099) (p = 0.125), with respect to the CG. Moreover, the results of the EQ-VAS showed an increase of 6.0 (95% CI: 0.4, 11.7) in favour of the intervention group (p = 0.036) (Table 3).

3.3. Changes in QoL Measured with the Cervantes Scale

Using the same ANCOVA model adjusted for the main determinants of QoL considered in this study, no significant changes were observed in the global score of QoL evaluated with the Cervantes scale (1.1 points; 95% CI: −4.5, 6.7; p = 0.478). Similarly, no changes were found when analysing menopause and health, mental domain, sexual relations and relationship; likewise, the subdimensions of vasomotor symptoms, health and ageing did not show any significant differences (Table 4).

Table 2. EQ-5D-3L variables in postmenopausal women participants.

	Intervention Group (*n* = 71)		**Control Group (*n* = 66)**	
	Baseline	**6 Months**	**Baseline**	**6 Months**
Mobility, *n* (%)				
No problems in walking about	65 (91.5)	69 (97.2)	62 (93.9)	60 (90.9)
Some problems in walking about	6 (8.5)	2 (2.8)	4 (6.1)	6 (9.1)
Confined to bed	-	-	-	-
Self-care, *n* (%)				
No problems with self-care	71 (100)	71 (100)	66 (100)	66 (100)
Some problems washing or dressing herself	-	-	-	-
Unable to wash or dress herself	-	-	-	-
Usual activities, *n* (%)				
No problems with performing her usual activities	66 (93.0)	69 (97.2)	65 (98.5)	63 (95.5)
Some problems with performing her usual activities	5 (7.0)	2 (2.8)	1 (1.5)	3 (4.5)
Unable to perform her usual activities	-	-	-	-
Anxiety and depression, *n* (%)				
Not anxious or depressed	51 (71.8)	55 (77.4)	57 (86.4)	59 (89.4)
Moderately anxious or depressed	19 (26.8)	15 (21.1)	9 (13.6)	7 (10.6)
Extremely anxious or depressed	1 (1.4)	1 (1.4)	-	-
Pain and discomfort, *n* (%)				
No pain or discomfort	46 (64.8)	52 (73.2)	50 (75.8)	46 (69.7)
Moderate pain or discomfort	23 (32.4)	19 (26.8)	15 (22.7)	20 (30.3)
Extreme pain or discomfort	2 (2.8)	-	1 (1.5)	-

Abbreviations: EQ-5D-3L, 3-level version of EQ-5D.

Table 3. Changes in the EQ-5D-3L score and EQ-VAS in postmenopausal women participants.

	Intervention Group (*n* = 71)		**Control Group (*n* = 66)**			
	Baseline	**6 Months**	**Baseline**	**6 Months**	**Adjusted Intergroup Diff**	***p***
EQ-5D-3L [2,3]	0.868 ± 0.159	0.901 ± 0.123	0.919 ± 0.124	0.907 ± 0.124	0.044 (−0.012, 0.099)	0.125
EQ-VAS [4]	74.7 ± 13.9	78.1 ± 14.0	78.3 ± 13.6	77.2 ± 14.9	6.0 (0.4, 11.7)	0.036

Values expressed as mean±standard deviation. Abbreviations: EQ-5D-3L, 3-level version of EQ-5D; EQ-VAS, EuroQoL visual analogue scale. [1] These values are adjusted for age, education level, marital status, time elapsed since the beginning of menopause, daily calorie intake (kcal), baseline consumption of chocolate with 70% cocoa, physical activity, alcohol consumption, smoking, depression and untreated dyslipidaemia. Results are based on analysis of covariance (ANCOVA). [2] Difference between groups at baseline ($p < 0.05$). [3] Range between 0 (worst quality of life) and 1 (best quality of life). [4] Range between 0 (worst quality of life) and 100 (best quality of life).

Table 4. Changes in the Cervantes scale score and dimensions in postmenopausal women participants.

	Intervention Group (IG) (*n* = 71)		**Control Group (CG) (*n* = 66)**			
	Baseline	**6 Months**	**Baseline**	**6 Months**	**Adjusted Intergroup Difference (IG–CG) [1]**	***p***
Total score (0–155) [2]	51.8 (2.5)	52.0 (2.4)	48.5 (2.4)	47.4 (2.5)	1.1 (−4.5, 6.7)	0.478
Dimensions						
Menopause and health (0–75)	26.2 (1.5)	26.1 (1.4)	24.9 (1.4)	24.9 (1.3)	0.1 (−2.8, 3.2)	0.927
Vasomotor symptoms (0–15)	6.8 (0.6)	6.5 (0.5)	6.3 (0.6)	5.7 (0.5)	0.3 (−0.8, 1.4)	0.622
Health (0–25)	7.7 (0.6)	8.0 (0.6)	7.8 (0.5)	7.8 (0.5)	0.3 (−1.0, 1.6)	0.652
Ageing (0–35)	11.7 (0.7)	11.6 (0.6)	10.8 (0.7)	11.3 (0.7)	−0.6 (−2.1, 0.9)	0.431
Mental domain (0–45)	8.5 (0.9)	9.6 (0.9)	8.2 (0.7)	8.3 (0.9)	1.0 (−1.1, 3.0)	0.357
Sexuality (0–20)	11.4 (0.5)	10.7 (0.6)	11.2 (0.6)	10.2 (0.6)	0.2 (−1.5, 1.8)	0.848
Relationship (0–15)	5.7 (0.6)	5.7 (0.6)	4.7 (0.6)	4.3 (0.6)	−0.2 (−1.5, 1.1)	0.775

Values expressed as mean (standard error) and differences are means (95% confidence interval). [1] These values are adjusted for age, education level, marital status, time elapsed since the beginning of menopause, daily calorie intake (kcal), baseline consumption of chocolate with 70% cocoa, physical activity, alcohol consumption, smoking, depression and untreated dyslipidaemia. Results are based on analysis of covariance (ANCOVA). [2] Range between 0 (best quality of life) and 155 (worst quality of life).

4. Discussion

The results of this clinical trial show that, in a sample of postmenopausal women, the daily consumption of 10 g of cocoa-rich chocolate produced a slight improvement in the score of the visual analogue scale of the EuroQol questionnaire (EQ-VAS), although there were no changes in the global score of QoL evaluated with the general questionnaire (EuroQoL-5D) or with a specific questionnaire for this population (Cervantes scale). Similarly, there were no changes in the dimensions or subdimensions analysed in the latter: menopause and health, mental domain, sexual relations, relationship, vasomotor symptoms, health and ageing.

The main tool used for the evaluation of health-related QoL in this study was the EuroQoL questionnaire. Within this questionnaire, there are two clearly differentiated sections with different purposes and objectives. The profile of the EQ-5D was developed to describe, in a quick and simple manner, the dimensions of health-related QoL (mobility, self-care, usual activities, pain and discomfort, anxiety and depression), as well as to estimate a single value that summarises all of these dimensions [26]. On the other hand, the EQ-VAS is aimed at obtaining the general health state of the participant, providing important and complementary information about the opinions of the patients regarding their own health. In a trial conducted in older individuals, an improvement in QoL measured with the EQ-5D was observed in the group that received a natural beverage made of flavonoid-rich cocoa powder, with a significant decrease in their perception toward problems in mobility and pain/discomfort, and without changes in the rest of the dimensions [33]. In our study, the proportion of women in each of the categories of the dimensions analysed in the EQ-5D-3L remained similar after the intervention. However, there were differences in the values of the EQ-VAS. The intake of chocolate is anecdotally associated with an increase of happiness, although few experimental studies have analysed this effect. Its sensory characteristics, nutritional composition and the presence of psychoactive components, such as tyramine and theobromine, have been suggested as the agents that cause the effects of chocolate on mental health and mood [34]. Another study revealed that chocolate seems to increase positive mood, particularly when eaten mindfully [35]. The EQ-VAS comprises all the aspects of health-related QoL and not only the content of the five dimensions measured in the EQ-5D. Therefore, the differences observed in the results of the EQ-VAS could help to value the general health status in further agreement with the perspective of the patient [36]. The results of the effect of a controlled and moderate dose of chocolate (10 g daily) in postmenopausal women observed in our study could be consistent with the effect attributed to chocolate on mood. The amount of 10 g of chocolate used in the intervention taken every day complies with the recommendations of the European Food Safety Authority [37] to maintain endothelium-dependent vasodilation [37], which is relevant since the main outcome of the trial that this study is part of was blood pressure, and Nurk et al. [38], who highlighted the attainment of a maximal beneficial effect on mental health with this daily amount of such product. Furthermore, it was reported that consumption of 14 g of milk chocolate enhanced positive mood [35]. Though the product used in this trial was dark chocolate, which is known to contain higher quantity of cocoa than milk chocolate, and the consumption of a similar amount of it was expected to have a greater effect on quality of life, it may not be sufficient to show changes in this outcome.

No changes were detected in the different dimensions of QoL evaluated with the Cervantes scale. This scale, validated in a Spanish population of perimenopausal women of 45–64 years of age [29], has been used in some studies [39,40]. The consumption of chocolate (including dark chocolate, milk chocolate and white chocolate) as a baseline condition in the study groups was 68 g/week (median (interquartile range): 42 (9–109) g/week in the IG and 50 (21–80) g/week in the CG), and this consumption was not restricted during the study. Therefore, the aim was to analyse whether an additional contribution of 10 g/day of dark chocolate improved the study variables. This was a condition of the design that could partly justify the absence of additional beneficial results on the dimensions of the Cervantes scale, especially on sexuality. In previous studies, the consumption of chocolate had a significant impact on sexual desire in women [11], although after adjusting for age,

such association disappeared. However, it is necessary to further investigate the impact of the intake of chocolate on specific aspects of QoL.

Although it was slight, the change found in the score of the EQ-VAS in favour of the experimental group, which received the additional amount of dark chocolate, could reflect a better perception toward the general health state after the intervention. Considering the clinical context posed by a decrease of QoL after menopause, those interventions, especially the non-pharmacological ones, which can improve the health state could be clinically relevant. This is especially important in people over 60 years of age, in whom the changes in QoL are considered as potential predictors of mortality [41].

Chocolate is a natural source of caffeine. The amount of this component present in chocolate varies depending on the percentage of cocoa it contains [42]. Caffeine has multiple effects on health, some of which could affect quality of life. Among the effects exerted by caffeine, it has to be highlighted its potential vasoconstricting and anti-inflammatory effects, which can have a positive influence on pain relief [42]. Moreover, the benefits of chocolate on mood seem to be mainly exerted by caffeine [43]. Also, caffeine consumption could reduce sleep quality [44], which has been shown to be associated with hot flashes, loss of sexual interest and depressed mood [45], having a negative impact on quality of life. Nonetheless, our results did not show any significant change in these domains, and none of the questionnaires used explore sleep quality as a component of quality of life.

Modifications in the dietary pattern and eating habits usually followed by participants could have possibly altered the results, hence all subjects were asked to not modify these during the study period. Results showed that daily energy intake, nutrients of habitual diet and chocolate intake remained unchanged in both the IG and CG, which suggests that these parameters were not altered during the intervention.

Another point to discuss is the mean time from menopause onset, which was 6.9 years in both study groups. This has to be considered, as many of the physiological changes during menopause take place during the period of menopausal transition. Avis et al. observed that the duration of vasomotor symptoms was more than 7 years during the menopausal transition for more than half of the participants in the Study of Women's Health Across the Nation (SWAN), and persisted for 4.5 years after the final menstrual period [46]. These vasomotor symptoms are associated with poorer quality of life, negative mood and sleep problems [47]. Considering this, carrying out this trial earlier in the menopause period could have had a greater impact on quality on life and should be taken into account in future research.

There are some limitations that must be highlighted. Firstly, the design of the trial that this study is part of does not establish the improvement of QoL as the main objective, and it is taken as a secondary variable. Thus, the sample size could be insufficient for the contrast of this variable. Secondly, there was no blinding in the participants due to the nature of the intervention, which may have influenced the findings about QoL. The participants of the study had no restrictions on the consumption of cocoa or chocolate in terms of presentation, which may underestimate the effect of the intervention; however, this approach is more in line with the eating habits of the population. Another limitation could be the lack of control of polyphenol intake during the intervention. This was not possible since the tool used to assess the nutritional composition of the habitual diet does not provide data on the polyphenol content of the diet or the specific foods consumed. We could assume that randomisation had balanced the groups with respect to dietary intake as well as polyphenol intake; nonetheless, this should be considered in future studies.

5. Conclusions

We can conclude that the additional daily contribution of 10 g of cocoa-rich chocolate in postmenopausal women could have a slight impact on their perception toward their health state, although without modifying the health-related QoL or the dimensions that compose it. Despite this, further analytical studies are required to delve specifically into these associations.

Author Contributions: Conceptualization, J.I.R.-R., J.A.M.-F., L.G.-O. and I.A.G.-Y.; methodology, L.G.-O., J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; validation, M.A.G.-M. and E.R.-S.; formal analysis, J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; investigation, M.A.G.-M., E.R.-S. and O.T.-M.; resources, O.T.-M.; data curation, E.R.-S. and O.T.-M.; writing—original draft preparation, I.A.G.-Y. and J.I.R.-R.; writing—review and editing, L.G.-O., M.A.G.-M., E.R.-S., O.T.-M. and J.A.M.-F.; supervision, J.I.R.-R., J.A.M.-F. and L.G.-O.; project administration, J.I.R.-R., J.A.M.-F. and I.A.G.-Y.; funding acquisition, J.A.M.-F. and J.I.R.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported in part by grants funded by Gerencia Regional de Salud de Castilla y León (GRS 1583/B/17). It was also supported by the Instituto de Salud Carlos III of the Ministerio de Ciencia e Innovación (Spain) through the Red de Investigación en Actividades Preventivas y Promoción de la Salud (redIAPP, RD16/0007), co-financed with the European Union's ERDF.

Acknowledgments: The authors are grateful to all the volunteers for their participation, and the professionals involved in the study: José I Recio-Rodríguez, José A Maderuelo-Fernández, Luis García-Ortiz, Manuel A Gómez-Marcos, Irene A García-Yu, Rosario Alonso-Domínguez, Sara Mora-Simón, Natalia Sánchez-Aguadero, Jesús González-Sánchez, Cristina Agudo-Conde, Cristina Lugones-Sánchez, Benigna Sánchez-Salgado, Carmen Castaño-Sánchez, Emiliano Rodríguez-Sánchez, Susana González-Manzano, Olaya Tamayo-Morales and Susana González-Sánchez.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. Lindt and Sprüngli provided the necessary chocolate for the implementation of the study. This company did not play any role in the design of the study, the data analysis, the reporting of results, or the decision to present the manuscript for publication.

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Discusión

Este trabajo explora el efecto sobre la salud de una intervención nutricional, consistente en la administración de chocolate disponible comercialmente, sobre una población especial, como son las mujeres posmenopáusicas. La menopausia tiene múltiples implicaciones en diferentes aspectos de la salud de las mujeres, entre los que destaca el riesgo cardiovascular aumentado de este grupo de población.

Tanto la PAS como la PAD se mantuvieron sin cambios entre los grupos de estudio tras la intervención, aunque se observó un descenso en la PP entre grupos favorable al GI. Los demás factores de riesgo cardiovascular y los parámetros relativos a la estructura y función vascular evaluados tampoco se modificaron.

En relación con la composición corporal, la intervención logró reducir la masa grasa, así como el porcentaje de grasa corporal del GI respecto al GC, sin que se observaran cambios en los demás parámetros de la composición corporal.

Por otro lado, se halló un descenso en el tiempo de ejecución del TMT-B, lo que podría indicar una leve mejoría en el rendimiento cognitivo relacionados con la flexibilidad cognitiva y velocidad de procesamiento, como componentes de las funciones ejecutivas.

Por último, no se observaron cambios en la calidad de vida ni en las dimensiones y subdimensiones que la componen. Sin embargo, se observó una leve mejora en la puntuación de la escala visual analógica del cuestionario EuroQoL (EQ-VAS).

1. Efecto de la intervención sobre las variables de presión arterial, rigidez arterial y factores de riesgo cardiovascular

Resultados principales

Los resultados no mostraron diferencias en la PAS ni en la PAD entre grupos, aunque se observó un descenso en la PP en el GI en comparación con el GC. Los niveles de CT, cLDL y cHDL, así como el peso corporal fueron similares en los dos grupos. Asimismo, no se encontraron diferencias en los valores de glucosa, insulina u HOMA-IR. No se observaron cambios relevantes en relación con las variables de medida de la estructura y función vascular.

Discusión de resultados de presión arterial

Okamoto et al. (119), en cuyo estudio se administró un compuesto de cacao todos los días o a días alternos a mujeres posmenopáusicas aleatorizadas en dos grupos, observaron que los valores de PAS, PAD, PAM, y PP disminuyeron de forma significativa respecto al GC.

La disminución en la PP en relación con un mayor consumo de chocolate también se ha observado en estudios en adultos sanos (193). Además, en un metanálisis de ensayos clínicos aleatorizados se concluyó que el chocolate y el cacao parecen reducir tanto la PAS como la PAD tras la ingesta crónica (194). En relación con esto, los resultados de este estudio están en línea con la evidencia previa.

El descenso en la PAS y la PP observado en las participantes con sobrepeso u obesidad del GI respecto al GC, contrasta con los resultados de otro trabajo en el que se observó que el consumo agudo de cacao aumentó 4 mmHg la PA en reposo en sujetos sanos con sobrepeso (195). No obstante, debemos tener en cuenta que en nuestro trabajo no se evaluaron los efectos agudos sino a medio plazo y que la población de estudio solo incluyó a mujeres posmenopáusicas.

A pesar de que no se hallaron diferencias estadísticamente significativas, los resultados sugieren que la ingesta del chocolate de forma aislada o de forma conjunta con otros alimentos podría influir en los posibles efectos que pudiera ejercer este compuesto, en el sentido de que la ingesta de otros alimentos junto con el chocolate podría interferir en los posibles efectos del chocolate sobre la salud. No obstante, sería necesario llevar a cabo otros estudios que pudieran aportar evidencia sobre esto.

El tamaño muestral de este ensayo clínico se estimó en base a la potencial modificación en la PAS, pero no fue dirigido a la detección de diferencias en los análisis por subgrupos. Por tanto, es posible que los tamaños de los subgrupos en base a la presencia o ausencia de sobrepeso u obesidad como condición basal y al modo de ingesta del chocolate fuesen insuficientes para dichos análisis.

Discusión de resultados de otros factores de riesgo cardiovascular

En relación con factores de riesgo metabólico, no se observaron cambios en el perfil lipídico de forma similar a los hallazgos de otros estudios en mujeres posmenopáusicas (119). No obstante, en un estudio en pacientes con hipertensión que presentaban intolerancia a la glucosa se observó una disminución significativa en los niveles de CT y cLDL tras el consumo de chocolate negro (196). Por otro lado, Okamoto et al. observaron un descenso significativo en los triglicéridos y en la glucosa tras la ingesta de cacao (119), mientras que nuestros resultados no mostraron cambios en estos parámetros. Estudios previos indican una mejora en los niveles de insulina y en el HOMA-IR (118,196); sin embargo, los hallazgos de este ensayo no mostraron diferencias en estos parámetros en ninguno de los dos grupos o entre ellos. El peso corporal tampoco se modificó tras el

consumo de chocolate negro al igual que en otros estudios en poblaciones con un riesgo aumentado (119,133,195).

Discusión de resultados de marcadores de rigidez arterial y función vascular

Existe evidencia que apoya la mejora de la rigidez arterial mediante la reducción de la VOP tras el consumo de cacao tanto en sujetos sanos (169,193) como en mujeres posmenopáusicas (119). En cambio, los resultados de este estudio no mostraron cambios significativos.

Estudios previos sugieren que el consumo de chocolate con alta concentración de cacao puede mejorar la función vascular en mujeres posmenopáusicas (120), aunque Marsh et al. evaluaron los efectos de forma aguda, mientras que nuestro estudio evaluó los efectos a medio plazo. Además, los efectos beneficiosos de un mayor consumo de chocolate sobre el índice de aumento se han observado en sujetos sanos (193) y en mujeres con sobrepeso (195). Por el contrario, los parámetros de función vascular medidos en nuestro estudio no mostraron cambios relevantes.

2. Efecto de la intervención sobre las variables de composición corporal

El principal hallazgo de este trabajo es el efecto beneficioso de añadir 10 g diarios de chocolate con alto porcentaje de cacao sobre la composición corporal en mujeres posmenopáusicas. Tras los 6 meses de intervención, se consiguió una reducción tanto de la masa grasa como del porcentaje de grasa corporal respecto al GC. No se observaron diferencias relevantes en relación con el peso corporal, IMC u otros aspectos de la composición corporal. Tampoco se encontraron cambios en la ingesta dietética habitual.

Este es de uno de los primeros trabajos realizados en humanos que muestra los efectos beneficiosos que los componentes del cacao podrían tener sobre marcadores de composición corporal y distribución de la grasa. Entre los principales componentes presentes en el cacao, los polifenoles han mostrado una alta capacidad antioxidante y una potencial capacidad como coadyuvantes en ciertos mecanismos metabólicos. En un ensayo en ratas, dosis bajas de suplementos de extracto de cacao fueron suficientes para contrarrestar la obesidad y la diabetes tipo 2, proporcionando nuevas ideas sobre la posible aplicación de suplementos de cacao en el manejo del síndrome metabólico(197). De la misma manera, en otro ensayo en laboratorio se concluyó que el cacao y sus flavanoles podrían mejorar la disfunción endotelial y contribuir a su efecto reductor de la presión arterial (198). Aunque existe aún cierto desconocimiento acerca de los polifenoles y su

impacto sobre la obesidad, algunos trabajos han sugerido que los polifenoles pueden afectar favorablemente a la regulación de la glucosa, la adipogénesis, la lipolisis, el metabolismo de los lípidos y el control del apetito (199–202).

En estudios previos realizados en personas sanas se ha observado una mejora en la sensibilidad a la insulina, con niveles reducidos de insulina y HOMA-IR, después del consumo de chocolate negro (203). En cambio, otros no han mostrado diferencias en estos parámetros tras la ingesta de chocolate (131), lo que está en consonancia con nuestros resultados. Desideri et al. (149) observaron una mejora en el HOMA-IR pero no modificaciones en los niveles plasmáticos de insulina tras la ingesta de flavanoles del cacao en personas mayores con deterioro cognitivo leve. Esta discrepancia se podría explicar por las diferencias en los métodos utilizados en cada investigación. Estos hallazgos significativos podrían explicarse por otros efectos sobre hormonas peptídicas gastrointestinales o la regulación neurohormonal de la función gastrointestinal y de saciedad, aunque, estos no se midieron en este estudio. Sería interesante explorar estos como posibles mecanismos del efecto en futuras investigaciones.

Existe evidencia de un incremento en el porcentaje de grasa corporal, así como de una redistribución central y visceral de la masa grasa con el envejecimiento (204). Además, la menopausia supone un gran cambio para la mujer en relación con su composición corporal. El estudio de Toth et al. (205,206) sugieren que la menopausia está asociada con un incremento en la grasa abdominal y cuantifican estos cambios en un 49% más de grasa abdominal en comparación con mujeres premenopáusicas. El metanálisis realizado por Ambikairajah et al. (207) mostró un incremento en el porcentaje de grasa corporal (2,88%; IC 95% 2,13 a 3,63) y porcentaje de grasa en el tronco (5,49%; IC 95% 3,91 a 7,06) entre mujeres premenopáusicas y posmenopáusicas, aunque el cambio en la cantidad de masa grasa se atribuyó fundamentalmente al aumento de la edad. Además, sus resultados mostraron un descenso en el porcentaje total de grasa en las extremidades inferiores y un incremento en las medidas de grasa central, lo que sugiere la aparición de cambios en la distribución de la masa grasa durante la menopausia. Adicionalmente, es necesario mencionar que Mahabir et al. (208) reportaron que un cambio de una unidad del porcentaje de masa corporal está asociado con un cambio relevante en la leptina en suero. Existe evidencia que sugiere que esta hormona podría ser un posible biomarcador del riesgo de cáncer de mama en mujeres, especialmente en aquellas con sobrepeso u obesidad y en mujeres posmenopáusicas (209), y que mayores niveles de leptina circulante se han asociado con una mayor gravedad de enfermedad del hígado graso no alcohólico (210). A su vez, estos cambios en la distribución regional de grasa se han correlacionado con un

aumento del riesgo cardiometabólico en esta población (211). Por tanto, es necesario conocer la variación en la distribución de grasa en mujeres posmenopáusicas como consecuencia de intervenciones como la que presentamos en este estudio. La actividad física aeróbica combinada con ejercicios de resistencia en forma de tres sesiones semanales ha demostrado reducir la masa grasa, y el porcentaje de grasa corporal en todos los segmentos corporales, es decir, tronco, brazos y piernas (129). La intervención dirigida por Choquette et al. (129) también incluyó un grupo al que se le administró un suplemento de polifenoles (isoflavonas) que logró esa reducción únicamente en las piernas. Es destacable, por tanto, la reducción lograda en nuestro estudio tanto en el porcentaje de grasa corporal general como la reducción de este en tronco, brazos y piernas, lo que resalta la magnitud de los resultados que se presentan en este manuscrito. Los hallazgos de nuestro ensayo pueden ser clínicamente relevantes y más aun teniendo en cuenta que estos cambios se han producido tras una intervención nutricional con un chocolate disponible comercialmente. Además, esta intervención no mostró efectos adversos en ninguno de las variables medidas.

En este trabajo no se encontraron cambios significativos en el peso ni en el IMC después de los 6 meses de intervención. Los resultados coinciden con los de un reciente metanálisis de 35 ensayos clínicos aleatorizados sobre el impacto del cacao en el peso corporal y el IMC (212). Este metanálisis sugirió que los suplementos de cacao no ejercen ningún efecto significativo sobre el peso corporal (−0,108 kg, IC 95% −0,262 a 0,046; p = 0,168) o el IMC (−0,014 kg/m^2, IC 95% −0,105 a 0,077; p = 0,759). Sin embargo, un análisis por subgrupos reveló que el peso corporal y el IMC se redujeron con suplementos de cacao de ≥ 30 g de chocolate por día en ensayos con una duración de 4-8 semanas. En nuestro trabajo, observamos una modificación de la composición corporal, pero sin afectación del peso corporal, aunque se observó una mayor reducción (−0,49 kg [IC 95% −1,08 a 0,10]) que en los resultados del metanálisis realizado por Kord-Varkaneh H et al. (212). Por tanto, el aporte diario de calorías procedente del suplemento del chocolate (10 gramos; 59 Kcal) no parece haber influido negativamente en el peso corporal. Tampoco parece que haya alterado la ingesta calórica media de las participantes (18 Kcal [IC 95% −112 a 149]). Además, no se observó ningún incremento relevante ni en el total de agua corporal, ni en la masa libre de grasa ni en la masa musculo-esquelética. Todos estos parámetros mostraron un ligero incremento en el grupo de intervención.

3. Efecto de la intervención sobre las variables de rendimiento cognitivo

Los hallazgos de este estudio muestran un descenso en el tiempo de ejecución en el TMT-B, en el GI tras el consumo diario de 10 g de chocolate con alta concentración de cacao (99%), lo que podría sugerir una leve mejoría en el rendimiento cognitivo relacionado con la flexibilidad cognitiva y velocidad de procesamiento, como componentes de las funciones ejecutivas. Sin embargo, no se encontraron diferencias relevantes en la atención, la memoria verbal inmediata ni demorada, la fluidez fonológica ni categorial, ni la memoria de trabajo.

El efecto del cacao sobre el rendimiento cognitivo ha sido estudiado por diversos autores, aunque los resultados obtenidos son heterogéneos. Los hallazgos de este ensayo clínico, en el que se ha observado una mejora en el tiempo de ejecución de una prueba que explora la flexibilidad cognitiva con un tamaño del efecto moderado, están en consonancia con los aportados por otros estudios. Nurk et al. (148) reportaron que los consumidores habituales de chocolate tenían un mejor desempeño en las pruebas cognitivas, siendo las funciones ejecutivas unas de las más favorecidas. Asimismo, en otros trabajos se ha observado una mejora en las funciones ejecutivas, reflejada en la reducción del tiempo requerido para completar el TMT-A y el TMT-B, tras la ingesta durante 8 semanas de dos compuestos de cacao ricos en polifenoles frente a otro compuesto con una baja concentración, en personas mayores tanto sin deterioro cognitivo (138) como con deterioro cognitivo leve (149). Por el contrario, en otro ensayo clínico no se logró mostrar un beneficio a corto plazo en las funciones ejecutivas tras el consumo de chocolate (146).

Las causas que desencadenan estos efectos sobre el rendimiento cognitivo se desconocen con exactitud, aunque se han propuesto distintos mecanismos que pueden ser los responsables. Algunos estudios apuntan a que un aumento en el flujo de la arteria cerebral provocado por los polifenoles del cacao puede mejorar el desempeño en las tareas de rendimiento cognitivo (140,213). Asimismo, Scholey et al. (134) señalan que la mejora en la función endotelial y el flujo sanguíneo causada por los polifenoles podría estar relacionada con estos efectos. También se ha sugerido que el factor neurotrófico derivado del cerebro (BDNF, por sus siglas en inglés) podría actuar como mediador en la mejora cognitiva tras la ingesta de cacao (137). Otros autores sugieren que una disminución en la resistencia a la insulina puede estar implicada en la aparición de estos efectos sobre el rendimiento cognitivo (138,149). Además, dado que el cambio en los niveles de estrógenos puede afectar el estado cognitivo de las mujeres posmenopáusicas (97), se cree que el estrógeno puede tener un papel fundamental en los cambios cognitivos que tienen lugar durante la menopausia ejerciendo un efecto neuroprotector (214). La evaluación de los

niveles hormonales no se llevó a cabo en este estudio, aunque debería tenerse en cuenta en futuros estudios.

Respecto al nivel educativo, una alta proporción de las mujeres de ambos grupos presentaban un nivel de estudios alto, siendo este porcentaje mayor en el GI. No obstante, nuestros hallazgos no muestran diferencias estadísticamente significativas entre ambos grupos considerando su nivel de estudios, a diferencia de estudios previos que señalan que las personas con un mayor nivel de estudios presentan mejor rendimiento cognitivo (178,215).

La intervención no parece haber modificado otras funciones cognitivas evaluadas, de forma similar a lo mostrado en algunos ensayos clínicos, como el realizado en mujeres posmenopáusicas por Marsh et al. (120) o el dirigido por Pase et al. (145). Por el contrario, en otros trabajos sí se han observado efectos beneficiosos en otros aspectos cognitivos como la memoria de trabajo o la atención (134,142). Nurk et al. (148) reportaron una mejora en la memoria verbal relacionada con el consumo de chocolate en un estudio observacional. Igualmente, se observó una mejora en la fluidez fonológica tras la ingesta de compuestos de cacao ricos en polifenoles en personas mayores (138,149). Por otro lado, Karabay et al. (147) sugieren que los flavanoles del cacao pueden mejorar ciertos aspectos de la atención. Asimismo, Grassi et al. (139) hallaron un incremento en la memoria de trabajo tras el consumo de chocolate rico en flavonoles tras la privación del sueño.

Es importante tener en cuenta que la repetición de las pruebas después de 6 meses de la visita basal puede haber introducido un componente de aprendizaje que puede haber influido sobre un mejor desempeño en las pruebas en la visita de seguimiento de ambos grupos, a pesar de que este es el periodo de tiempo recomendado para evitar el efecto de aprendizaje.

Este ensayo clínico comprende un mayor número de participantes en comparación con otros estudios que evalúan el efecto del chocolate rico en cacao en la función cognitiva (120,216,217), aunque el tamaño muestral podría ser insuficiente para el contraste de esta variable dado que no fue estimado en base a este parámetro. La cantidad de 10 g de chocolate utilizada en la intervención se ajusta a las recomendaciones de la Autoridad Europea de Seguridad Alimentaria (43) y concuerda con los datos aportados por Nurk et al. (148), quienes señalaron la obtención de un efecto beneficioso máximo en el rendimiento cognitivo con un consumo diario de aproximadamente 10 g de chocolate.

La muestra de población sobre la que se ha realizado el estudio presenta características especiales. Las mujeres posmenopáusicas pueden presentar dificultades

cognitivas (97) que parecen estar vinculadas a los cambios hormonales propios de ese periodo. Por ello, resulta importante el desarrollo de intervenciones como la realizada en este ensayo que están dirigidas a mejorar la función cognitiva en este grupo de población sin causar efectos adversos.

4. Efecto de la intervención sobre las variables de calidad de vida

Los resultados de este ensayo clínico muestran que, en una muestra de mujeres posmenopáusicas, el consumo de 10 g diarios de chocolate rico en cacao produce una leve mejora en la puntuación de la escala visual analógica del cuestionario EuroQoL (EQ-VAS), aunque no se observan cambios en la puntuación global de calidad de vida evaluada con el cuestionario general (EuroQoL-5D) ni con un cuestionario específico para esta población (escala Cervantes). Tampoco se registraron cambios relevantes en las dimensiones y subdimensiones analizadas con este último: menopausia y salud, dominio psíquico, relaciones sexuales, relaciones de pareja, sintomatología vasomotora, salud y envejecimiento.

La principal herramienta utilizada para la evaluación de la calidad de vida relacionada con la salud en este estudio ha sido el cuestionario EuroQoL. Dentro de este cuestionario existen dos apartados claramente diferenciados cuyo propósito y objetivos son distintos. El perfil del EQ-5D fue desarrollado para describir de forma rápida y sencilla las dimensiones de la calidad de vida relacionada con la salud (movilidad, autocuidado, actividades habituales, dolor e incomodidad, ansiedad y depresión), además de estimar un único valor, a modo de índice que resuma todas ellas(184). Por otro lado, el EQ-VAS está orientado a buscar la calificación general del encuestado acerca de su salud, proporcionando información importante y complementaria sobre las opiniones de los pacientes sobre su propia salud. En un ensayo en personas mayores se observó una mejora en la calidad de vida medida con el EQ-5D en el grupo que recibió una bebida natural compuesta de polvo de cacao rico en flavonoides, con una reducción significativa en su percepción sobre los problemas de movilidad y el dolor/disconfort, sin cambios en las demás dimensiones (218). En nuestro estudio, la proporción de mujeres en cada una de las categorías de las dimensiones analizadas en el EQ-5D-3L fue similar tras la intervención. Sin embargo, se observaron diferencias en los valores del EQ-VAS. La ingesta de chocolate se asocia anecdóticamente con un aumento de la felicidad, pero pocos trabajos experimentales han examinado este efecto. Las características sensoriales, su composición nutricional y la presencia de componentes psicoactivos como la tiramina o la teobromina, se han postulado como posibles responsables de los efectos del chocolate en la salud mental o en la regulación

del estado de ánimo (219). Otro estudio mostró que el chocolate parece aumentar el estado de ánimo positivo, particularmente cuando se consume con atención plena (220). El EQ-VAS comprende todos los aspectos de la calidad de vida relacionada con la salud y no solo el contenido de las cinco dimensiones medidas en el EQ-5D. Por tanto, las diferencias observadas en los resultados del EQ-VAS podrían proporcionar un medio para valorar la salud general de manera más cercana a la perspectiva del paciente (221). Los resultados del efecto de una dosis controlada y moderada de chocolate (10 g diarios) en mujeres posmenopáusicas observados en nuestro trabajo podrían ser concordantes con el efecto atribuido al chocolate sobre el estado de ánimo. La cantidad de 10 g de chocolate aportados diariamente se ajusta a las recomendaciones de la EFSA para mantener la vasodilatación dependiente del endotelio (43), lo cual resulta relevante ya que el principal resultado de este ensayo fue la presión arterial, y de Nurk et at. (148), quienes señalaron que con esta cantidad diaria de dicho producto se lograría un efecto beneficioso máximo sobre la salud mental. Además, se ha apuntado a que el consumo de 14 g de chocolate con leche mejora el estado de ánimo positivo (220). Aunque el producto utilizado en este ensayo fue chocolate negro, que es conocido por contener una mayor cantidad de cacao que el chocolate con leche, y se esperaba que el consumo de una cantidad similar tuviera un mayor efecto sobre la calidad de vida, podría no ser suficiente para observar cambios en esta variable.

No se encontraron cambios en las diferentes dimensiones de la calidad de vida evaluadas con la escala Cervantes. Esta escala, validada en población española perimenopaúsica de 45-64 años (187), ha sido utilizada en algunos trabajos (222,223). El consumo de chocolate (incluyendo chocolate negro, chocolate con leche y chocolate blanco) como condición basal en los grupos de estudio fue 68 g/semana (mediana (rango intercuartílico): 42 (9–109) g/semana en el GI y 50 (21–80) g/semana en el GC), y este consumo no se restringió durante el estudio. El objetivo fue, por tanto, analizar si un aporte adicional de 10 g/día de chocolate negro mejoraba las variables de estudio. Esta es una condición del diseño que podría explicar, en parte, que no se encontraran resultados beneficiosos adicionales en las dimensiones en las que está estructurada la escala Cervantes, especialmente sobre la sexualidad. En estudios previos, el consumo de chocolate tuvo un impacto significativo sobre el deseo sexual en mujeres (159), aunque tras un ajuste por la edad, esta asociación desaparecía. Sin embargo, es necesario seguir investigando el impacto de la ingesta de chocolate sobre aspectos específicos de la calidad de vida.

Aunque discreto, el cambio encontrado en la puntuación del EQ-VAS favorable al grupo experimental, que recibió la cantidad adicional de chocolate negro podría reflejar una mejor percepción del estado de salud general tras la intervención. Teniendo en cuenta el

contexto clínico que implica una disminución de la calidad de vida tras la menopausia, aquellas intervenciones, especialmente las no farmacológicas, que logren mejoras en el estado de salud podrían ser clínicamente relevantes. Esto cobra especial importancia en las personas mayores de 60 años, en quienes los cambios en calidad de vida son considerados como potenciales predictores de mortalidad (224).

El chocolate es una fuente natural de cafeína. La cantidad de este componente presente en el chocolate varía en función del porcentaje de cacao que contenga (225). La cafeína ejerce múltiples efectos sobre la salud, algunos de los cuales pueden afectar a la calidad de vida. Entre los efectos que ejerce la cafeína, cabe destacar sus posibles efectos vasoconstrictores y antiinflamatorios, que pueden tener una influencia positiva en el alivio del dolor (225). Además, parece que la cafeína es la principal responsable de los beneficios del chocolate sobre el estado de ánimo (226). También, el consumo de cafeína podría reducir la calidad del sueño (227), lo que parece estar asociado con sofocos, pérdida del apetito sexual y estado de ánimo depresivo (228), teniendo un impacto negativo sobre la calidad de vida. No obstante, nuestros resultados no mostraron cambios significativos en estas dimensiones, y ninguno de los cuestionarios utilizados explora la calidad del sueño como un componente de la calidad de vida.

Otra cuestión a discutir es la media del tiempo desde el inicio de la menopausia, que fue de 6,9 años en ambos grupos. Esto se ha de tener en cuenta debido a que muchos de los cambios fisiológicos que ocurren en la menopausia acontecen durante el periodo de transición menopáusica. Avis et al. observaron que la duración de los síntomas vasomotores era de más de 7 años durante la transición menopáusica en más de la mitad de las participantes del estudio SWAN *(Study of Women's Health Across the Nation)*, y persistieron durante 4,5 años después de la última menstruación (87). Estos síntomas vasomotores se asocian a una peor calidad de vida, estado de ánimo negativo y alteraciones del sueño (229). Teniendo en cuenta esto, realizar este estudio más pronto en el periodo menopáusico podría haber tenido un mayor impacto en la calidad de vida y debería considerarse en estudios futuros.

5. Fortalezas y limitaciones

El estudio en el que se basa esta tesis doctoral presenta diversas fortalezas, así como algunas limitaciones, que son objeto de discusión.

A través de un diseño de ensayo clínico aleatorizado, esta intervención tenía como objetivo evaluar los efectos del consumo a largo plazo de chocolate rico en cacao en una población con características especiales, como lo son las mujeres posmenopáusicas. Dicho ensayo clínico cuenta con una muestra más amplia de mujeres posmenopáusicas que otros estudios similares con un tamaño muestral relativamente pequeño (119,120). Además, el tiempo de seguimiento de 6 meses contrasta con el de otros trabajos en los que la intervención dura pocas semanas, lo cual nos permitió la evaluación de los efectos a medio plazo de la intervención.

Los cambios en el patrón dietético y los hábitos alimenticios habitualmente seguidos por los participantes podrían haber alterado los resultados, por lo que se pidió a todas las mujeres que no los modificaran durante el periodo de estudio. De este modo, en este ensayo no se realizaron modificaciones ni restricciones en la dieta habitual de las mujeres participantes a diferencia de otros estudios (119); sino que únicamente se añadió el chocolate a la dieta de aquellas que pertenecían al GI. Los resultados mostraron que el consumo energético diario, los nutrientes de la dieta habitual y el consumo de chocolate permanecieron sin cambios tanto en el GI como en el GC, lo que sugiere que estos parámetros no se alteraron durante la intervención.

En cuanto a la adherencia al consumo del chocolate alcanzada en este ensayo, es muy alta, prácticamente total, y semejante a la reportada por estudios de características similares (138,146,169,230).

Cabe destacar que la intervención del estudio consistió en la adición a la dieta habitual de una cantidad de chocolate disponible comercialmente y, por tanto, accesible a la población general, que no fue diseñado específicamente para este propósito y que presenta características concretas y no modificables. Esto proporciona un contexto clínico real y permitió la evaluación de los posibles beneficios, así como los posibles efectos perjudiciales, de la ingesta de este producto en su conjunto, tal como se pretendía. Por consiguiente, esto hace que los resultados de este ensayo sean más accesibles y aplicables a la práctica clínica habitual que los de otros estudios (120,138) en los que utilizan compuestos no comerciales que son elaborados específicamente para fines de investigación y no se encuentran disponibles en un contexto real. A los participantes en el estudio no se les restringió el consumo de cacao o chocolate en cualquier forma de presentación, lo que podría subestimar

el efecto de la intervención; sin embargo, este enfoque es más acorde con los hábitos de consumo de la población. Además, el aporte de 10 g de chocolate se ajusta a las recomendaciones de la Autoridad Europea de Seguridad Alimentaria (EFSA, por sus siglas en inglés) que indica que el consumo de esta cantidad de chocolate negro con alto contenido de flavanoles incluido en una dieta equilibrada podría ayudar a mantener la vasodilatación dependiente del endotelio (43). En cambio, otros ensayos utilizan una mayor cantidad de chocolate, de 50 a 100 g/día (194), o un producto elaborado específicamente para los propósitos de la investigación, logrando compuestos con un contenido muy elevado en polifenoles (120,169). De este modo, los posibles efectos se pueden ver potenciados por el gran aporte de polifenoles, pero este tipo de intervenciones no se ajustan a un contexto clínico real y fácilmente reproducible.

Aunque la media de la ingesta de chocolate en la visita basal fue de aproximadamente 70 g/semana (10 g/día) en ambos grupos (mediana (rango intercuartílico): 42 (9–109) g/semana) en el GI y 50 (21–80) g/semana en el GC), es importante mencionar que esto incluye el chocolate negro, el chocolate con leche y el chocolate blanco. Sin embargo, la media de la ingesta de chocolate con >70% de cacao (chocolate negro) fue menor de 20 g/semana en ambos grupos (mediana (rango intercuartílico): 0 (0–26) g/semana en el GI y 0 (0–24) en el GC). El consumo diario de 10 g de chocolate rico en cacao (99%) añadido a la dieta habitual en el GI se ajusta a las recomendaciones de la EFSA y asegura que todos los participantes de este grupo cumplen con estas recomendaciones. Sin embargo, como se ha comentado anteriormente, el chocolate utilizado en este ensayo contiene una menor concentración de polifenoles en comparación con los compuestos especialmente diseñados para ese fin utilizados en otros estudios (134,138). Además, el consumo de polifenoles en la dieta puede ser muy alto, como se ha observado en estudios previos, con una estimación de la ingesta media total de 820 ± 323 mg de polifenoles/día en la cohorte PREDIMED (231), y 1193 ± 510 mg/día en la cohorte SU.VI.MAX (232). La contribución de polifenoles del cacao presentes en la cantidad de chocolate utilizado en la intervención parece ser limitada en comparación con la cantidad de polifenoles consumidos en la dieta. Por tanto, aunque la intervención logra el objetivo del estudio al proporcionar un preparado comercial a la dieta habitual, la cantidad de polifenoles aportada puede ser insuficiente para observar cambios importantes en la magnitud del efecto y se debería considerar en estudios futuros.

Otra limitación a tener en cuenta podría ser la falta de consideración de la cantidad de polifenoles consumidos por cada participante como un criterio de inclusión y la falta de control sobre el consumo de polifenoles durante la intervención. Esto no fue posible debido a que la herramienta utilizada para evaluar la composición nutricional de la dieta habitual

no proporciona información acerca del contenido de polifenoles de la dieta ni de los alimentos específicos que se han consumido. La ingesta de suplementos dietéticos tampoco se registró. Aunque podemos asumir que la aleatorización distribuyó los participantes en ambos grupos de forma equilibrada respecto a la ingesta dietética, así como al consumo de polifenoles, estas cuestiones se deberían tener en cuenta en futuros ensayos.

Además, hubiera sido interesante evaluar la biodisponibilidad de polifenoles a través de la medición, por ejemplo, de los niveles de epicatequina en plasma, con el fin de establecer una correlación con los resultados; sin embargo, esto no fue factible.

Por otro lado, en los estudios nutricionales se debe procurar el uso de un control con placebo si es posible. Estudios similares que evalúan los efectos del consumo de chocolate han utilizado chocolate blanco como un placebo adecuado. Proporcionar un compuesto placebo al GC podría haber controlado algunos factores de confusión, tales como el efecto de la precarga sobre las calorías consumidas durante la comida posterior y, probablemente, habría permitido evaluar los efectos teniendo en cuenta la composición polifenólica del chocolate. Sin embargo, es posible que esto también hubiese influido en otras variables metabólicas y no hubiese permitido evaluar los efectos del consumo del chocolate en su conjunto. En línea con esto, Almoosawi et al. (233) señalaron la posibilidad de aparición de efectos adversos con un placebo de chocolate pobre en polifenoles, indicando que en la ausencia de polifenoles, los productos con alto contenido en grasa, como el chocolate, podrían afectar negativamente al metabolismo causando efectos deletéreos. No obstante, este ensayo clínico tenía como objetivo evaluar el efecto de añadir una cantidad diaria de 10 g de chocolate con alto contenido en cacao (99%) y polifenoles a la dieta habitual sobre la presión arterial, la función vascular, el rendimiento cognitivo, la calidad de vida y la composición corporal en mujeres posmenopáusicas.

Cabe señalar también como una limitación que el enmascaramiento de las participantes no fue posible debido a la naturaleza de la intervención, lo cual podría haber influido en los hallazgos sobre algunas de las variables medidas, especialmente en la calidad de vida. Sin embargo, se aseguró el enmascaramiento de los investigadores que tomaron las mediciones y de aquellos que realizaron el análisis estadístico.

Por último, el propio diseño del estudio establece como objetivo principal la diferencia en la presión arterial y el tamaño muestral se estimó teniendo en cuenta este parámetro, por lo que este podría resultar insuficiente para el contraste de las variables secundarias.

6. Implicaciones clínicas y futuras líneas de investigación

El consumo de chocolate rico en cacao a largo plazo parece ofrecer ciertos beneficios sobre la salud en las mujeres posmenopáusicas, sin producir efectos desfavorables a largo plazo. De este modo, el aporte de los 10 g de chocolate utilizados en la intervención no parece afectar negativamente ni al perfil lipídico ni al peso corporal.

Aunque no se encontraron mejoras relevantes en la salud cardiovascular, los resultados de este ensayo clínico muestran que el consumo de chocolate rico en cacao podría mejorar algunos aspectos del rendimiento cognitivo en la población de estudio. Asimismo, los hallazgos relativos a la composición corporal apuntan a un efecto beneficioso del consumo de este producto, causando una disminución de la masa grasa y del porcentaje de grasa corporal sin modificar el peso corporal. Las investigaciones futuras deberían centrarse en los mecanismos mediante los cuales los componentes principales del cacao ejercen estos efectos, para determinar la cantidad más adecuada y la duración del consumo de estos compuestos. En cuanto a la calidad de vida relacionada con la salud y las dimensiones que la componen, no se observaron cambios, aunque esta intervención podría ejercer un ligero impacto en la percepción hacia el estado de salud de la población de estudio. A pesar de ello, son necesarios más estudios de carácter analítico que profundicen de manera específica en estas asociaciones.

Una intervención nutricional de estas características con un producto que se encuentra disponible comercialmente puede ser útil y fácilmente aplicable en la práctica clínica. No obstante, a pesar de estos resultados prometedores, es necesario llevar a cabo ensayos clínicos adicionales que incluyan un mayor número de participantes, que de igual forma sean fácilmente reproducibles en la práctica clínica habitual y que evalúen los efectos de la ingesta de chocolate rico en cacao a largo plazo en poblaciones con un riesgo cardiovascular aumentado, como lo son las mujeres posmenopáusicas.

Conclusiones

- En relación con la presión arterial, otros factores de riesgo cardiovascular y las variables relativas a la estructura y función vascular, se halló un descenso en la PP de −2,05 mmHg (IC 95% −4,08 a −0,02) en el GI comparado con el GC.

  En conclusión, la ingesta diaria de 10 g de chocolate comercial rico en cacao parece proporcionar una discreta mejora en la salud cardiovascular, aunque tampoco provoca efectos adversos en los parámetros evaluados en mujeres posmenopáusicas a largo plazo.

- En cuanto a la composición corporal, tras la intervención se observó una reducción favorable al GI tanto en la masa grasa corporal (−0,63 kg [IC 95% −1,15 a −0,11]) como en el porcentaje de grasa corporal (−0,79% [IC 95% −1,31 a −0,26]).

  Por tanto, la adición diaria a la dieta habitual de 10 g de chocolate comercial rico en cacao en mujeres posmenopáusicas disminuye la masa grasa y el porcentaje de grasa corporal sin alterar el peso corporal.

- Los hallazgos sobre el rendimiento cognitivo mostraron un descenso en el tiempo de ejecución del Trail Making Test B de −12,08 s (IC 95% −23,99 a −0,18) en el GI en comparación con el GC.

  De este modo, el consumo diario de 10 g chocolate comercial rico en cacao añadido a la dieta habitual podría estar asociado a una leve mejora en el rendimiento cognitivo con respecto a la flexibilidad cognitiva y la velocidad de procesamiento en mujeres posmenopáusicas, sin producir cambios en las demás variables de rendimiento cognitivo evaluadas.

- Respecto a la calidad de vida, el GI mostró un incremento de 6,0 puntos (IC 95% 0,4 a 11,7) en el EQ-VAS en comparación con el GC.

  Por ello, el aporte adicional de 10 g de chocolate comercial rico en cacao en mujeres posmenopáusicas podría tener un ligero impacto en su percepción de su estado de salud, pero sin modificar la calidad de vida relacionada con la salud ni las dimensiones que la componen.

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Anexos

Anexo I.

Calendario de recogida de tomas del chocolate de la intervención

Mes Año						
Lun	**Mar**	**Mié**	**Jue**	**Vie**	**Sáb**	**Dom**
	1 Toma chocolate ☐ Hora: _____	**2** Toma chocolate ☐ Hora: _____	**3** Toma chocolate ☐ Hora: _____	**4** Toma chocolate ☐ Hora: _____	**5** Toma chocolate ☐ Hora: _____	**6** Toma chocolate ☐ Hora: _____
7 Toma chocolate ☐ Hora: _____	**8** Toma chocolate ☐ Hora: _____	**9** Toma chocolate ☐ Hora: _____	**10** Toma chocolate ☐ Hora: _____	**11** Toma chocolate ☐ Hora: _____	**12** Toma chocolate ☐ Hora: _____	**13** Toma chocolate ☐ Hora: _____
14 Toma chocolate ☐ Hora: _____	**15** Toma chocolate ☐ Hora: _____	**16** Toma chocolate ☐ Hora: _____	**17** Toma chocolate ☐ Hora: _____	**18** Toma chocolate ☐ Hora: _____	**19** Toma chocolate ☐ Hora: _____	**20** Toma chocolate ☐ Hora: _____
21 Toma chocolate ☐ Hora: _____	**22** Toma chocolate ☐ Hora: _____	**23** Toma chocolate ☐ Hora: _____	**24** Toma chocolate ☐ Hora: _____	**25** Toma chocolate ☐ Hora: _____	**26** Toma chocolate ☐ Hora: _____	**27** Toma chocolate ☐ Hora: _____
28 Toma chocolate ☐ Hora: _____	**29** Toma chocolate ☐ Hora: _____	**30** Toma chocolate ☐ Hora: _____	**31** Toma chocolate ☐ Hora: _____			

Anexo II.

Recomendaciones para el consumo del chocolate de la intervención

Estudio **ECCAMP**

Efecto de añadir chocolate con alto porcentaje de cacao y polifenoles a la dieta habitual sobre la presión arterial, función vascular, calidad de vida y rendimientos cognitivos en mujeres postmenopáusicas. Ensayo clínico aleatorio. GRS 1583/B/17.

RECOMENDACIONES PARA EL CONSUMO DEL CHOCOLATE:

(a) El chocolate del estudio tiene un 99% de cacao por lo que su sabor es ligeramente más amargo y menos dulce al del chocolate al que estamos acostumbrados. Conforme al consumo diario se irá habituando al sabor y sensaciones que experimente.

(b) Se recomienda consumir el chocolate todos los días a la misma hora aproximadamente.

(c) La cantidad de chocolate que se debe consumir es de 10 gramos al día, que es el equivalente a una quinta parte de una tableta de las que va a recibir. De esa manera, una tableta durará 5 días.

(d) Después del consumo de la cantidad diaria debe anotar la hora de consumo y marcarla en el calendario que se le ha entregado. Ese calendario tendrá que devolverlo a la Unidad de Investigación en cada visita de reabastecimiento que realice.

(e) Disfrute del placer que supone el consumo de este producto.

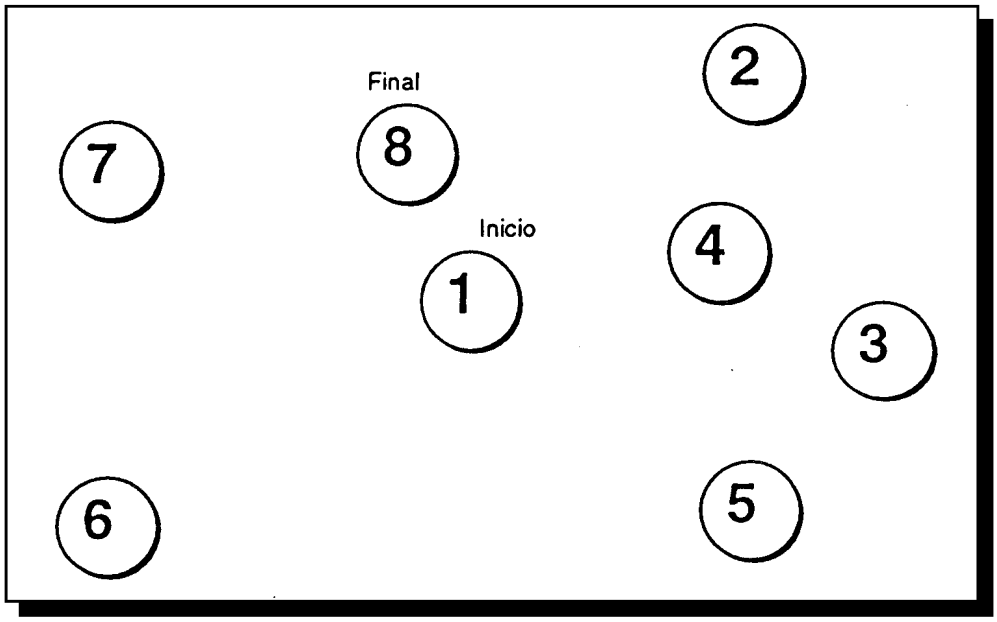
Anexo III.

Trail Making Test

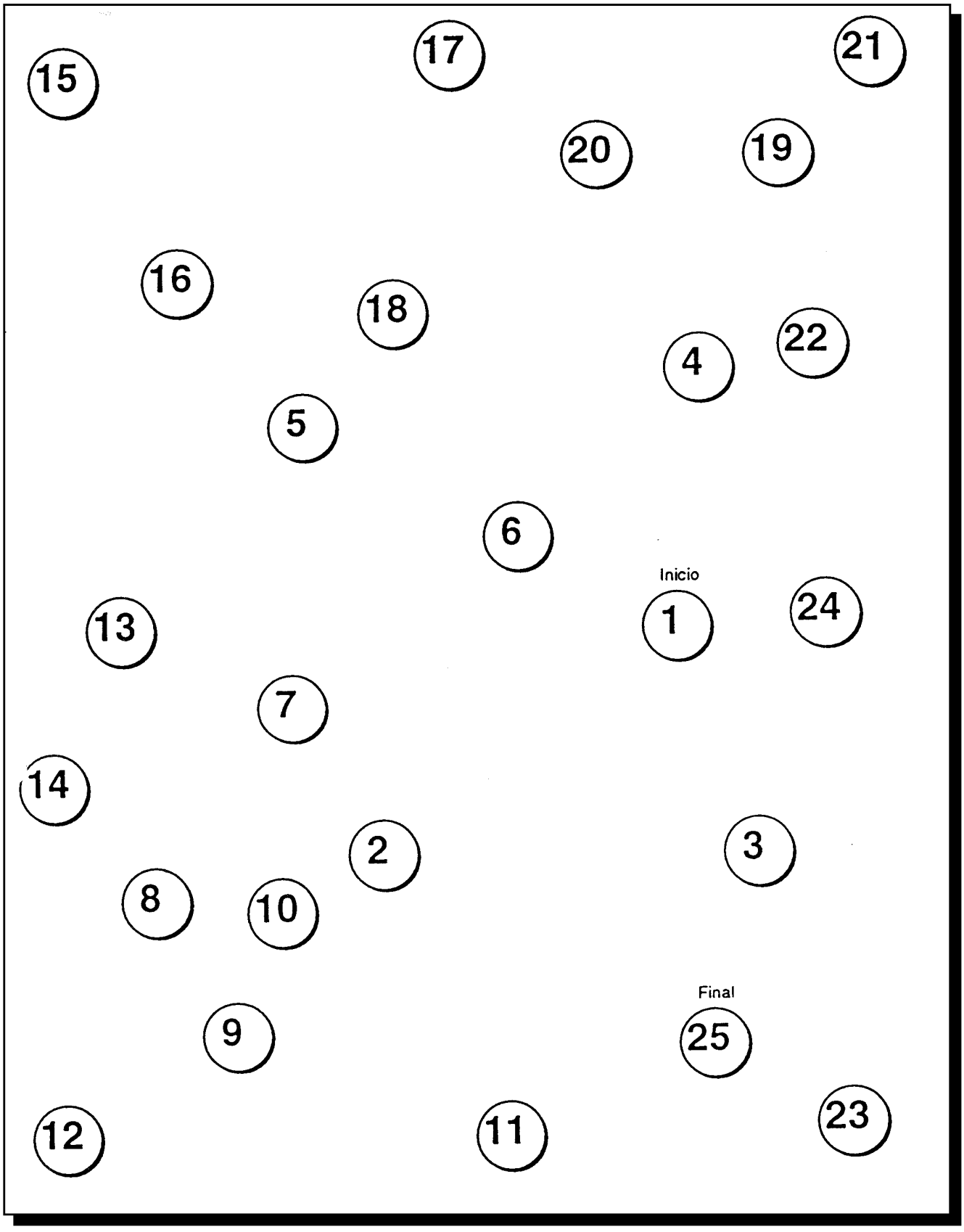
TRAIL MAKING TEST
(FORM A)

Nombre: Varón [ ] Mujer [ ]
Fecha: F. nacimiento: Edad:
Estudios/Profesión: N. Hª:
Lateralidad:
Observaciones:

ENSAYO



TEST

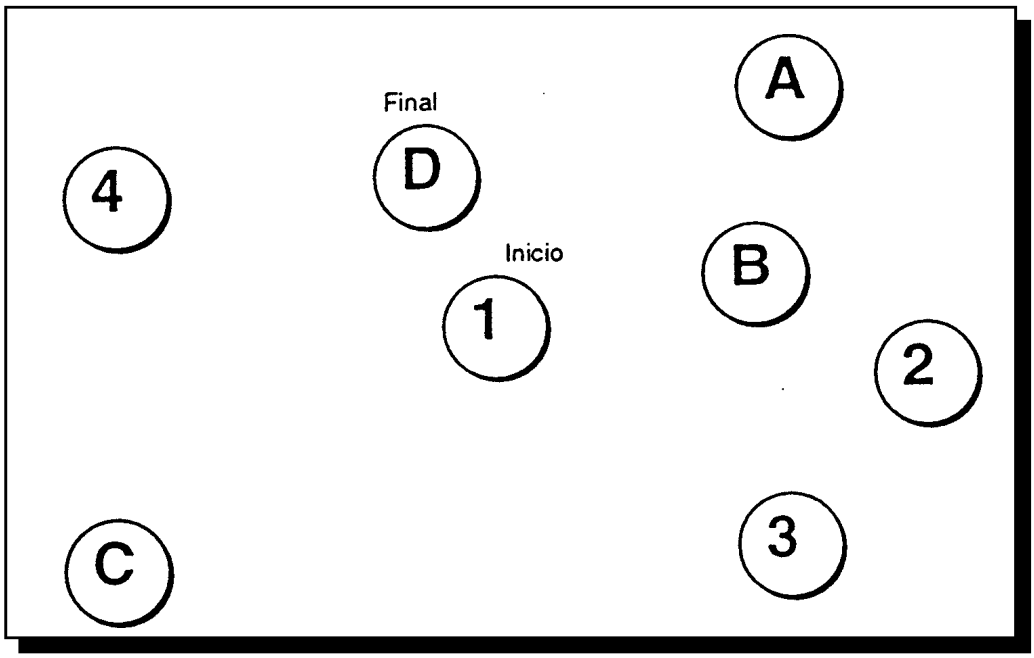

15
17
21
20
19
16
18
22
4
5
6
Inicio
1
24
13
7
14
2
3
8
10
Final
9
25
12
11
23

a.e.g. (1999)

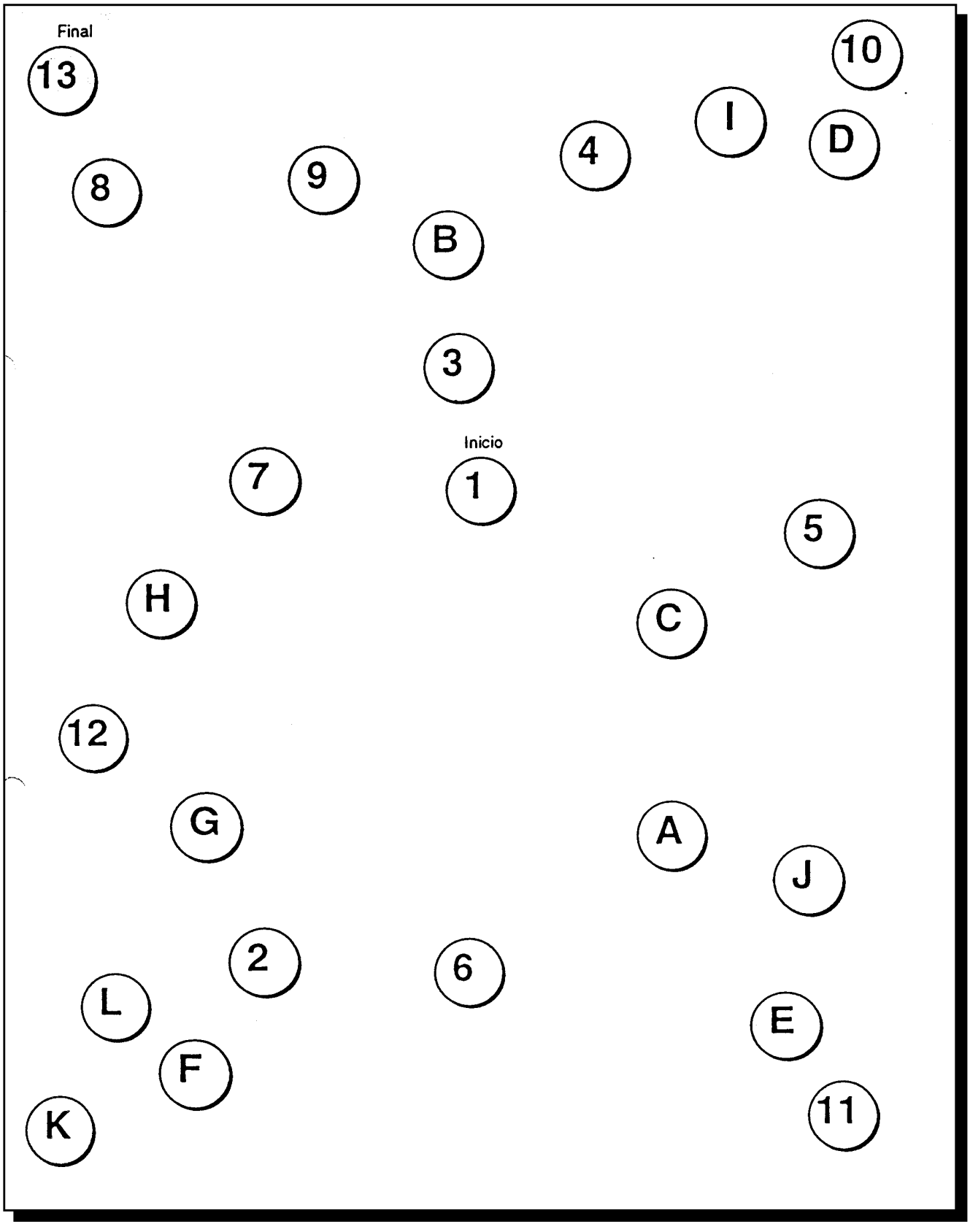
TRAIL MAKING TEST
(FORM B)

Nombre: Varón [ ] Mujer [ ]
Fecha: F. nacimiento: Edad:
Estudios/Profesión: N. Hª:
Lateralidad:
Observaciones:

ENSAYO



TEST



Anexo IV.

Cuaderno de recogida de datos

Efecto de añadir chocolate con alto porcentaje de cacao y polifenoles a la dieta habitual sobre la presión arterial, función vascular, calidad de vida y rendimientos cognitivos en mujeres posmenopáusicas. Ensayo clínico aleatorio. GRS 1583/B/17.

*__ID participante__ _ _ _

*__Fecha de visita__ _ _ / _ _ /_ _ _ _

* __Fecha de nacimiento__ _ _ / _ _ /_ _ _ _

*__Grupo de investigación__:

☐ Control

☐ Intervención

*__Visita:__

☐ Basal
☐ Seguimiento 3 meses
☐ Seguimiento 6 meses

Datos sociodemográficos (basal)

Estado civil

☐ Soltera ☐ Casada / cohabita ☐ Otros (comunidades religiosas, colegios…)
☐ Viuda ☐ Separada / divorciada

¿Cuántas personas viven con usted (incluyéndose usted misma)? _ _ personas

¿Cuál es su situación laboral actual?

☐ Está trabajando ☐ Trabaja pero tiene una baja laboral >3 meses ☐ Estudiante
☐ Ama de casa ☐ Incapacidad permanente ☐ Desempleada
☐ Jubilada

¿Qué tarea concreta realiza o realizaba? _____

Clase social _ _ _ _

¿Cuál es el nivel más alto de escolarización que ha completado?

☐ Doctorado, máster, licenciada ☐ Diplomada, ingeniero técnico ☐ Secundaria, bachillerato, FP
☐ Estudios primarios ☐ No sabe leer ni escribir

Antecedentes personales (basal y 6 meses)

¿Qué edad tenía cuando inició la menopausia? _ _

Diabetes gestacional	☐ No	☒ Sí	Año _ _ _ _
Dislipemia	☐ No	☐ Sí	Año _ _ _ _
Hipertensión arterial	☐ No	☐ Sí	Año _ _ _ _
Diabetes mellitus	☐ No	☐ Sí	Año _ _ _ _
Depresión/ansiedad	☐ No	☐ Sí	Año _ _ _ _

TRATAMIENTO FARMACOLÓGICO

Antiagregantes	☐ No	☐ Sí
Anticoagulantes	☐ No	☐ Sí
Tratamiento hormonal tiroideo	☐ No	☐ Sí
Hipolipemiantes	☐ No	☐ Sí
Antihipertensivos	☐ No	☐ Sí
Antidiabéticos	☐ No	☐ Sí
AINEs (últimas 2 semanas)	☐ No	☐ Sí
Ansiolíticos	☐ No	☐ Sí

CONSUMO DE TABACO

¿Fuma usted actualmente?

☐ Nunca fumador
☐ Fumador ocasional
☐ Sí, regularmente
☐ Ex-fumador de 0 a 1 año
☐ Ex-fumador de > 1 año

Solo fumadores y exfumadores

¿Qué edad tenía cuando empezó a fumar? _ _
¿Qué edad tenía cuando dejó de fumar? _ _
¿Aproximadamente cuántos cigarrillos fuma o fumaba al día? _ _

CONSUMO DE ALCOHOL en los últimos 7 días

Vasos vino (100cc)		Cervezas		Copas (coñac, whisky, vodka, otros licores)	
Vino tinto	_ _	Botellín (125 cc)	_ _	Copas (50 cc)	_ _
Otros vinos	_ _	Caña (200 cc)	_ _	Chupitos/carajillos (25 cc)	_ _
Cava	_ _	Mediana (330 cc)	_ _		

Antecedentes familiares (basal y 6 meses)

¿Algún familiar directo (padres, hermanos, hijos) que haya tenido algún evento cardiovascular (angina de pecho, infarto de miocardio, embolia, ictus)?

☐ No ☐ Sí Edad del familiar: _ _

Exploración física

Talla _ _ _ cm **Peso** _ _ _ , _ Kg **Cintura** _ _ _ cm **Cadera** _ _ _ cm

PRESIÓN ARTERIAL Y FRECUENCIA CARDIACA

	Primera		Segunda		Tercera	
	PAS	PAD	PAS	PAD	PAS	PAD
Presión arterial **derecha**	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _
Frecuencia cardiaca	_ _ _		_ _ _		_ _ _	
Presión arterial **izquierda**	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _	_ _ _
Frecuencia cardiaca	_ _ _		_ _ _		_ _ _	

VASERA

R-ABI (derecho)	_ , _ _	R-CAVI (derecho)	_ _ , _ _	RbaPWV (derecho)	_ _ , _ _
L-ABI (izquierdo)	_ , _ _	L-CAVI (izquierdo)	_ _ , _ _	LbaPWV (izquierdo	_ _ , _ _

AURORA → incorporar archivo 2.5-3 MG ☐

Datos de laboratorio (basal y 6 meses)

BIOQUÍMICA		**ORINA**	
Glucosa en ayunas (mg/dL)	_ _ _	Creatinina orina (mg/dL)	_ _ _ , _
Hemoglobina glicada (%)	_ _ , _	Microalbuminuria (mg/dL)	_ _ _ , _ _
Colesterol total (mg/dL)	_ _ _		
Triglicéridos totales (mg/dL)	_ _ _ _		
Colesterol HDL (mg/dL)	_ _ _		
Colesterol LDL (mg/dL)	_ _ _		
Creatinina (mg/dL)	_ , _ _		
Insulinemia (mg/dL)	_ _ _ , _		

IPAQ (Cuestionario Internacional de Actividad Física) (basal y 6 meses)

1. Durante los últimos 7 días, ¿en cuántos realizó actividades físicas intensas tales como levantar pesos pesados, cavar, ejercicios aeróbicos o andar rápido en bicicleta?

Días por semana (indique el número) _ Ninguna actividad física intensa (pase a la pregunta 3)

2. Habitualmente, ¿cuánto tiempo en total dedicó a una actividad física intensa en uno de esos días?

Indique cuántas horas por día _ _ Indique cuántos minutos por día _ _ _ _

3. Durante los últimos 7 días, ¿en cuántos días hizo actividades físicas moderadas tales como transportar pesos livianos, o andar en bicicleta a velocidad regular? (No incluya caminar)

Días por semana (indicar el número) _ Ninguna actividad física moderada (pase a la pregunta 5)

4. Habitualmente, ¿cuánto tiempo en total dedicó a una actividad física moderada en uno de esos días?

Indique cuántas horas por día _ _ Indique cuántos minutos por día _ _ _ _

5. Durante los últimos 7 días, ¿en cuántos días caminó por lo menos 10 minutos seguidos?

Días por semana (indicar el número) _ Ninguna caminata (pase a la pregunta 7)

6. Habitualmente, ¿cuánto tiempo en total dedicó a caminar en uno de esos días?

Indique cuántas horas por día. _ _ Indique cuántos minutos por día _ _ _ _

7. Durante los últimos 7 días, ¿cuánto tiempo pasó sentado durante un día hábil?

Indique cuántas horas por día _ _ Indique cuántos minutos por día _ _ _ _

Consumo de chocolate en los últimos 7 días:

	Cantidad	¿Cuántos gramos?	Equivalencias de cacao	
☐ Chocolate negro >90%		_ _ _ g		
☐ Chocolate negro 80-89%		_ _ _ g		
☐ Chocolate negro 70-79%		_ _ _ g	Tableta	100 g
☐ Chocolate con leche		_ _ _ g	Onza	28 g
☐ Chocolate blanco		_ _ _ g	Cuadradito	6 g
☐ Otros chocolates (negro <70% cacao, cremas de untar de cacao, barritas de chocolate…)		_ _ _ g		

	Cantidad	¿Cuántos gramos?	Equivalencias de cacao	
☐ Chocolate en taza		_ _ _ g	Cucharadita	
☐ Cacao en polvo		_ _ g	De café	4 g
☐ Bombones		_ _ g	De postre	9 g
☐ Barrita de chocolate		_ _ g	Sopera	20 g
☐ Crema de cacao		_ _ g	Crema de cacao	30 g/porción tostada
			Barrita de chocolate	50 g
			Taza	100 g
			Bombón	15 g

Evaluación rendimientos cognitivos (basal y 6 meses)

Memoria verbal

	I SI	II SI	III SI	Rec. Demorado SI
Pupitre	( )	( )	( )	( )
Pastor	( )	( )	( )	( )
Gorrión	( )	( )	( )	( )
Zapato	( )	( )	( )	( )
Pipa	( )	( )	( )	( )
Montaña	( )	( )	( )	( )
Gafas	( )	( )	( )	( )
Esponja	( )	( )	( )	( )
Lámina	( )	( )	( )	( )
Barco	( )	( )	( )	( )
Cordero	( )	( )	( )	( )
Fusil	( )	( )	( )	( )
Lápiz	( )	( )	( )	( )
Iglesia	( )	( )	( )	( )
Peces	( )	( )	( )	( )
Total Palabras Recordadas	_ _	_ _	_ _	_ _

Atención y velocidad de procesamiento (Trail Making Test A y B)

	Tiempo (s)	Nº de errores
PARTE A	_ _ _	_ _
PARTE B	_ _ _	_ _

Memoria de trabajo

DÍGITOS INVERSOS			
		ACIERTO	ERROR
1	2 - 4	☐	☐
	5 - 7	☐	☐
2	6 - 2 - 9	☐	☐
	4 - 1 - 5	☐	☐
3	3 - 2 - 7 - 9	☐	☐
	4 - 9 – 6 - 8	☐	☐
4	1 - 5 - 2 - 8 - 6	☐	☐
	6 - 1 - 8 - 4 - 3	☐	☐
5	5 - 3 - 9 - 4 - 1 - 8	☐	☐
	7 - 2 - 4 - 8 - 5 - 6	☐	☐
6	8 - 1 - 2 - 9 - 3 - 6 - 5	☐	☐
	4 - 7 - 3 - 9 - 1 - 2 - 8	☐	☐
7	9 - 4 - 3 - 7 - 6 - 2 - 5 - 8	☐	☐
	7 - 2 - 8 - 1 - 9 - 6 - 5 - 3	☐	☐

¿Ha completado correctamente todas las categorías? ☐ ☐

Fluidez fonológica

Total de palabras que empiecen por la letra **F** _ _

(Registrar palabras en este espacio de abajo):

_____
_____
_____
_____

Fluidez categorial

Total Puntos: _ _

«Dígame todos los **animales** que sepa hasta que yo le diga basta»:

_____
_____
_____
_____
_____

Cuestionario de salud EUROQOL 5D (basal y 6 meses)

Marque con una X la respuesta de cada apartado que mejor describa su estado de salud hoy:

1. **Movilidad**

   ☐ No tengo problemas para caminar

   ☐ Tengo algunos problemas para caminar

   ☐ Tengo que estar en la cama

2. **Cuidado personal**

   ☐ No tengo problemas con el cuidado personal

   ☐ Tengo algunos problemas para lavarme o vestirme

   ☐ Soy incapaz de lavarme o vestirme

3. **Actividades cotidianas (trabajar, estudiar, hacer tareas domésticas, actividades familiares o actividades durante el tiempo libre)**

   ☐ No tengo problemas para realizar mis actividades cotidianas

   ☐ Tengo algunos problemas para realizar mis actividades cotidianas

   ☐ Soy incapaz de realizar mis actividades cotidianas

4. **Dolor/malestar**

   ☐ No tengo dolor ni malestar

   ☐ Tengo moderado dolor o malestar

   ☐ Tengo mucho dolor o malestar

5. **Ansiedad/depresión**

   ☐ No estoy ansioso ni deprimido

   ☐ Estoy moderadamente ansioso o deprimido

   ☐ Estoy muy ansioso o deprimido

TERMÓMETRO EUROQOL DE AUTOVALORACIÓN DEL ESTADO DE SALUD

Para ayudar a la gente a describir lo bueno o malo que es su estado de salud hemos dibujado una escala parecida a un termómetro en el cual se marca con un 100 el mejor estado de salud que pueda imaginarse y con un 0 el peor estado de salud que pueda imaginarse

Nos gustaría que nos indicara en esta escala, en su opinión, lo bueno o malo que es su estado de salud en el día de HOY. Por favor, dibuje una línea desde el casillero donde dice «Su estadode salud hoy» hasta el punto del termómetro que en su opinión indique lo bueno o malo que es su estado de salud en el día de HOY.

Su estado de salud hoy

Escala Cervantes de calidad de vida relacionada con la salud (basal y 6 meses)

1. Durante el día noto que la cabeza me va doliendo cada vez más	Nunca	0	1	2	3	4	5	Todos los días
2. No puedo más de lo nerviosa que estoy	Nunca	0	1	2	3	4	5	Constantemente
3. Noto mucho calor de repente	Nunca	0	1	2	3	4	5	En todo momento
4. Mi interés por el sexo se mantiene como siempre	Mucho menos	0	1	2	3	4	5	Igual o más
5. No consigo dormir las horas necesarias	Nunca me ocurre	0	1	2	3	4	5	Constantemente
6. Todo me aburre, incluso las cosas que antes me divertían	No es cierto	0	1	2	3	4	5	Cierto
7. Noto hormigueos en las manos y/o los pies	No, en absoluto	0	1	2	3	4	5	Insoportable
8. Me considero feliz en mi relación de pareja	Nada	0	1	2	3	4	5	Completamente
9. De pronto noto que empiezo a sudar sin que haya hecho ningún esfuerzo	Nunca	0	1	2	3	4	5	Constantemente
10. He perdido la capacidad de relajarme	No, en absoluto	0	1	2	3	4	5	Completamente
11. Aunque duermo, no consigo descansar	Nunca me ocurre	0	1	2	3	4	5	Constantemente
12. Noto como si las cosas me dieran vueltas	Nada	0	1	2	3	4	5	Mucho
13. Mi papel como esposa o pareja es...	Nada importante	0	1	2	3	4	5	Muy importante
14. Creo que retengo líquido, porque estoy hinchada	No, como siempre	0	1	2	3	4	5	Sí, mucho más
15. Estoy satisfecha con mis relaciones sexuales	Nada	0	1	2	3	4	5	Completamente
16. Noto que los músculos o las articulaciones me duelen	No, en absoluto	0	1	2	3	4	5	Dolor insoportable
17. Creo que los demás estarían mejor sin mí	No, en absoluto	0	1	2	3	4	5	Cierto
18. Me da miedo hacer esfuerzos porque se me escapa la orina	No, en absoluto	0	1	2	3	4	5	Mucho
19. Desde que me levanto me encuentro cansada	Nada	0	1	2	3	4	5	Mucho
20. Tengo tan buena salud como cualquier persona a mi edad	No, en absoluto	0	1	2	3	4	5	Igual o mejor
21. Tengo la sensación de que no sirvo para nada	Nunca	0	1	2	3	4	5	En todo momento
22. Tengo relaciones sexuales tan a menudo como antes	Mucho menos	0	1	2	3	4	5	Igual o más
23. Noto que el corazón me late muy deprisa y sin control	Nada	0	1	2	3	4	5	Mucho
24. A veces pienso que no me importaría estar muerta	Nunca	0	1	2	3	4	5	Constantemente
25. Mi salud me causa problemas con los trabajos domésticos	En absoluto	0	1	2	3	4	5	Constantemente
26. En mi relación de pareja me siento tratada de igual a igual	Nunca	0	1	2	3	4	5	Siempre
27. Siento picor en la vagina, como si estuviera demasiado seca	Nada	0	1	2	3	4	5	Mucho
28. Me siento vacía	Nunca	0	1	2	3	4	5	Siempre
29. Noto sofocaciones	Nunca	0	1	2	3	4	5	En todo momento
30. En mi vida el sexo es...	Nada importante	0	1	2	3	4	5	Extremadamente importante
31. He notado que tengo más sequedad de piel	No, como siempre	0	1	2	3	4	5	Sí, mucho más

REGISTRO DIETÉTICO 24 HORAS (basal y 6 meses). Incluir alimentos ricos en polifenoles como té, café, tipos de aceite etc…

	PRIMER DIA Fecha:	SEGUNDO DIA Fecha:	TERCER DIA Fecha:
DESAYUNO			
ALMUERZO			
COMIDA			
MERIENDA			
CENA			

Anexo V.

Informe del Comité de Ética de la Investigación con Medicamentos del Área de Salud de Salamanca

COMPLEJO ASISTENCIAL UNIVERSITARIO EISALAMANCA
Paseo de San Vicente, 58-182
37007 Salamanca
Comité Ético de Investigación Clínica
Teléfono: 923 29 11 00- Ext. 55 515

Sacyl
SANIDAD DE CASTILLA Y LEÓN

E-mail: comite.etico.husa@saludcastillayleon.es

EL COMITE DE ETICA DE LA INVESTIGACION CON MEDICAMENTOS DEL AREA DE SALUD DE SALAMANCA,

INFORMA

Que el Proyecto de Investigación presentado por D. JOSE A. MADERUELO FERNANDEZ,

Titulado:

"Efecto de añadir chocolate con alto porcentaje de cacao y polifenoles a la dieta habitual sobre la presión arter ial, función vascular, calidad de vida y rendimientos cognitivos en mujeres postmenopáusicas. Ensayo clínico aleatorio".

Que presenta como Investigador responsable, SE AJUSTA A LAS NORMAS ÉTICAS Y DE BUENA PRÁCTICA CLÍNICA, establecidas para tales estudios.

Código CEIC: PI11812/2017.

Y para que conste lo firma en Salamanca con fecha 26 de febrero de 2018.

LA SECRETARIA TÉCNICA

COMPLEJO ASISTENCIAL UNIVERSITARIO SALAMANCA
COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS

Fdo.: D.ª Belén Vidri L .cfüillé

Anexo VI.

Consentimiento informado y hoja de información al paciente

HOJA DE INFORMACIÓN

Título del estudio: **Efecto de añadir chocolate con alto porcentaje de cacao y polifenoles a la dieta habitual sobre la presión arterial, función vascular, calidad de vida y rendimientos cognitivos en mujeres posmenopáusicas. Ensayo clínico aleatorio**

Usted ha sido invitada a participar en un estudio de investigación. Antes de confirmar su participación, es importante que entienda en qué consiste. Por favor, lea detenidamente este documento y pregunte todas las dudas que le puedan surgir.

Objetivo del estudio:
Evaluar el efecto de una intervención consistente en suplementar la dieta habitual con una dosis de chocolate con alto porcentaje de cacao y polifenoles sobre la presión arterial, función vascular, calidad de vida y rendimientos cognitivos en mujeres posmenopáusicas.

Procedimientos del estudio:
El médico/investigador valorará si usted es una candidata adecuada para este estudio. Una vez haya otorgado su consentimiento y el investigador haya verificado que cumple los criterios para participar en el presente estudio, realizará una visita de 1 hora y media de duración que consistirá en unas preguntas sobre su salud, actividad física, alimentación y calidad de vida y se realizarán las exploraciones que se detallan a continuación: determinación de la composición corporal, medidas de la función vascular (velocidad de la onda de pulso e índice cardio-tobillo) y medición de la presión arterial clínica. Además, se le hará una extracción de sangre para la determinación de parámetros analíticos básicos y se evaluarán los rendimientos cognitivos mediante una serie de cuestionarios breves. Estas mediciones se realizarán también a los 3 y 6 meses desde la primera visita.
Tras la primera visita, las participantes serán distribuidas aleatoriamente (al azar) en dos grupos:
Grupo 1: Las participantes de este grupo no recibirán ninguna cantidad de chocolate y continuarán con su atención habitual.
Grupo 2: Las participantes de este grupo recibirán chocolate (99% de cacao) con las instrucciones de tomar diariamente 10 g añadidos a su alimentación habitual durante 6 meses. Este chocolate, debido a su alto contenido en cacao tiene un sabor menos dulce y algo más amargo de lo habitual.

Beneficios y riesgos esperados:
El beneficio para usted será conocer su presión arterial, otros factores de riesgo cardiovascular y el envejecimiento de sus arterias. Se le informará de los resultados de las exploraciones realizadas. Las exploraciones que se realizan no conllevan riesgo vital alguno, únicamente la incomodidad que pueda suponer la duración de la realización de las pruebas (una hora y media), ninguna de ellas invasiva, a excepción de la extracción de sangre.

Confidencialidad:

Si usted accede a colaborar en este estudio, debe saber que algunos datos recogidos serán incorporados a una base de datos informatizada sin incluir su nombre u otro elemento que permita su identificación.

Ninguna participante será identificada personalmente en la comunicación y publicación de los resultados. Sus documentos médicos podrían ser revisados por personas dependientes de las autoridades sanitarias, miembros de comités éticos independientes y otras personas designadas por ley para comprobar que el estudio se está llevando a cabo correctamente. Todos sus datos se mantendrán estrictamente confidenciales, y no podrán ser divulgados por ningún medio, conservando en todo momento la confidencialidad investigadora/participante (Ley de Protección de datos 15/1999).

Se atenderá cualquier imprevisto, urgencia o problema sobreañadido o de nueva aparición durante el curso del estudio.

Preguntas / Información

Si desea hacer alguna pregunta o aclarar algún tema relacionado con el estudio, o si precisa ayuda por cualquier problema de salud relacionado con este estudio, por favor, no dude en ponerse en contacto con el personal del estudio en el centro donde fue atendido o llamando por teléfono al 923231859.

HOJA DE CONSENTIMIENTO INFORMADO

D/Dña....., médico/investigador he informado de todo lo anterior al firmante, aclarando sus dudas y apreciando su entendimiento de todos los términos expuestos.

Firma Fecha

Dña.:....., habiendo recibido toda la información pertinente, comprendo que mi participación es voluntaria y que puedo retirarme del estudio cuando quiera, sin tener que dar explicaciones y sin que esto repercuta en mi atención médica. Doy libremente mi conformidad para participar en el estudio en el día de hoy,

En....., a..... de..... de 20….

Firma Fecha

Anexo VII.

Comunicaciones presentadas a congresos

ESC
European Society
of Cardiology

CERTIFICATE

This is to certify that

Assistant Professor JI Recio-Rodriguez

(Salamanca - Spain)

has presented an abstract entitled

Effect of adding chocolate with high cocoa content to the usual diet of postmenopausal women on blood pressure and arterial stiffness markers. Randomized clinical trial

in the session entitled

Moderated poster session - Public health and cardiac rehabilitation

On Friday 03 May 2019, from 10:00 to 11:30, in Moderated Poster Area -

during the

EuroHeartCare 2019

in Milan - Italy

European Society of Cardiology

Isabel Bardinet
Chief Executive Officer

Abstract: **25**

Effect of adding chocolate with high cocoa content to the usual diet of postmenopausal women on blood pressure and arterial stiffness markers. Randomized clinical trial

Authors:
J I Recio-Rodriguez[1], I Garcia-Yu[2], B Sanchez-Salgado[3], L Garcia-Ortiz[3], C Agudo-Conde[3], M Gomez-Marcos[3], O Tamayo-Morales[3], J Gonzalez-Sanchez[3], E Rodriguez-Sanchez[3], C Lugones-Sanchez[3], J A Maderuelo-Fernandez[3], [1]Universidad de Burgos, Facultad de Ciencias de la Salud - Burgos - Spain, [2]Gerencia de Atencion Primaria - Burgos - Spain, [3]Primary Health Care Research Unit, La Alamedilla Health Center, IBSAL - Salamanca - Spain,

On behalf: La Alamedilla Primary Care Research Unit

Topic(s):
Hypertension – Treatment

Citation:

Funding Acknowledgements:
This study was supported in part by grants funded by la Gerencia Regional de Castilla y León (GRS 1583/B/17)

BACKGROUND

The intake of polyphenols has shown certain effects on cardiovascular health, especially in populations with an increased cardiovascular risk.

PURPOSE

This work evaluates the effect of adding 10g. of chocolate with 99% cocoa content to the usual diet, on blood pressure and arterial stiffness markers in postmenopausal women.

METHODS

Randomized clinical trial with two parallel groups that included 61 women (26 control and 35 intervention) between 50-64 years in postmenopausal period contrasted by amenorrhea of 12 months. Personal history of cardiovascular disease and presence of high blood pressure, diabetes mellitus or dyslipidemia were exclusion criteria. Blood pressure was measured with an oscillometric device and the brachial-ankle pulse wave velocity (baPWV) and the cardio-ankle vascular index (CAVI). The intervention group received instructions for the daily intake of 10 g. of chocolate with 99% of cocoa added to your usual diet, for 3 months. The nutritional contribution of this product is 59 kcal and 65.4 mg of polyphenols per day. There was no intervention in the control group. All the variables were measured in the baseline assessment and at 3 months post-randomization. The recruitment began in June 2018.

RESULTS

The mean age was 58.3±3.6 years. The values of systolic blood pressure-SBP 109.0±13.9, baPWV 7.95±1.00 and CAVI 4.57±0.52 ($p>0.05$ in all, with the exception of CAVI (Intervention 4.39±0.47, control 4.79±0.50, $p=0.002$). No differences were found between groups in the evolution of any of the variables studied after adjustment for the baseline value (SBP $p=0.290$, baPWV $p=0.782$, CAVI $p=0.502$). The control group decreased SBP -2.9 (-0.4 to 6.2), $p>0.05$, and baPWV -0.26 (-0.04 to -0.49), $p<0.05$. The intervention group decreased SBP -5.0 (-1.1 to -8.9), $p=0.012$, and the baPWV -0.22 (-0.04 to -0.40), $p=0.016$. Both groups slightly increase their CAVI values without reaching statistical significance.

CONCLUSIONS Add 10 g. of chocolate with a high proportion of cocoa to the usual diet of postmenopausal women for 3 months, does not provide additional benefits against non-supplementation, in the blood pressure figures, baPWV and CAVI. Although the reduction is stronger in the case of the experimental group for the SBP and the baPWV. More studies, with more women and more follow-up, are necessary to evaluate the effects of cocoa polyphenols on the cardiovascular health of postmenopausal women.

Anexo VIII.

Índices de calidad de las publicaciones aportadas

Índices de calidad de las publicaciones (JCR)

Revista	Factor de impacto	Categoría	Nº. de revistas en la categoría	Puesto en la categoría	Cuartil	Publicación
BMJ Open (2018)	2.376	Medicine, General & Internal	160	50	Q2	Garcia-Yu IA, Garcia-Ortiz L, Gómez-Marcos MA, Alonso-Dominguez R, Gonzalez-Sanchez J, Mora-Simon S, González-Manzano S, Rodriguez-Sanchez E, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: a study protocol for a randomised clinical trial. BMJ Open. 2018 Dec 14;8(12):e024095. doi: 10.1136/bmjopen-2018-024095.
Nutrients (2019)	4.546	Nutrition & Dietetics	89	17	Q1	Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Agudo-Conde C, Gonzalez-Sanchez J, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Effects of Cocoa-Rich Chocolate on Blood Pressure, Cardiovascular Risk Factors, and Arterial Stiffness in Postmenopausal Women: A Randomized Clinical Trial. Nutrients. 2020 Jun 12;12(6):1758. doi: 10.3390/nu12061758.
Nutrients (2019)	4.546	Nutrition & Dietetics	89	17	Q1	Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Tamayo-Morales O, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Cocoa-Rich Chocolate and Quality of Life in Postmenopausal Women: A Randomized Clinical Trial. Nutrients. 2020 Sep 10;12(9):2754. doi: 10.3390/nu12092754.
British Journal of Nutrition (2019)	3.334	Nutrition & Dietetics	89	40	Q2	Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Lugones-Sanchez C, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Cocoa-rich chocolate and body composition in postmenopausal women: a randomised clinical trial. Br J Nutr. 2021 Mar 14;125(5):548-556. doi: 10.1017/S0007114520003086. Epub 2020 Aug 4.
Nutritional Neuroscience (2019)	4.028	Neurosciences	272	88		Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, Rodriguez-Sanchez E, Mora-Simon S, Maderuelo-Fernandez JA, Recio-Rodriguez JI. Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial. Nutr Neurosci. 2020 Nov 15:1-12. doi: 10.1080/1028415X.2020.1840119. Epub ahead of print.
		Nutrition & Dietetics	89	25	Q2	